X doesn't mark the spot: Role of the X chromosome in improved immunity in *Drosophila melanogaster*

Amisha Agarwala
MS15052

A dissertation submitted for the partial fulfilment of
BS-MS dual degree in Science

Indian Institute of Science Education and Research Mohali
May 2020

Certificate of Examination

This is to certify that the dissertation entitled "**X doesn't mark the spot: Role of the X chromosome in improved immunity in *Drosophila melanogaster***" submitted by Amisha Agarwala (Reg No. MS15052) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Manjari Jain Dr. Rhitoban Ray Choudhury Dr. N.G. Prasad

(Supervisor)

Dated:

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. N.G. Prasad at the Indian Institute of Science Education and Research, Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Amisha Agarwala

(Candidate)

Dated:

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge

Dr. N.G. Prasad

(Supervisor)

Acknowledgement

I'll begin by thanking IISER Mohali and the members of my thesis committee, Dr. Manjari Jain and Dr. Rhitoban Ray Choudhury for the guidance and support I received through the year of the thesis. I'd also like to thank DST for the KVPY Fellowship.

To Dr. N.G Prasad, for being my supervisor for this thesis, and an additional five years of mentoring, thank you for making the lab a family.

The other parts of the "we" that makes this thesis are Manas, TJ, Jigisha and Mayank, without whom there is no earthly way I would have finished the experiments described within. There are many other reasons to thank them and this is perhaps only the most relevant.

I would like to thank Dr. Pierre Cornelis of the Free University of Brussels, Belgium, who provided us with the bacterial strain used here, Prof. Adam Chippindale for generously providing us with the Clone Generator flies.

The PhDs of the lab who've taught me various fly (and life) skills deserve a lot of gratitude and credit for keeping the lab running: Manas, TJ, Rohit, Neetika, Komal, Ruchika and AKB. The same credit is due to many past members of EBL: Radhika, Martik, Karan Bhatt, Prakhar, Aatashi, Harsha and Zeeshan. And next, the best lab mates anybody could ask for- Aaditya, Abhishek, Jigisha, Imran, Manu, Paresh, Santy, Sohit, Mayank, Broti and Poddar. From playing far too much UNO to cooking ridiculous amounts of fly food, help and fun were always around. Our lab would grind to a halt without Nagender and Negi bhaiya, who wash enough vials, bottles and cages to keep us going.

When the fly work brings me to the brink of insanity, to friends who keep me sane outside the lab: Lakshmi, Lipika, Pankhuri, Nikhil, Aditya, Fidha, Dhanvin, Hari, y'all are amazing. In the same vein, I also have a lot of appreciation for the incredible people who inhabit my floor: Ardra, Ruchira, Shradha and Soumya.

And the final thanks to family. My father- for showing me the many wonders of science (though I imagine it was physics he had in mind), my mother- for the help in getting here, with the belief that I could, and Aryan- for occasionally fetching me whatever I've left in the other room and putting up with my cabin fever while writing this thesis.

List of Figures

Figure 1: *Drosophila melanogaster* life cycle 3

Figure 2: Karyotype of *D. melanogaster.* 4

Figure 3: IUS Maintenance regime 6

Figure 4: Schematic representation of the required crosses. 8

Figure 5: Experimental Protocol for the survivorship assay 10

Figure 6: Survivorship Curves 14

Figure 7: Effect of Sex and Selection Regime on Median Time to Death 15

Figure 8: Effect of Sex and Selection Regime on Proportion Survivorship 16

Figure 9: Male- Female correlation (Median Time to Death) 17

Figure 10: Male- Female Correlation (Proportion Survivorship) 18

List of Tables

Table 1: Composition of 1 litre banana-jaggery food 7

Table 2: Composition of 1 litre cornmeal-molasses-yeast food 7

Table 3: Logistic Regression 19

Table 4: Median Time to Death (GLMM) 19

Table 5: Proportion Survivorship (GLMM) 19

Table 6: Cox's Proportional Hazards (combined blocks) 20

Table 7: Cox's Proportional Hazards 21

Table 8: Male- Female Correlation (Median Time to Death) 22

Table 9: Male- Female Correlation (Proportion Survivorship) 23

Contents

List of Figures i

List of Tables iii

Abstract vii

Chapter 1 : Introduction 1

Chapter 2 : Experimental System 3

Chapter 3 : Methods 9

Chapter 4 : Statistical Analysis 11

Chapter 5 : Result 13

Chapter 6 : Discussion 25

References 27

Abstract

We investigated X-linked variation for immune response, and its role in sexually dimorphic immune defenses. Immunity has been shown to be subject to Intralocus Sexual Conflict (IaSC), and it is reported that sexually antagonistic variation is likely to be concentrated on the X chromosome. We used laboratory-based populations of *Drosophila melanogaster* selected for increased survivorship against *Pseudomonas entomophila*, a gram-negative bacterial pathogen. After 160 generations of selection, X chromosomes were cloned from I (selected) and S (control) populations, and expressed in flies where the other chromosomes came from the ancestral baseline population to create 30 X chromosome lines respectively. To determine the result of selection on the X chromosome in these populations, we subjected male and female flies from these lines to a *P. entomophila* infection and assayed their survivorship for 96 hours post-infection.

We were unable to detect any effect of the X chromosome on the immune response in these populations as there was no difference in survivorship post-infection of flies carrying the X chromosome from the selected or control population.

Chapter 1 : Introduction

In sexually reproducing organisms, the evolutionary interests of males and females often diverge, leading to sexual conflict. Sexual conflict is broadly categorised as Inter-locus (IeSC) or Intra-locus (IaSC) sexual conflict (Chapman et al., 2003; reviewed by Schenkel et al., 2018). This project focuses on IaSC, where fitness optima for shared phenotypes with a common genetic basis differ in males and females. The variation at such loci is called sexually antagonistic variation. One of the ways IaSc can be resolved is the sex-specific expression of antagonistic alleles, which drives the evolution of sexual dimorphism. (Bonduriansky & Chenoweth, 2009)

Multiple laboratory studies have demonstrated sexual antagonism by finding a negative correlation between the fitness of the two sexes in a ground cricket (Fedorka & Mousseau, 2004), Red deer (Foerster et al., 2007), plants (Delph et al., 2004), side-blotched lizards (Calsbeek & Sinervo, 2003), mountain goats (Mainguy et al., 2009), collared flycatchers (Brommer et al., 2007) and zebrafinches (Price & Burley, 1993). A large number of studies perform this analysis in *Drosophila melanogaster* by hemiclonal analysis (Bedhomme et al., 2008; Chippindale et al., 2001; Innocenti & Morrow, 2010; Long & Rice, 2007; Pischedda & Chippindale, 2006; Prasad et al., 2007). Multiple traits under IaSC have also been identified: locomotor activity in *D. melanogaster* (Long & Rice, 2007), bill colour and fitness in zebra finches (Simons et al., 2012), body size in flycatchers (Merilä et al., 1997).

Immunity has also been demonstrated to be under IaSC, majorly in two studies. Vincent & Sharp (2014) demonstrated a negative genetic correlation between the two sexes for resistance and tolerance, two key components of immunity in *D. melanogaster*; Another study found that in side blotched lizards, *Una stansburiana*, orange throats and high antibody responses enhanced survival in males, but reduced fitness in females (Svensson et al., 2009).

