UNCOVERING THE DYNAMICS OF *WOLBACHIA*-ASSOCIATED PLASTIC RECOMBINATION IN *DROSOPHILA MELANOGASTER*

by

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A DISSERTATION

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DISSERTATION ABSTRACT

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Wolbachia pipientis is an endosymbiotic bacterium that infects 40%-60% of arthropod species and manipulates the reproduction and fitness of its host. *Wolbachia* also infects the model fruit fly, *Drosophila melanogaster*, in a symbiotic host-microbe relationship. Recently, *Wolbachia* infection was shown to induce plastic recombination in *D. melanogaster*, but the dynamics of this phenomenon are unknown. In my dissertation work, I investigated how microbe and host affect this phenomenon, in addition to illuminating where this phenomenon occurs across the genome. In Chapter I, I tested the effect of *Wolbachia* titer on recombination rate and found that bacterial titer did not affect the magnitude of recombination rate increase in *Wolbachia*-associated recombination. In Chapter II, I examined the effect of *Wolbachia* infection on transposable element expression in *D. melanogaster* and compared the effect of *Wolbachia* to other types of infections. Together, these findings provide a crucial foundation for future work investigating the mechanisms of *Wolbachia*-associated plastic recombination.

This dissertation includes previously published and unpublished co-authored material.

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CHAPTER I

INTRODUCTION

Wolbachia pipientis is a maternally transmitted, endosymbiotic bacterium that infects the somatic and gametic tissues of numerous arthropod and nematode species. *Wolbachia* is well-known for its ability to manipulate host phenotypes and reproduction, including cytoplasmic incompatibility, parthenogenesis, resistance to viral infections, and more. Recently, work in the Singh lab has found that *Wolbachia* is associated with changes in recombination rate in *Drosophila melanogaster*. Though many of *Wolbachia's* other effects on its hosts have been studied, substantially less is known about how *Wolbachia* plastically manipulates recombination in *D. melanogaster*.

Recombination is phenotypically plastic in a number of taxa, where the rate of recombination changes in response to environmental stressors. In *D. melanogaster*, recombination plasticity has been studied in response to stressors such as temperature, maternal age, starvation, and parasite infection. Recombination plasticity is well-characterized at the descriptive level, but the genetic and molecular mechanisms behind this plasticity are still unknown.

The host-microbe relationship between *D. melanogaster* and *Wolbachia* offers an ideal model system to answer these questions about plastic recombination. Understanding how *Wolbachia* and host affect one another, and how changes to host and microbe affect plastic recombination, could provide insight into the mechanisms that drive *Wolbachia*-associated plastic recombination. Therefore, my doctoral work explores the relationship between *D. melanogaster* and *Wolbachia* in order to understand the dynamics of *Wolbachia*-associated plastic recombination.

In Chapter I, I investigate how differences in *Wolbachia* titer influence the magnitude of plastic recombination in *D. melanogaster*. Both plastic recombination and *Wolbachia*-associated phenotypes have been shown to vary depending on the strength of the inducer. Does *Wolbachia*-associated plastic recombination also follow this pattern, where the magnitude of plastic recombination depends on bacterial titer? To answer this question, I used different host diets to manipulate *Wolbachia* titer and measured the resulting recombination rate in *D. melanogaster*. Contrary to what has been found for other *Wolbachia*-associated phenotypes, my results demonstrate that the magnitude of plastic recombination did not change significantly in response to changes in *Wolbachia* titer. These findings suggest that *Wolbachia*-associated plastic recombination responds discretely to *Wolbachia* infection, rather than continuously. This chapter has been published and is co-authored with Dr. Nadia Singh.

In Chapter II, I explore how *Wolbachia* infection affects transposable element (TE) expression in *D. melanogaster*. TEs are mobile, repetitive elements that originated from ancient viral infections and make up 5-90% of eukaryotic genomes. Over time, some TEs have evolved to become integrated in host gene expression networks and affect several cellular processes, including the immune system and recombination. How does *Wolbachia* infection affect TE expression, and are these TEs associated with changes in recombination? To answer this, I analyzed several gene expression datasets of *D. melanogaster* infected with *Wolbachia* and other types of pathogens. My results find that pathogen species significantly affects what types of TEs are affected during infection, and that infection with *Wolbachia* increases expression of DNA TEs, which have been associated with increased recombination rate in other species. Findings from this chapter are among the first to suggest a potential genetic mechanism for *Wolbachia*-

associated plastic recombination in *D. melanogaster*. This chapter is co-authored with Dr. Nadia Singh.