In XY systems, it is predicted that sexually antagonistic variation is concentrated on the X chromosome. Specifically, male beneficial recessive alleles and female beneficial dominant alleles are predicted to accumulate on the X chromosome (Fitzpatrick, 2004; Gibson et al., 2002; Lindholm & Breden, 2002). Charlesworth et al., (1987) showed that provided mutations are recessive, or partially recessive, adaptation fixes favourable alleles on X- and Y- linked loci faster than autosomal loci. Rice, (1984) found that X chromosomes are likely to disproportionately accumulate sexually antagonistic alleles compared to autosomes (But see Fry (2010)).

Vanika Gupta (2015) established laboratory populations of *D. melanogaster* selected against a systemic infection by *Pseudomonas entomophila.* In response to the selection, immune response improved (in terms of survivorship post infection), but in a sex specific manner. Females evolved increased resistance, while males evolved increased tolerance, prompting the hypothesis that a significant fraction of the loci involved in the improvement were located on the X chromosome. Manas Samant (2015) tested this hypothesis by setting up crosses between the control and selected populations and testing the immune response of the F1 hybrid males. He detected no effect of the X chromosome on immune response, as males from the two reciprocal crosses had indistinguishable immune response. This method assumes that the Y chromosome does not carry genes that control the immune response. However, Kutch & Fedorka (2015) report Y-linked variation that regulates X-linked and autosomal immune response genes, which means the results of the study could be confounded by the effects of the Y chromosome.

In this study we examine the role of the X chromosome in adaptation against a pathogenic challenge by *Pseudomonas entomophila*. We use cytogenetic cloning to sample X chromosomes from the selected and control populations and express them flies that otherwise carry the genome of the ancestral baseline population. The immune response of these flies is assayed, quantified as their survivorship post an infectious challenge by *P. entomophila.*

Chapter 2 : Experimental System

For this project, we use the fruit fly *Drosophila melanogaster* (Phylum: Arthropoda, Class: Insecta, Order: Diptera, Family: Drosophilidae) as our model system.

In the wild, *D. melanogaster* adults feed on overripe or rotten fruit. Eggs are laid on fruits as well and larvae eat the food they were laid on. *D. melanogaster* is a holometabolous insect - its life cycle has four stages: egg, larva, pupa and the adult fly (Figure 1).

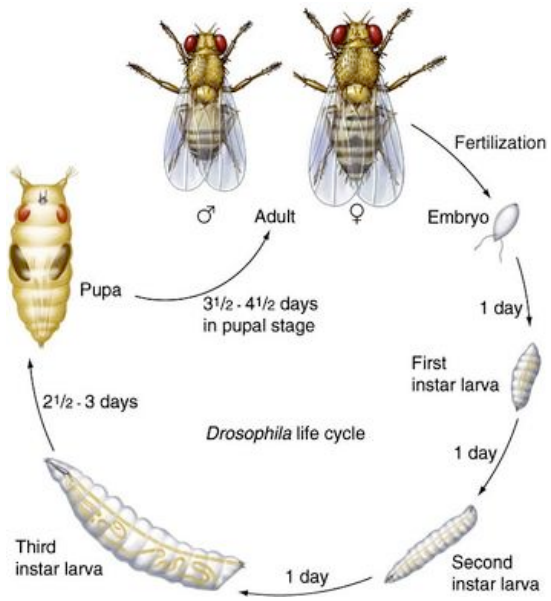

Figure 1: *Drosophila melanogaster* life cycle

D. melanogaster is a widely used model organism in genetic studies. Apart from its short generation time, low maintenance cost and small size, the use of *D. melanogaster* historically has led to well-established genetic tools for research (like balancer chromosomes). The abundance of phenotypic markers and its genetic tractability are critical to this study.

The genome of *D. melanogaster* is approximately 180 Mb (Adams, 2000), organised into 4 pairs of chromosomes (Figure 2) : the first pair is sex chromosomes, the remaining 3 are autosomes. Chromosomes 2 and 3 are large metacentric chromosomes while chromosome 4 is a small dot chromosome (Deng et al., 2007). Sex determination is governed by the "dosage" of X chromosomes, i.e., by an X counting mechanism. Normal females are XX and males are XY, however, unlike in humans, the Y chromosome does not directly determine sex (Bridges, (1925) but see Erickson & Quintero, (2007)).

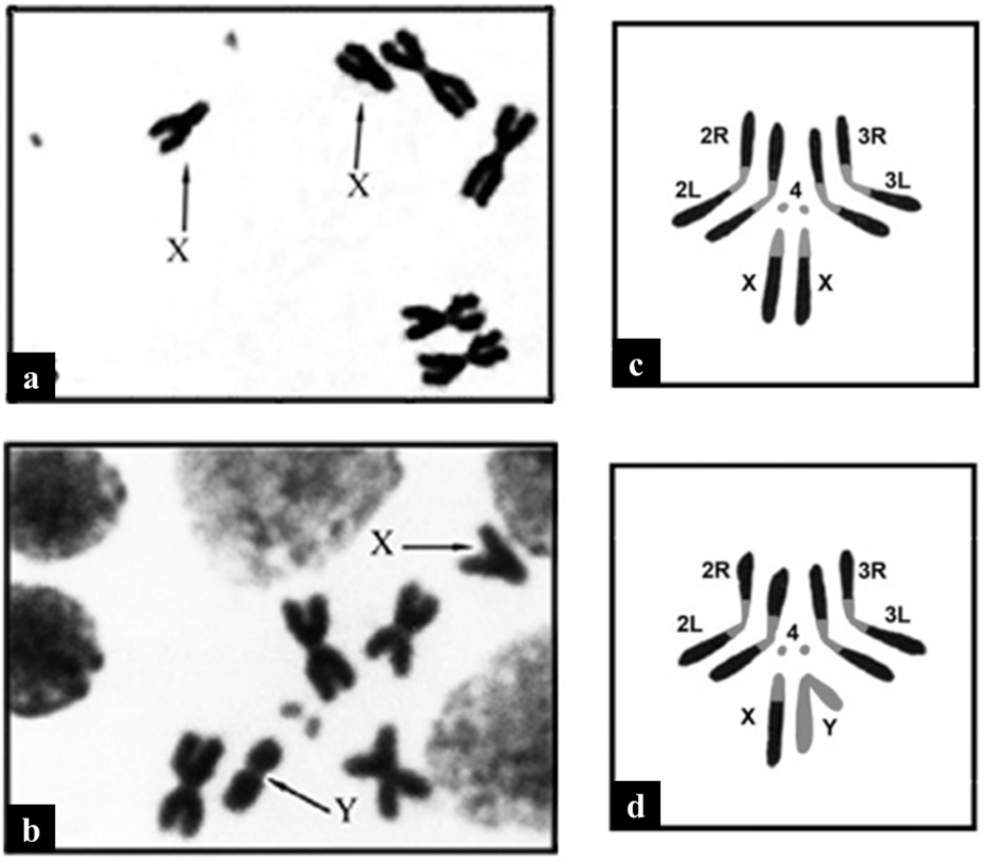

Figure 2: Karyotype of *D. melanogaster.* Metaphase chromosomes of a) D. melanogaster ♀ b) D. melanogaster ♂. Representation of karyotype of c) D. melanogaster ♀ d) D. melanogaster ♂. Modified from (Deng et al., 2007; Kaufman, 2017)

Fly stocks:

All stocks used are maintained on banana-jaggery food (Table 1) unless mentioned otherwise.

a. **BRB:** BRB was established in 2011 at IISER Mohali by combining 100 males and females from 19 isofemale lines (initially maintained in the laboratory of Dr. Daniel Promislow at University of Washington). BRB is an outbred population, maintained on a 14 day discrete generation cycle, 12:12 Light:Dark regime, 25°C and 60-70% Relative Humidity. After 10 generations of maintenance, 5 replicates, BRB 1-5, were derived. These replicates are independently maintained under the above laboratory conditions. (Singh et al., 2015)
b. **IUS:** At the time of the experiment, IUS had undergone over 160 generations of selection**.** The IUS_{1-4} populations were derived from the respective BRB_{1-4} population. They are maintained under the same laboratory conditions as the BRB populations. The maintenance of IUS populations has been detailed by Gupta et al., (2016)**.** On the 12^{th} day post egg collection, flies are anaesthetised using CO_2 and subjected to the required selection pressure. I flies are infected with *Pseudomonas entomophila* (as per the infection protocol described in Chapter 3) at an OD_{600} such that mortality is maintained at 33%. S flies are sham infected, and U flies are simply sorted under anaesthesia (summarized in Figure 3). Populations with the same numerical subscript are handled on the same day and are related by ancestry. They therefore also compromise statistical blocks.

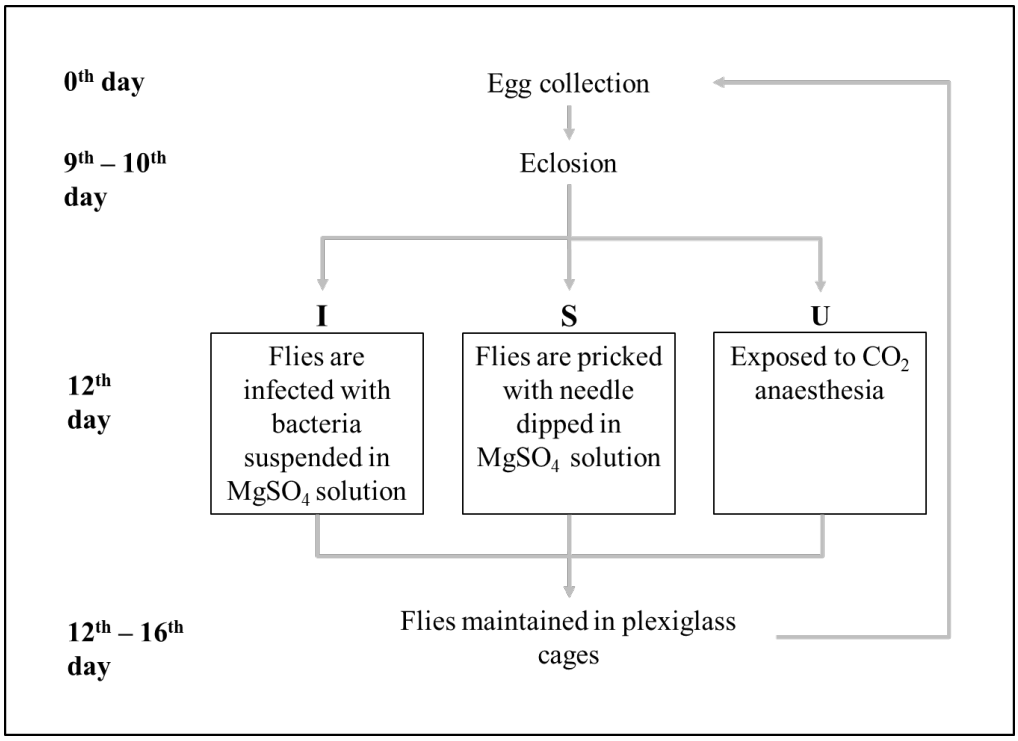

Figure 3: IUS Maintenance regime

c. **DxBRB:** The DxBRB population was created by backcrossing the compound X chromosome from Clone Generators into BRB-1. This population is maintained like BRB populations and is regularly backcrossed with BRB-1 to maintain genetic homogeneity.

d. **Clone generators (CG):** Clone generator females carry a compound X [C(1)DX *y f*] chromosome, Y chromosome, and a homozygous viable translocation of two autosomes [T(2;3) *rdgC st in ri* p^P bw^D]

   Males have an X [*sn su(b)*] chromosome, Y chromosome and the same translocated autosomes. (Rice, 1996)

   In this system, females inherit the compound X chromosome from their mother and a Y chromosome from their father. Males inherit the Y chromosome from their mother and the X chromosome from their father. Clone generators are maintained on cornmeal-molasses-yeast food (Table 2).

Table 1: Composition of 1 litre banana-jaggery food

Ingredient	Amount	
Water	1180	ml
Banana	205	g
Barley flour	25	g
Jaggery	35	g
Yeast	36	g
Agar	12.4	g
Ethanol	45	ml
p-hydroxymethyl benzoate	2.4	g

Table 2: Composition of 1 litre cornmeal-molasses-yeast food

Ingredient	Amount	
Water	1100	ml
Cornmeal	100	g
Molasses	100	g
Yeast	41.2	g
Agar	14.8	g
p-hydroxymethyl benzoate	2.4	g
Ethanol	45	ml
Propionic Acid	8	ml

Figure 4: Schematic representation of the required crosses. The translocated 2nd and 3rd chromosomes are represented by a black bar since they must be inherited together. X, Y, II and III represent the respective chromosomes. X.X represents the compound X chromosome. The subscript represents the fly stock/selection regime from which the chromsome originates.

Chapter 3 : Methods

Cytogenetic Cloning: Through appropriate crosses, the chromosomal constructs in the Clone Generator (CG) flies allow for replacement of cII and cIII. Therefore the required sex chromosome can be represented in a neutral background where 99.5% of the genome of the flies is controlled (excluding cIV, the dot chromosome). The following properties of the system allow such manipulation of the genome:

1) There is no molecular recombination in *Drosophila melanogaster* males
2) The two translocated autosomes must be inherited together; zygotes that do not are inviable
3) Zygotes carrying no X chromosomes or three X chromosomes are inviable
4) Zygotes with the compound X chromosome and a Y chromosome are female

Sampling X chromosomes: After 160 generations of selection, X chromosomes were sampled from selected (I) and control (S) populations. Initially, 30 X chromosomes were randomly sampled from each population (and replicate block), each used to create a single X chromosome line. Corresponding replicates of I and S were always handled together. In order to clone the X chromosome into a BRB autosomal background, the following crosses were made:

1. IUS ♂ x CG ♀ ⟶ XResult1 (Males) + other progeny
2. XR1 ♂ x DxBRB ♀ ⟶ XResult2 (Brown eyed males) + other progeny
3. XR2 ♂ x DxBRB ♀ ⟶ XResult3♂ (Red eyed males) + other progeny
4. XR3 ♂ x DxBRB ♀ ⟶ XResult4♂ (Males) + other progeny

   XR3 ♂ x BRB-1 ♀ ⟶ XResult4♀ (Females) + other progeny

1. A single I or S male was combined with 10 CG females in a food vial supplied with yeast granules, and allowed to interact for 48 hours, during which females oviposited. DxBRB flies required for the next cross were collected as eggs the same day.
2. For the 2nd, 3rd and 4th crosses, the required progeny from the previous crosses were combined with 5 DxBRB or BRB-1 females in fresh food vials, and allowed a 48 hour window to mate and oviposit, after which they were discarded. Flies required for the next cross were collected as eggs the same day

A single vial per X-line was maintained for all crosses. Egg densities were maintained such that viable eggs numbered 70 eggs/vial. Crosses were set on the 12th day post egg collection. All flies were maintained on standard banana jaggery food (Table 1) when crossed. A detailed account of the genomes involved in each cross is detailed in Figure 4.