Together, the work described in this dissertation forms a critical foundation for uncovering the dynamics of *Wolbachia*-associated plastic recombination in *D. melanogaster.* My doctoral work has contributed significantly to both *Wolbachia*-associated plastic recombination specifically and to *Wolbachia* host-microbe associations more generally.

CHAPTER II

DIET-INDUCED CHANGES IN TITER SUPPORT A DISCRETE RESPONSE OF *WOLBACHIA*-ASSOCIATED PLASTIC RECOMBINATION IN *DROSOPHILA MELANOGASTER*

From Mostoufi SL, Singh ND. 2022. Diet-induced changes in titer support a discrete response of *Wolbachia*-associated plastic recombination in *Drosophila melanogaster*. *G3 Genes|Genomes|Genetics* 12(1): jkab375.

Introduction

Phenotypic plasticity is the phenomenon by which a single genotype may produce multiple phenotypes in response to variable environmental stimuli. Plasticity is pervasive in nature, affecting a range of phenotypes like morphology, development, behavior, and reproduction in bacteria, plants, and animals (Fusco and Minelli 2010; Forsman 2015; Fox *et al.* 2018). Meiotic recombination has also been shown to be phenotypically plastic, where the proportion of recombinant offspring increases in response to environmental stimuli. Plastic recombination has been observed in a number of taxa and in response to different stimuli: yeast experience elevated recombination rates under nutrient stress (Abdullah and Borts 2001), *Arabidopsis* displays recombination plasticity when exposed to extreme temperatures (Francis *et al.* 2007; Saini *et al.* 2017; Lloyd *et al.* 2018; Modliszewski *et al.* 2018), infection causes increased recombination in mosquitoes (Zilio *et al.* 2018) and plants (Chiriac *et al.* 2006; Andronic 2012), and social stress is associated with plastic recombination in male mice (Belyaev and Borodin 1982).

Plastic recombination also has a rich history of study in the fruit fly, *Drosophila melanogaster*. Temperature was the first condition associated with plastic recombination in *D.*

melanogaster, a phenomenon which has been well-characterized over the last century (Plough 1917; Plough 1921; Stern 1926; Hayman and Parsons 1960; Grell 1978; Kohl and Singh 2018). Several other factors have been identified which induce plastic recombination in *D. melanogaster*, including maternal age (Bridges 1927; Priest *et al.* 2007; Hunter *et al*. 2016a), starvation (Neel 1941), heat shock (Zhong and Priest 2011; Jackson *et al*. 2015), and parasite infection (Singh *et al.* 2015).

More recently, infection with the bacteria *Wolbachia pipientis* has been associated with plastic recombination in *D. melanogaster* (Singh 2019). *Wolbachia* is a Gram-negative endosymbiont that infects approximately 40% of terrestrial arthropod species including insects, spiders, and mites (Zug and Hammerstein 2012). Though *Wolbachia* is found throughout the somatic and germline tissues of its hosts (for review see Pietri *et al.* 2016), it is particularly abundant in germ cells and is maternally inherited through the oocyte (Dobson *et al.* 1999; Clark *et al.* 2002). Different *Drosophila* species are infected with unique strains of *Wolbachia*, each with varied effects on host biology (for review see Serbus *et al*. 2008; Werren *et al.* 2008; Correa and Ballard 2016; Kaur *et al*. 2021). One of the most well-studied *Wolbachia*-associated phenotypes is cytoplasmic incompatibility, which causes certain mating pairings between infected and uninfected flies to produce nonviable embryos (Turelli and Hoffmann 1995). Other strains of *Wolbachia* can cause phenotypes like male offspring killing or decreased lifespan in *Drosophila* (Hurst *et al*. 2000; Chrostek and Teixeira 2015). The native *Wolbachia* strain in *D. melanogaster*, *w*Mel, has been shown to provide protection against viral pathogens (Hedges *et al*. 2008; Teixeira *et al.* 2008), increase host fecundity (Fry *et al.* 2004; Fast *et al.* 2011), and now is associated with plastic increases in recombination rate (Singh 2019; Bryant and Newton 2020).