Protocol for infections: For the infection treatment, flies anaesthetised with CO_2 are pricked in the thorax with a needle (Minutein pin 0.1 mm, Fine Science Tools, CA) dipped in bacterial solution. For these experiments, flies were infected with *Pseudomonas entomophila* (OD_{600}=1, suspended in 10mM $MgSO_4$). The sham infection treatment is similar, except the needle is dipped in sterile $MgSO_4$ solution.

Survivorship assay: Progeny flies from the 4th cross were sorted on the 11th day post oviposition, and maintained in same sex vials at a density of 10/ vial.

On the 12th day, 4 infectors infected experimental flies and transferred to fresh vials at a density of 8/vial. 20 X-lines of each selection regime (I and S) were infected, with 3 vials of the infected treatment and 1 control sham treatment per X-line. Mortality was monitored for 96 hours following the infection. Flies were transferred to fresh food after 2 days.

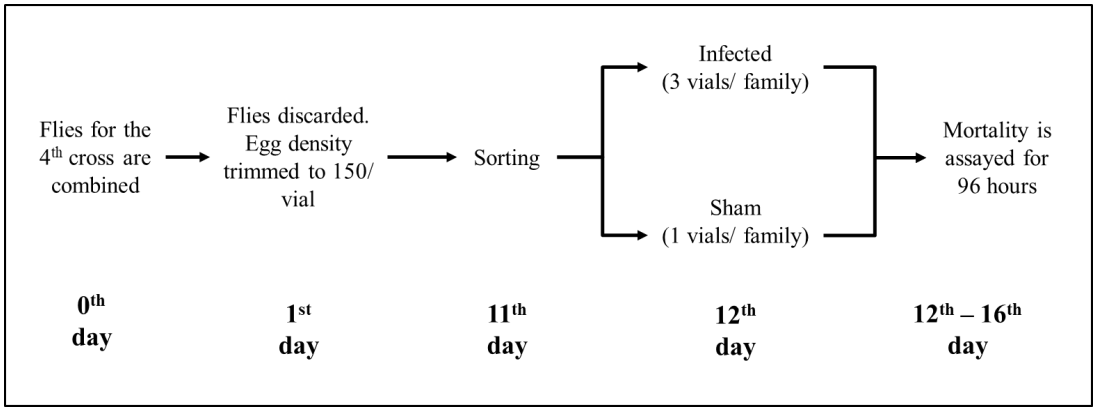

Figure 5: Experimental Protocol for the survivorship assay

Chapter 4 : Statistical Analysis

We performed four different analyses for this data.

We calculated proportion survivorship at the end of the 96-hour observation window and the median time to death for each vial. We then fit the following linear mixed effects model for these two quantities using the R package lme4:

Y ~ SelectionRegime + Sex + SelectionRegime:Sex + (1 | Infector) + (1 | Block) + (1 | X-line:SelectionRegime:Block)

We fit the following logistic regression on the status (dead or alive) of each fly at the end of the 96-hour observation window:

Status ~ Selection Regime + Sex + SelectionRegime:Sex + (1 | Infector) + (1 | Block) + (1 | X-line:Block:SelectionRegime)

We also fit the following cox's proportional hazards model:

Time to Death ~ SelectionRegime+ Sex + (1 | Block) +(1 | Xline:SelectionRegime:Block) + (1 | Infector)

And the following cox's proportional hazards model separately for each block:

Time to Death ~ SelectionRegime + Sex + SelectionRegime:Sex + (1 | Infector) + (1 | X-line:SelectionRegime)

Lastly, we calculated the average median time to death and proportion survivorship for each X-line in both the sexes. Average median time to death was calculated as the mean of median time to death across the all vials of an X-line.

For these two read-outs of immunity, we calculated the correlation between male and female immunity.

We fit the following linear model separately for each combination of selection regime and block:

FemaleMeasure ~ MaleMeasure

We also calculated correlation using Spearman's Rank Correlation

Chapter 5 : Result

For the Cox Proportional Hazards model, Wald's test did not find any effect of selection regime, sex or their interaction in any of the four blocks (Figure 6, Table 6, Table 7)

The logistic regression and the linear mixed effects model for proportion survivorship show a significant effect of sex, where males have a slightly higher survivorship than females, but no effect of selection or its interaction with sex (Figure 8, Table 3, Table 5).

The linear mixed effects model for median time to death did not find identify any effect of selection, sex or their interaction (Figure 7, Table 4). The linear mixed effects model for median time to death and proportion survivorship also did not find any effect of X chromosome line. A large part of the variation seen is explained by Block and Infector effects.

Spearman's rank correlation did not detect a significant correlation between male and female (of the same X chromosome line) proportion survivorship (Table 9) or median time to death (Table 8) in any of the eight selection regime × block combinations. Similarly, the linear models of median time to death (Figure 9, Table 8) and proportion survivorship (Figure 10, Table 9) did not find a significant effect of the male measure on the female measure (except for proportion survivorship in I_2).

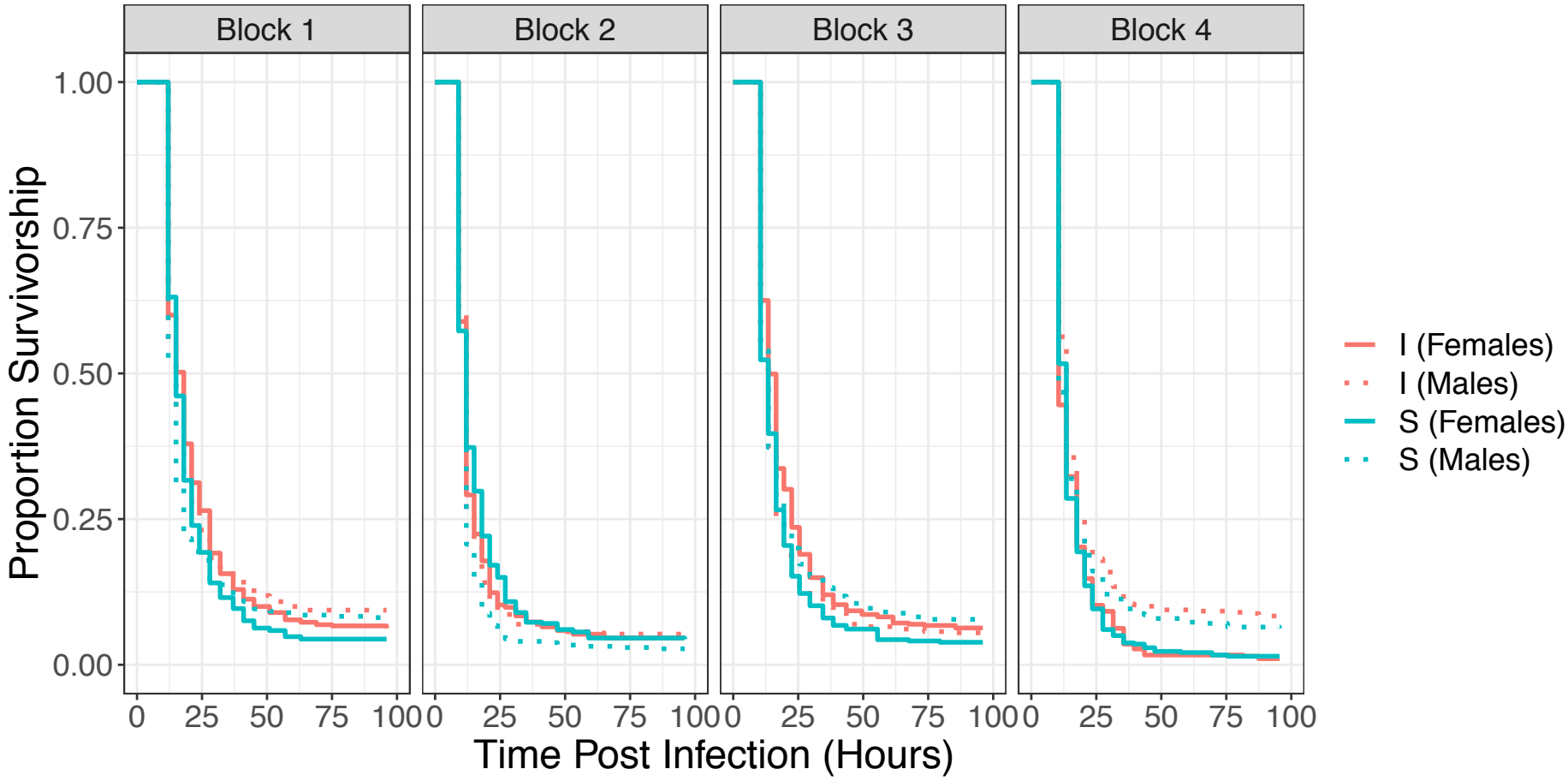

Figure 6: Survivorship Curves.

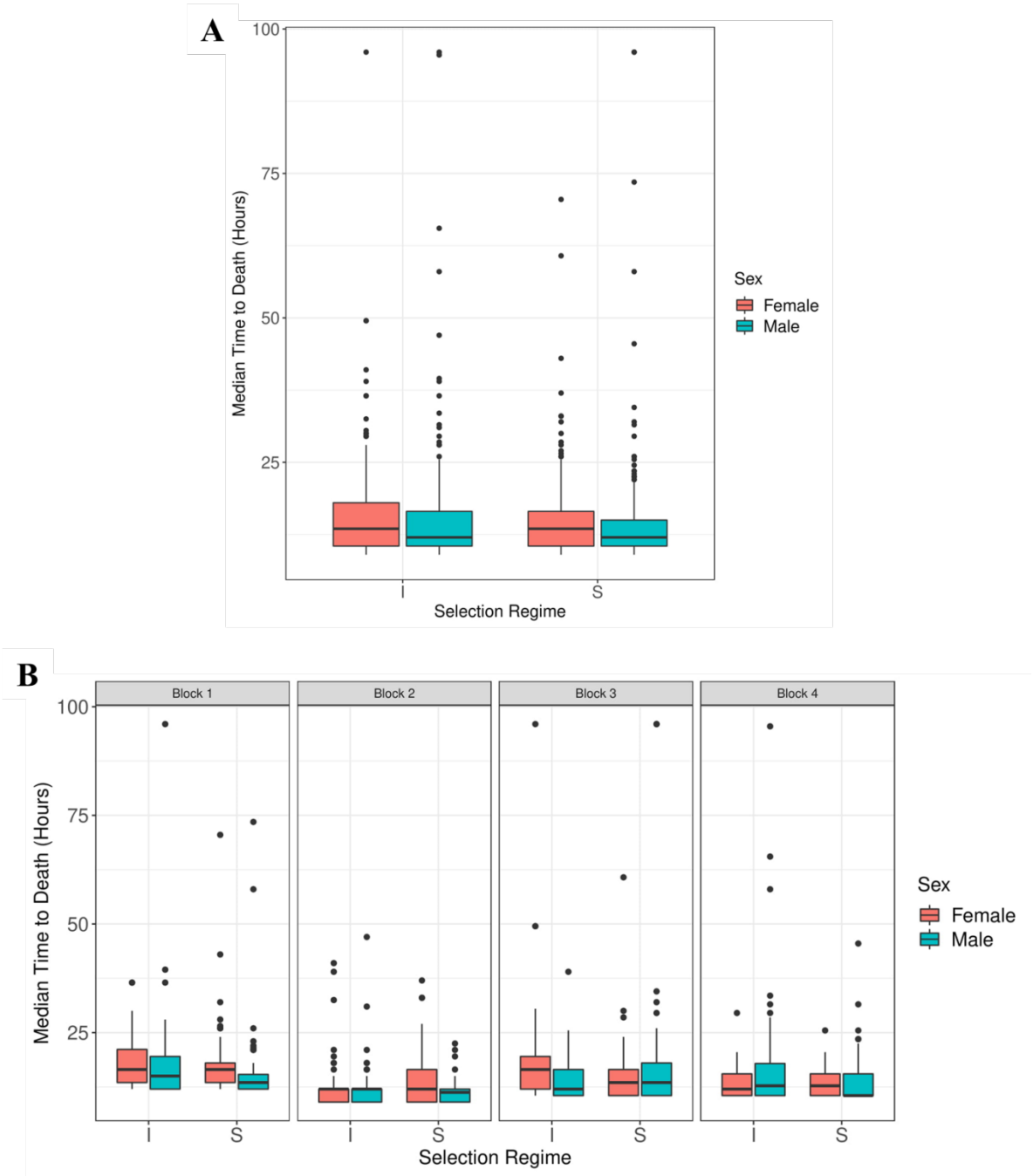

Figure 7: Effect of Sex and Selection Regime on Median Time to Death A) Data from all blocks pooled B) All blocks analysed separately