Since *Wolbachia*'s role in plastic recombination is a recent discovery, there remains a large gap in our understanding of this interaction. One of the first papers to identify this phenomenon observed a correlation between *Wolbachia* infection and increased recombination across an interval of the X chromosome, but not on chromosome 3 (Hunter *et al*. 2016b). This finding was experimentally validated and expanded upon to demonstrate that *Wolbachia*'s effect on recombination was plastic and occurred in multiple strains of *D. melanogaster* (Singh 2019). Yet the scope, magnitude, and mechanisms behind this phenomenon are unclear.

Of particular interest is the potential effect of magnitude in *Wolbachia*-associated plastic recombination. Plastic phenotypes can often be described as either categorical, where the phenotype exists in discrete forms, or continuous, where the phenotype may display dose-dependency and scale with the magnitude of extrinsic or intrinsic factors (Sheiner and Levis 2021). Plastic recombination in *D. melanogaster* has displayed dose-dependency in response to temperature changes, where increased exposure time to heat shock continuously increased the magnitude of plastic recombination (Jackson *et al*. 2015). This raises an interesting question of how plastic recombination may be influenced by the strength of *Wolbachia* infection.

An obvious candidate for testing this question of magnitude is the number of bacteria present within a cell, referred to as titer. *Wolbachia*-associated phenotypes can vary according to bacterial titer, including cytoplasmic incompatibility (Calvitti *et al*. 2015), lifespan reduction (Chrostek and Teixeira 2015), and viral pathogen protection (Chrostek *et al.* 2013; Ye *et al*. 2016). These phenotypes are considered dose-dependent because the strength of the phenotype continuously scales with the number of *Wolbachia* cells present within the host. However, some *Wolbachia*-associated phenotypes may display both categorical and continuous responses; at low bacterial titer, the phenotype exists in discrete forms which are not expressed until a certain

Wolbachia titer has been reached, after which the response scales continuously with increasing bacterial titer. This has been observed in both male-killing (Hurst *et al*. 2000) and lifespan reduction (Reynolds *et al*. 2003), where the phenotypes display both discrete and continuous responses.

If both plastic recombination and *Wolbachia*-associated phenotypes can display dose-dependency, this suggests that *Wolbachia*-associated plastic recombination may also follow the same pattern. Recently, Bryant and Newton (2020) tested this by using flies infected with two *Wolbachia* strains that maintain different titers and found that flies infected with a higher titer of *Wolbachia* also had a higher recombination rate. Though these results are consistent with the idea that *Wolbachia*-associated plastic recombination responds continuously to bacterial titer, it is difficult to determine since different *Wolbachia* strains were used. Because titer and *Wolbachia* strain were conflated, the distinct contributions of *Wolbachia* genotype and titer cannot be determined. Thus, additional research is needed to discern whether plastic recombination responds continuously to *Wolbachia* titer. It is also certainly possible that the response is continuous under some environmental conditions and discrete under others.

To address this question, we tested the effect of *Wolbachia* titer on plastic recombination in *D. melanogaster*. We used host diet to manipulate *Wolbachia* titer in fly ovaries under control, yeast-enriched, and sucrose-enriched conditions to evaluate the effect of titer on plastic recombination. Recombination rate was measured using classic genetic approaches in *Wolbachia*-infected and uninfected flies across a genomic interval on the X chromosome. Our data recapitulate that *Wolbachia* infection is associated with increased recombination rate and find that diet-induced changes in titer had no effect on the magnitude of plastic recombination.

These findings demonstrate that *Wolbachia*-associated plastic recombination displays discrete phenotypes in response to diet-induced changes in *Wolbachia* titer in *D. melanogaster.*

Materials and Methods

Fly Strain and Rearing

The *D. melanogaster* strain used in this experiment was RAL306, which comes from the *Drosophila* Genetics Reference Panel (DGRP) (Mackay *et al.* 2012; Huang *et al.* 2014). We used the RAL306 strain because it is naturally infected with *Wolbachia* and exhibits *Wolbachia*-associated plastic recombination (Hunter *et al*. 2016b; Singh 2019). To generate uninfected controls, we raised flies on tetracycline-containing media for two generations to remove *Wolbachia*. Tetracycline-containing media was created using standard cornmeal/molasses media mixed with ethanol-dissolved tetracycline at a final concentration of 0.25 mg/mL media (Holden *et al.* 1993). Following two generations of tetracycline treatment, flies were raised on standard media for over ten generations to allow passive recolonization of the gut microbiome via the fly's external microbiome.