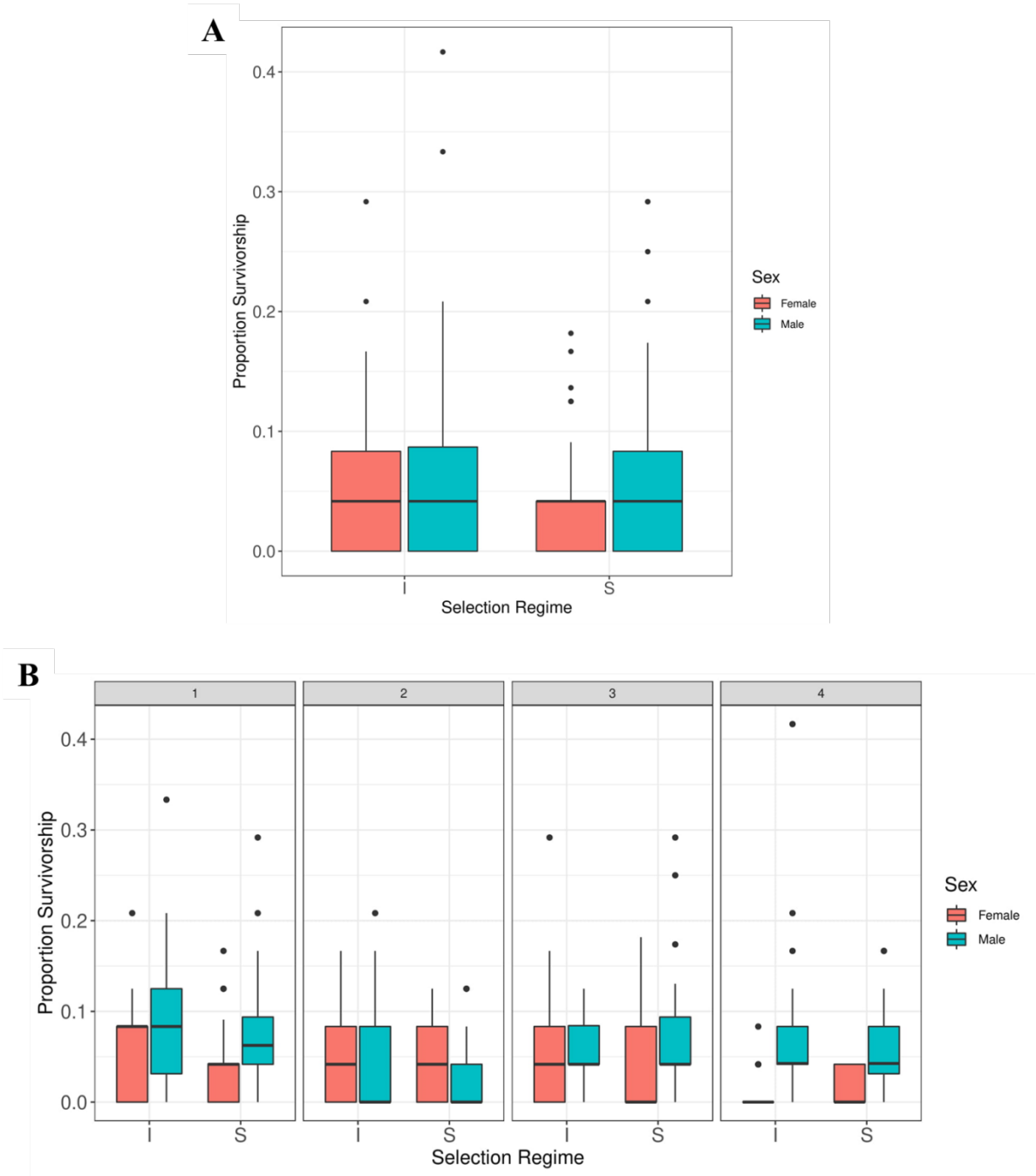

Figure 8: Effect of Sex and Selection Regime on Proportion Survivorship A) Data from all blocks pooled B) All blocks analysed separately.

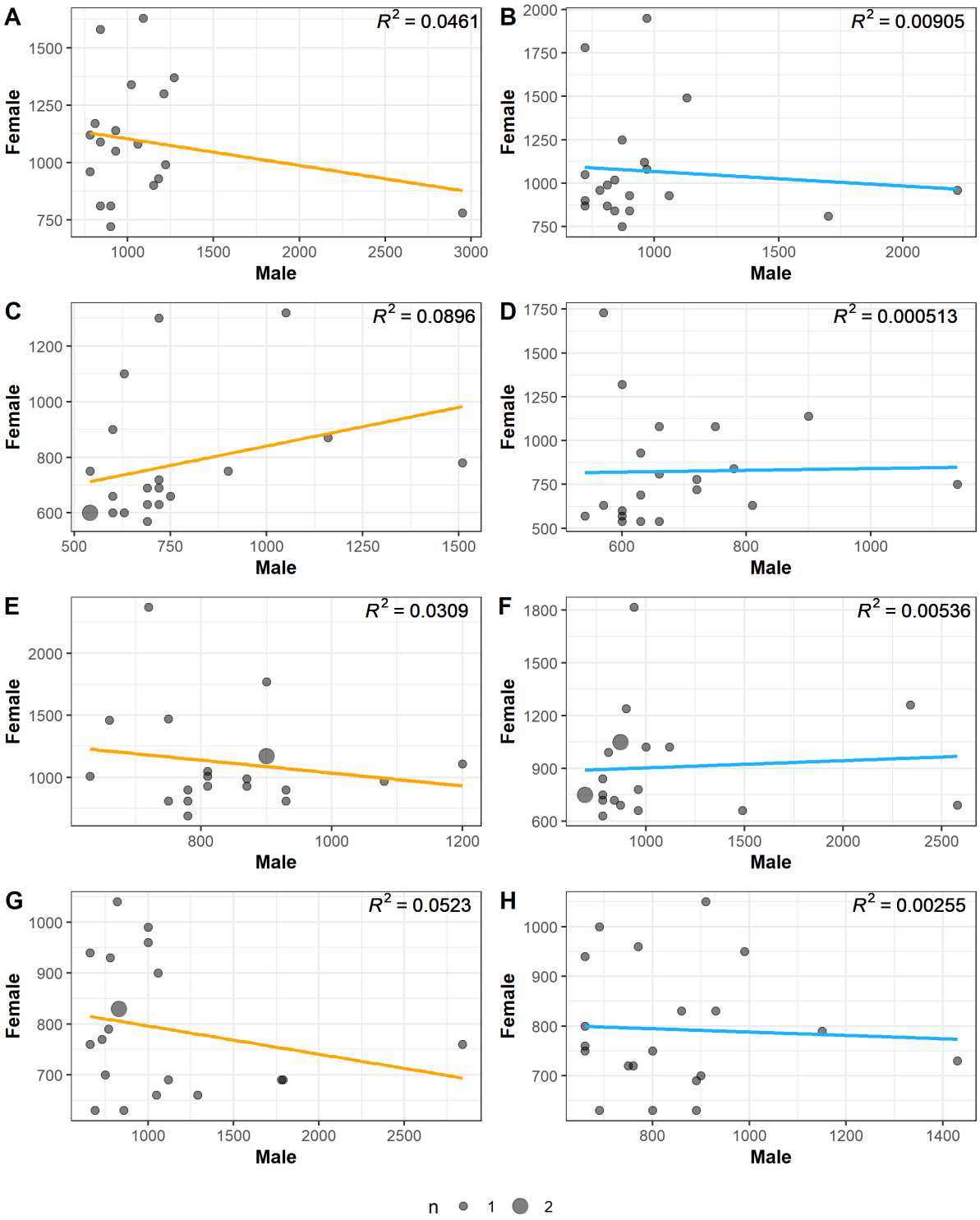

Figure 9: Male- Female correlation (Median Time to Death)

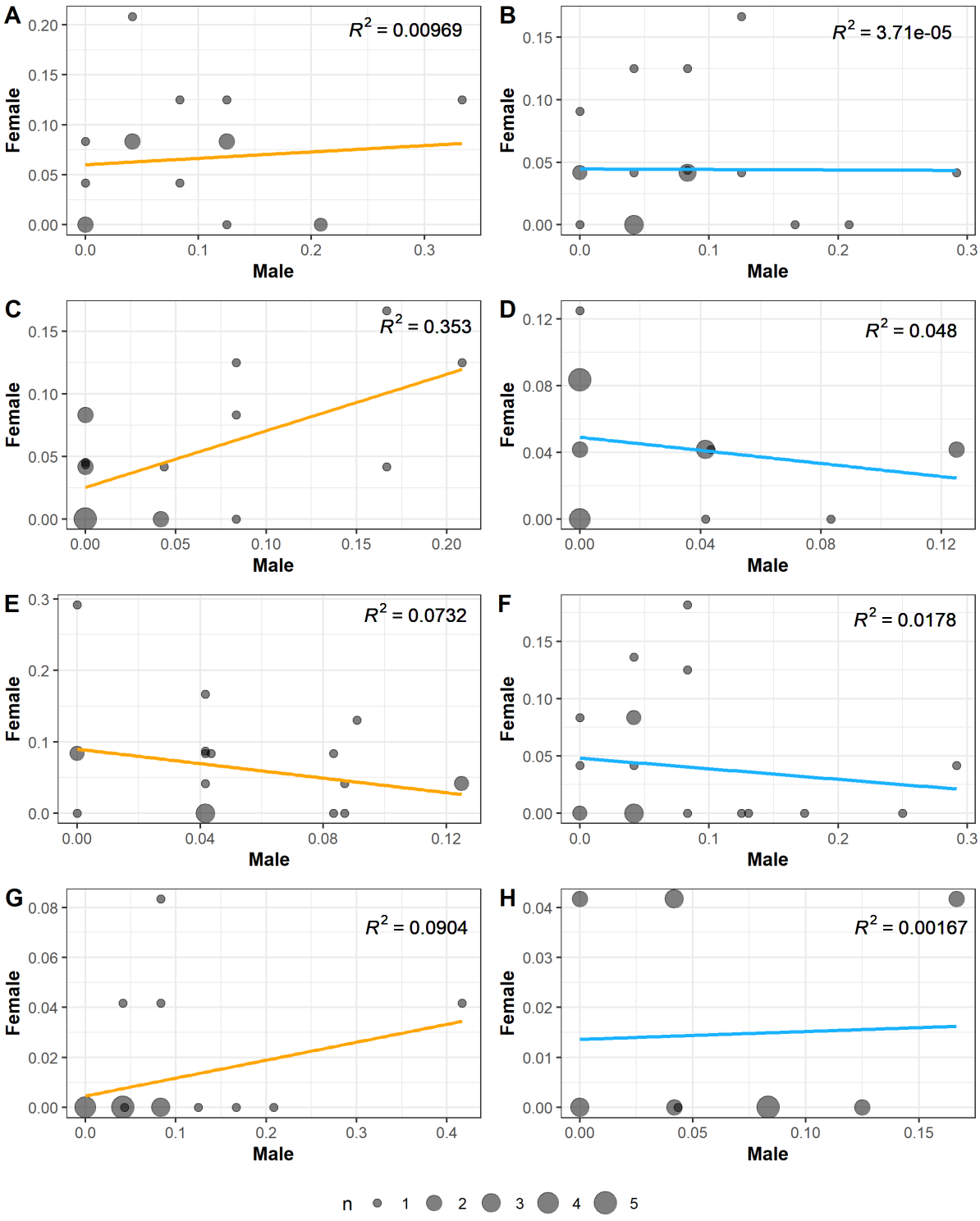

Figure 10: Male- Female Correlation (Proportion Survivorship)

Table 3: Logistic Regression						
Fixed effects		Estimate	Std. Error	z value	Pr(>|z|)	
	(Intercept)	-3.2541	0.2689	-12.1	**<0.0001**	***
	SelectionS	-0.2443	0.1857	-1.315	1.88E-01	
	Sexmale	0.4386	0.1401	3.13	**0.00175**	**
	SelectionS:Sexmale	0.1502	0.2069	0.726	0.4678	

Random effects	Groups	Name	Variance	Std.Dev.
	Xline:Block:Selection	Intercept	0.30504	0.5523
	Block	Intercept	0.05664	0.238
	Replicate	Intercept	0.12347	0.3514

Table 4: Median Time to Death (GLMM)								
Fixed effects		Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)	
	Selection	439354	439354	1	156.13	1.6122	2.06E-01	
	Sex	11070	11070	1	801.11	0.0406	0.8403	
	Selection:Sex	387	387	1	801.11	0.0014	0.9699	

Random effects		Npar	logLik	AIC	LRT	Df	Pr(>Chisq)	
	<none>	8	-7360.8	14738				
	(1 | Replicate)	7	-7374.8	14764	28.0106	1	**<0.0001**	***
	(1 | Block)	7	-7373.9	14762	26.2017	1	**<0.0001**	***
	(1 | Xline:Selection:Block)	7	-7360.9	14736	0.1795	1	0.6718	

Table 5: Proportion Survivorship (GLMM)								
Fixed Effects		Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)	
	Selection	0.0054	0.005367	1	155.07	1.4284	0.2338	
	Sex	0.0476	0.04758	1	158.01	12.6633	**0.0005**	***
	Selection:Sex	0.0002	0.000159	1	158.01	0.0423	0.8374	

Random Effects		Npar	logLik	AIC	LRT	Df	Pr(>Chisq)	
	<none>	7	422.33	-830.66				
	(1 | Xline:Block:Selection)	6	422.33	-832.66	0.0029	1	0.9572	
	(1 | Block)	6	419.92	-827.84	4.8251	1	**0.0281**	*

Table 6: Cox's Proportional Hazards (combined blocks)						
Fixed coefficients		Coef	exp(coef)	se(coef)	Z	P
	SelectionS	0.0583	1.0601	0.0558	1.05	0.3000
	Sexmale	0.0131	1.0131	0.0342	0.38	0.7000
	SelectionS:Sexmale	0.0293	1.0297	0.0483	0.61	0.5400
Random effects	Group	Variable		StdDev	Variance	
	Block/Selection/Xline	Intercept		0.196452	0.0777	
	Block/Selection	Intercept		0.136931	**<0.0001**	
	Block	Intercept		0.019731	0.0827	
	Infector	Intercept		0.019346	0.0368	