We used PCR to confirm *Wolbachia* infection status prior to the start of the experiment. Briefly, single females were collected from stock vials of *Wolbachia*-infected and uninfected RAL306 flies. DNA was extracted with a standard squish protocol (Gloor and Engels 1992) and used in PCR with primers for the *Wolbachia* gene, *Wolbachia* surface protein (*wsp*), to identify the presence of *Wolbachia* (Jeyaprakash and Hoy 2000; Singh 2019).

Diet Treatments

For both *Wolbachia*-infected and uninfected groups, F1 virgin females were raised on one of three diet treatments: control, yeast-enriched, or sucrose-enriched. After three days, males

were added to diet treatment vials with virgin females for crossing. We set up ten replicate vials for each experimental group in a single block, repeated for four total blocks.

To produce the sucrose-enriched diets, we made a 40% sucrose mixture following Serbus *et al*. (2015). Initially, we crossed flies in pure sucrose-enriched vials, but larvae raised on sucrose media showed increased mortality and slower development (unpublished observations). Therefore, we devised a strategy to allow adult flies to feed on the sucrose-enriched media while also promoting normal larval development by using "sucrose patties." Sucrose-enriched mixture was poured into vials and allowed to cool before being sliced into 1 cm patties, which were placed on top of control diet vials. This strategy allowed adult flies to feed on the sucrose-enriched media while larvae could burrow down to feed on control media after hatching.

To make the yeast-enriched diets, we made a standard yeast paste by mixing dry active yeast and deionized water (Serbus *et al*. 2015). Approximately 2 mL of paste was added to control diet vials for the yeast-enriched treatments. Similar to the sucrose-enriched patties, this allowed adult flies to feed on yeast-enriched media while larvae could develop on control media.

Experimental Crosses

Since *Wolbachia* have been shown to increase recombination on the X chromosome (Singh 2019), we measured recombination with a standard two-step backcrossing scheme using the markers *yellow* (*y*) and *vermillion* (*v*) (33 cM apart) (Figure 1.1). In the first cross, roughly 20 RAL306 females and 20 *yv* males were crossed in 8oz bottles. Heterozygous F1 virgin female offspring were collected from these bottles. For the second cross, 5 F1 females were backcrossed to 5 *yv* males in a vial, with approximately 10 vials per diet treatment per block, repeated for a total of 4 blocks. BC1 offspring (Figure 1.1) were counted to estimate recombination rate in F1 females by calculating the recombinant fraction (cM/100), which is the proportion of

recombinant types to the total number of offspring. For these crosses, recombinant types were heterozygous (female BC1) or hemizygous (male BC1) for either the *y* or *v* allele (Figure 1.1).

All crosses were conducted at 25°C with a 12:12 hour light: dark cycle. Virgins were age-matched at approximately 48 hours before crossing. In each cross, flies were allowed to mate and lay eggs for four days before being removed.

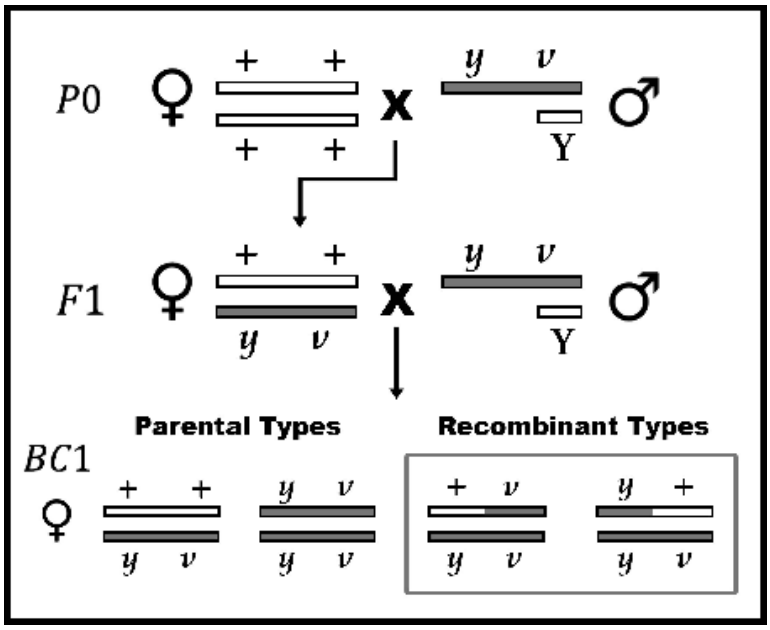

Figure 1.1. A two-step crossing scheme to measure recombination. Recombination rate can be estimated on the X chromosome using the recessive visible markers *yellow* (*y)* and *vermillion* (*v*) (33 cM). Males with the *y v* markers are crossed to wildtype (+ +) females. Heterozygous F1 females are backcrossed to the same male strain to produce BC1 progeny. Progeny which display either the yellow or vermillion phenotype are considered recombinant. Male BC1 genotypes are not shown, but males are heterogametic and require only one copy of the *yellow* or *vermillion* marker to display a phenotype.

Measuring *Wolbachia* Titer

We collected and froze F1 females at -20°C after egg-laying for ovary dissections and DNA extraction. Flies from blocks 1-3 were dissected in 1x PBS for a total of 22 ovaries per experimental group. DNA was extracted from ovary samples using the DNeasy® Blood & Tissue Kit (Qiagen) following insect and Gram-negative bacteria protocols. Quantitative PCR

(qPCR) was conducted to amplify the *Wolbachia* gene, *wsp,* and estimate the average ovarian titer within each experimental group relative to two host genes, *αTub84B* and *CG15365*. Each experimental group consisted of seven technical replicates for *wsp* and four technical replicates for each of the host genes, along with non-template controls to assess qPCR reaction efficacy. We used the SYBR Green Mastermix and standard manufacturer's protocols for qPCR on a QuantStudio3 Real-Time PCR System (Life Technologies). We also estimated primer efficiencies using a 1:5 serial dilution standard curve with five dilutions using DNA extracted from *Wolbachia*-infected flies.

Statistical Analyses

Recombination rate between groups was compared using a logistic regression model to evaluate statistical significance of the effect of *Wolbachia* infection (W_j), diet (D_i), or *Wolbachia* by diet interaction effects ($D_i \times W_j$). The full model is as follows, where *Y* refers to observed recombination data, μ refers to overall mean recombination rate, and ε refers to random variation: $Y_{i,j} = \mu + D_i + W_j + D_i \times W_j + \varepsilon$, (for $i = 1 \ldots 3,\ j = 1 \ldots 2$). We used the statistical software JMP Pro (v16.0.0) for logistic regression modeling, using a general linear model with binomial distribution and link logit function.

All other statistical analyses were carried out in RStudio (v1.2.5033). Mutant markers were tested for viability defects using G-tests for goodness of fit. A one-way ANOVA and Tukey's multiple comparisons test were used to analyze differences in fly fecundity between experimental groups. A post-hoc analysis of recombination rate variance was conducted using a modified robust Brown-Forsythe Levene-type test and Tukey's multiple comparisons test. For qPCR, raw Cq scores were analyzed using the Livak and Pfaffl methods (Pfaffl 2001) and

differences between groups were tested using a one-way ANOVA and Tukey's multiple comparisons test. The significance threshold for all statistical tests was set at 0.05.
Power analyses for recombination rate comparisons were conducted using the R package "SIMR" to validate experimental results (Green and MacLeod 2016). Simulated data were generated in R to produce a range of differences in mean recombination rate between groups, which were tested using repeated simulations in SIMR to calculate statistical power, where 80% power or greater is considered ideal.

Results

Fly Fecundity

To assess the effect of *Wolbachia* titer on plastic recombination, we set up crosses for *Wolbachia*-infected and uninfected flies on three diet treatments and measured recombination between the *yellow* and *vermillion* interval on the X chromosome. In total, 22,228 BC1 flies were scored for recombination (Table 1.1). For flies fed a control diet, the number of progeny per vial for *Wolbachia*-infected flies averaged 110 flies/vial, while uninfected flies averaged 111 flies/vial. On a sucrose-enriched diet, *Wolbachia*-infected flies produced an average of 117 flies/vial, compared to uninfected flies which produced an average of 132 flies/vial. Finally, the number of progeny per vial for flies fed a yeast-enriched diet averaged 225 flies/vial, while uninfected flies averaged 208 flies/vial (Figure 1.2). Results from a one-way ANOVA test demonstrated that diet treatment ($P < 2e\text{-}16$, ANOVA (N = 150, df = 2)), but not *Wolbachia* infection ($P = 0.942$, ANOVA (N = 150, df = 1)) significantly affected fly fecundity. Further analysis with a Tukey's multiple comparisons test found that the yeast-enriched diet increased

fecundity significantly compared to the control (P < 1.3e-13) and sucrose diet treatments (P < 3.1e-14).