Table 7: Cox's Proportional Hazards						
Block 1						
Fixed coefficients		coef	exp(coef)	se(coef)	Z	p
	SelectionS	0.0952	1.0999	0.0953	1	0.3200
	Sexmale	0.0246	1.0249	0.0691	0.36	0.7200
	SelectionS:Sexmale	0.0907	1.0949	0.0970	0.93	0.3500
Random effects	Group	Variable		StdDev	Variance	
	Selection/Xline	Intercept		0.2136	0.0456	
	Selection	Intercept		0.0058	<0.0001	
	Infector	Intercept		0.0660	0.0044	
Block 2						
Fixed coefficients		coef	exp(coef)	se(coef)	Z	p
	SelectionS	-0.0605	0.9413	0.1280	-0.47	0.6400
	Sexmale	0.0849	1.0887	0.0678	1.25	0.2100
	SelectionS:Sexmale	0.1789	1.1959	0.0961	1.86	0.0630
Random effects	Group	Variable		StdDev	Variance	
	Selection/Xline	Intercept		0.3402	0.1157	
	Selection	Intercept		0.0123	0.0002	
	Infector	Intercept		0.0785	0.0062	
Block 3						
Fixed coefficients		coef	exp(coef)	se(coef)	Z	p
	SelectionS	0.2444	1.2769	0.0946	2.58	**0.0097**
	Sexmale	0.2315	1.2605	0.0677	3.42	**0.0006**
	SelectionS:Sexmale	-0.3304	0.7186	0.0963	-3.43	**0.0006**
Random effects	Group	Variable		StdDev	Variance	
	Selection/Xline	Intercept		0.2044	0.0418	
	Selection	Intercept		0.0104	0.0001	
	Infector	Intercept		0.4130	0.1706	
Block 4						
Fixed coefficients		coef	exp(coef)	se(coef)	z	p
	SelectionS	-0.0219	0.9783	0.0953	-0.23	0.8200
	Sexmale	-0.2665	0.7660	0.0679	-3.92	**<0.0001**
	SelectionS:Sexmale	0.1465	0.1577	0.0951	1.54	0.1200
Random effects	Group	Variable		StdDev	Variance	
	Selection/Xline	Intercept		0.2147	0.0461	
	Selection	Intercept		0.0085	<0.0001	
	Infector	Intercept		0.3822	0.1461	

Table 8: Male- Female Correlation (Median Time to Death)						
GLM						
		Estimate	Std. Error	t value	Pr(>|t|)	
I_1	(Intercept)	1219.7436	151.8339	8.033	3.45E-07	***
	Male	-0.1162	0.1281	-0.907	0.377	
I_2	(Intercept)	561.978	164.5289	3.416	0.00308	**
	Male	0.2787	0.2094	1.331	0.19975	
I_3	(Intercept)	1554.2376	584.9196	2.657	0.016	*
	Male	-0.5193	0.6858	-0.757	0.459	
I_4	(Intercept)	851.76194	66.03246	12.899	1.56E-10	***
	Male	-0.05562	0.05579	-0.997	0.332	
S_1	(Intercept)	1150.92809	213.75331	5.384	4.07E-05	***
	Male	-0.08347	0.20587	-0.405	0.69	
S_2	(Intercept)	789.79295	368.20716	2.145	0.0459	*
	Male	0.05041	0.52461	0.096	0.9245	
S_3	(Intercept)	860.80819	154.40877	5.575	2.72E-05	***
	Male	0.04127	0.1325	0.312	0.759	
S_4	(Intercept)	821.31778	135.24689	6.073	9.71E-06	***
	Male	-0.03361	0.15679	-0.214	0.833	

Spearman's Rank Correlation			
	S	Rho	p-value
I_1	1142.5	-0.002200712	0.9929
I_2	722.04	0.4571129	0.04273
I_3	1430.9	-0.07584403	0.7506
I_4	1643.7	-0.2358497	0.3168
S_1	1255	0.05641851	0.8132
S_2	987.65	0.2574068	0.2732
S_3	1098.8	0.1738147	0.4636
S_4	1321.9	0.006058322	0.9798

Table 9: Male- Female Correlation (Proportion Survivorship)						
GLM						
		Estimate	Std. Error	t value	Pr(>|t|)	
I_1	(Intercept)	0.06006	0.01954	3.073	0.00689	**
	Male	0.06378	0.15635	0.408	0.68844	
I_2	(Intercept)	0.02531	0.01144	2.214	0.04002	*
	Male	0.45294	0.14456	3.133	0.00575	**
I_3	(Intercept)	0.08984	0.02767	3.247	0.00447	**
	Male	-0.5081	0.42613	-1.192	0.24861	
I_4	(Intercept)	0.004579	0.006655	0.688	0.5	*
	Male	0.071768	0.053673	1.337	0.198	
S_1	(Intercept)	0.044528	0.016272	2.736	0.0136	*
	Male	-0.003901	0.150877	-0.026	0.9797	
S_2	(Intercept)	0.04909	0.01	4.908	0.000113	***
	Male	-0.19654	0.20628	-0.953	0.353311	
S_3	(Intercept)	0.0481	0.01794	2.681	0.0153	*
	Male	-0.09249	0.16204	-0.571	0.5752	
S_4	(Intercept)	0.013603	0.007338	1.854	0.0802	
	Male	0.015659	0.090286	0.173	0.8642	

Spearman's Rank Correlation			
	S	Rho	p-value
I_1	934.09	0.1806201	0.4593
I_2	810.33	0.3907283	0.0885
I_3	1486.8	-0.1178857	0.6206
I_4	932.02	0.2992304	0.2
S_1	1232.9	0.073006	0.7597
S_2	1657.3	-0.2461255	0.2955
S_3	1463.5	-0.1004129	0.6736
S_4	1441.4	-0.08378421	0.7254

Chapter 6 : Discussion

There is an abundance of evidence for immunity related sexually antagonism in *D. melanogaster.* Coupled with literature that shows X chromosomes to be hotspots of sexually antagonistic variation (Gibson et al., 2002; Rice, 1984), it led to the hypothesis that the improved immunity seen in selected populations should largely be because of X-linked loci. Further support from this hypothesis came from the prediction that X chromosomes are more likely to facilitate adaptive evolution as compared to autosomes (Charlesworth et al., 1987)

Vincent & Sharp, (2014) found a negative correlation between the two sexes for resistance and tolerance (two components of immunity), but we failed to find any correlation between male and female survivorship in any of the eight selection regime × block combinations (4 selected, 4 control)

Hill-Burns & Clark, (2009) reported that variation at multiple SNPs in X-linked immune genes was associated with immune response phenotypes (like bacterial clearance ability and immune gene expression). They find that many of these associations act in a sexually antagonistic manner. It should be noted that bacterial clearance ability and immune gene expression do not necessarily translate to improved survivorship.

However, negative correlations between the two sexes for a trait does not necessarily imply a negative correlation for fitness, which is essential to show intralocus sexual conflict. A negative correlation for fitness is therefore essential for the predictions made by the model posited by Rice, (1984).

Samant, (2015) calculated an estimate of dominance coefficient for proportion survivorship, finding significant sex-specific dominance, which is predicted to alter the distribution of sexually antagonistic variation, making it increasingly autosomal (Fry, 2010; Spencer & Priest, 2016). In light of these observations, the lack of X-linked immune variation is not as surprising as first appears. Infact, Ruzicka et al., (2019) performed a GWAS to examine the

genetic basis of sexual antagonism in a laboratory population of *D. melanogaster*, and found no evidence that the X chromosome is a hot spot for sexually antagonistic variation. This is in contradiction to a previous study in the same population (Innocenti & Morrow, 2010). Other factors that can contribute to a shift from what classical theory predicts are epistasis between loci (Arnqvist et al., 2014) and assortative mating based on fitness (Arnqvist, 2011).

An additional reason for the lack of enrichment of X-linked sexually antagonistic variation, is genetic drift, that the classical theory does not take into account. Due to the smaller size of the X chromosome, it is excessively affected by drift (Caballero, 1995), which could disproportionately deplete X-linked sexually antagonistic variation

The results of this cytogenetic cloning experiment therefore support the conclusion of Samant, (2015) that X-linked loci are not responsible for the improved immunity in I populations. Considering the X chromosome forms approximately 19% of the genome (Bridges, 1935), the complete lack of loci that might aid in adaptation to a systemic pathogenic infection is significant.

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