Table 1.1. Offspring counts for experimental groups

Diet treatment	*Wolbachia*-infected	Uninfected	Total
Control	3284	3296	6580
Sucrose	2824	2888	5712
Yeast	4952	4984	9936
Total	11060	11168	22228

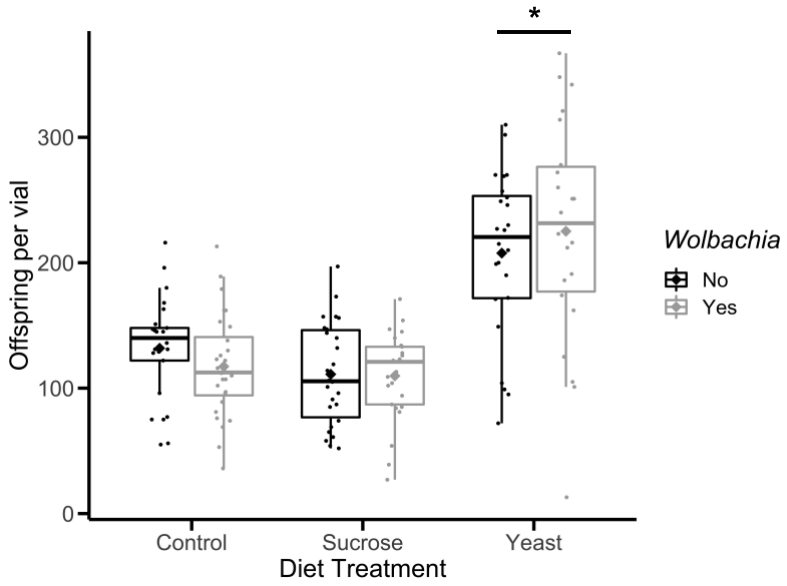

Figure 1.2. Fecundity, or number of offspring per vial, of experimental groups. Wolbachia-infected flies are shown in gray, while uninfected flies are shown in black. Each point corresponds to the total number of offspring in a single vial. Boxplots present summary statistics, where the top and bottom edges encompass the first to third quartiles and the middle bar represents the median for each group. Boxplot whiskers extend to the smallest and largest nonoutliers. The diamond in each boxplot represents the mean fecundity for each group. Statistically significant groups (P < 0.05) are denoted with an asterisk (*).

Viability Effects of Mutant Markers

To determine whether the viability of the mutant markers affected the ratios of offspring phenotypes, we performed G-tests for goodness of fit within each vial for the following ratios: males vs. females, wildtype (*wt*) flies vs. *yv* flies, and *yellow* flies vs. *vermillion* flies. The null

hypothesis is a 1:1 ratio for all phenotypic classes compared. Significant deviations from expected ratios would indicate that the markers affected the viability of certain phenotype combinations, which would negatively impact recombination rate estimates.

Similar to previous work (Hunter *et al*. 2016b, Singh 2019), we find small but nonsignificant viability defects associated with these markers. Out of 151 crosses, seven showed significant deviation with regards to the male-female ratio, eleven deviated from expected wildtype to *yv* ratios, and nine deviated from the expected ratio of *yellow* to *vermillion* flies. However, after using the Bonferroni correction for multiple tests, only one of the deviant crosses remained significant (P = 1.14 E-12, G-test). This specific cross had a ratio of 9.8 wildtype flies to *yv* flies and a recombinant fraction of 0.05. This likely stems from mating contamination and we discarded this cross from further analyses.

Host Diet and Quantitative PCR

To compare *Wolbachia* titer between diet treatment groups, we ran qPCR with DNA extracted from frozen female F1 flies collected after egg-laying. Results are shown in Table 2, where gene expression of *wsp* relative to host genes in *Wolbachia*-infected flies was calculated using the Livak and Pfaffl methods (Pfaffl 2001). Analysis of qPCR data using either the Livak or Pfaffl method produced similar results, where flies fed a sucrose-enriched diet had the highest relative gene expression of *wsp* compared to control group flies and flies on the yeast-enriched diet (Table 1.2). Since *wsp* expression is correlated with *Wolbachia* titer, this corresponds to a 3% increase in *Wolbachia* titer in flies on the sucrose diet treatment and a 23% decrease in *Wolbachia* titer in flies on the yeast diet treatment. Relative gene expression of *wsp* was significantly affected by diet treatment for both the Livak (P = 0.0019, ANOVA (N = 21, df = 2)) and Pfaffl analysis methods (P = 0.005, ANOVA (N = 21, df = 2)). A Tukey's multiple

comparisons test indicated that *Wolbachia* titer was significantly reduced in the yeast-enriched diet treatment compared to flies in the control ($P = 0.008$ Livak, $P = 0.018$ Pfaffl) and sucrose-enriched diets ($P = 0.003$ Livak, $P = 0.007$ Pfaffl).

Table 1.2. Relative gene expression of *wsp* in fly ovaries

Diet treatment	Livak	Pfaffl
Control	1.091	1.012
Sucrose	1.122	1.044
Yeast	0.842*	0.799*

* = $P < 0.05$, Tukey's multiple comparisons

The Effect of Infection and Diet on Recombination

We used logistic regression modeling to identify variables which significantly contributed to differences in mean recombination rate between experimental groups. Results are shown in Table 1.3, where *Wolbachia* infection ($P = 0.0008$, X^2 test (N = 150, df =1)) and experimental block ($P = 0.0001$, X^2 test (N = 150, df =3)) were significantly associated with differences in recombination rate. We measured recombination rate across the *y*-*v* interval (33 cM) of the *X* chromosome, with an expected recombination rate of 30-35 cM. The effect of *Wolbachia* infection can be seen clearly in Figure 1.3, where *Wolbachia*-infected flies display an average recombination rate of 37.1 cM while uninfected flies display an average rate of 34.7 cM, resulting in an average increase of 2.4 cM in recombination rate across all diet treatments. Neither host diet ($P = 0.42$, X^2 test (N = 150, df =2)) nor infection by diet interaction effects ($P = 0.43$, X^2 test (N = 150, df =2)) was significant. Based on the power of our tests, we would have been able to detect a difference of 5.8% or greater between group means, which corresponds to a difference in recombination rate of approximately 2 cM. This indicates that the effect of diet or *Wolbachia* titer, if present, was weaker than the effect of *Wolbachia* infection alone.

Table 1.3. Results of logistic regression modeling on recombinant fraction

Source	DF	L-R X^2	Prob X^2
Wolbachia	1	11.315	0.0008*
Diet	2	1.723	0.42
*Wolbachia**Diet	2	1.686	0.43
Block	3	20.435	0.0001*

* = $P < 0.05$, general linear model

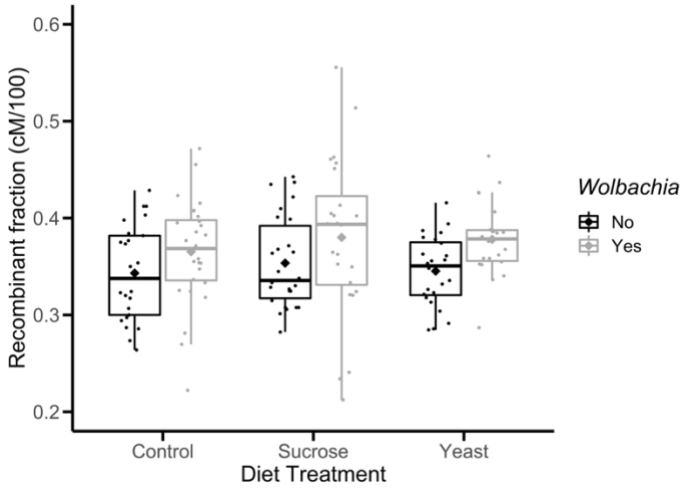

Figure 1.3. Recombination rate, reported as recombinant fraction, of experimental groups. The recombinant fraction is the proportion of recombinant progeny compared to the total number of progeny produced for each cross, which is equivalent to cM divided by 100. *Wolbachia*-infected flies are shown in gray, while uninfected flies are shown in black. Each point corresponds to the recombinant fraction of a single vial. Boxplots present summary statistics, where the top and bottom edges encompass the first to third quartiles and the middle bar represents the median for each group. Boxplot whiskers extend to the smallest and largest nonoutliers. The diamond in each boxplot represents the mean recombination rate for each group. Statistical significance was tested using a general linear model, where *Wolbachia* and experimental block significantly affected recombination rate ($P < 0.05$), while diet and *Wolbachia*-diet interactions were not significant.

Since the sucrose diet treatment did not significantly increase *Wolbachia* titer relative to the control diet, we performed additional logistic regression modeling on the control and yeast diet treatment groups. *Wolbachia* infection ($P = 0.0001$, X^2 test (N = 99, df =1)) and experimental

block ($P = 0.038$, X^2 test (N = 99, df =3)) were significantly associated with recombination rate differences, while host diet ($P = 0.26$, X^2 test (N = 99, df =1)) and infection by diet interaction effects ($P = 0.97$, X^2 test (N = 99, df =1)) were not significant.

We also tested for the effect of *Wolbachia* infection, titer, and diet on recombination rate variance, which was calculated as absolute residuals. Uninfected flies showed no significant difference in recombination rate variance between diet treatment groups ($P = 0.25$, Levene's test (N = 75, df = 2)), and a comparison between uninfected and *Wolbachia*-infected flies was also nonsignificant ($P = 0.11$, Levene's test (N = 150, df = 1)). However, *Wolbachia*-infected flies displayed significant differences in variance between diet treatment groups ($P = 0.007$, Levene's test (N = 75, df = 2)) and a Tukey's multiple comparisons test found that infected flies on a sucrose-enriched diet were significantly different from flies on a control ($P = 0.03$) and yeast-enriched diet ($P = 0.003$).

Discussion

Effect of *Wolbachia* Infection and Diet on Recombination

The goal of this experiment was to assess whether *Wolbachia*-associated plastic recombination in *D. melanogaster* is continuous or discrete in response to changes in bacterial titer. *Wolbachia* cannot currently be transgenically modified, making it impossible to use genetic engineering to test for differences in titer. Several other factors have been shown to alter *Wolbachia* titer, including temperature (Hurst *et al*. 2000; Moghadam *et al*. 2018), bacterial genotype (Chrostek and Teixeira 2015), and host diet (Serbus *et al*. 2015). However, both temperature (e.g., Plough 1917; Grell 1978; Jackson *et al.* 2015) and bacterial genotype (Singh *et al.* 2015; Bryant and Newton 2020) also affect recombination rate in *D. melanogaster*. Host diet

can alter *Wolbachia* titer within fly ovaries, specifically that a yeast-enriched diet decreases titer while a sucrose-enriched diet increases titer (Serbus *et al.* 2015; Camacho *et al.* 2017; Christensen *et al.* 2019). Therefore, we used host diet to manipulate *Wolbachia* titer and tested the effects of *Wolbachia* infection, host diet, and *Wolbachia* titer on recombination rate. We find that *Wolbachia* infection is associated with a significant increase in recombination rate across the *y – v* interval on the X chromosome (Table 1.3). Our data indicate that the *Wolbachia*-associated increase in recombination is robust with regards to variation in host diet, as *Wolbachia*-infected flies displayed a higher recombination rate than their uninfected counterparts in each diet treatment (Figure 1.3). This finding adds to a growing body of literature which supports *Wolbachia* as an inducer of plastic recombination in *D. melanogaster* (Hunter *et al.* 2016b; Singh 2019; Bryant and Newton 2020).

Since we used host diet to manipulate *Wolbachia* titer, we also assessed whether diet treatments had an impact on plastic recombination. We find that our diet treatments did not significantly affect recombination rate in *Wolbachia*-uninfected flies (Table 1.3, Figure 1.3). The effect of diet on plastic recombination in flies has been severely understudied, where one previous study reported that starvation in larvae was associated with increased recombination rate (Neel 1941). Differences between our study and the previous one may indicate that only severe changes in diet such as starvation are sufficient to induce plastic recombination in *D. melanogaster*. However, it should also be noted that Neel's study was carried out using markers on chromosome 3 (1941) while our study assessed recombination on the X chromosome. This may suggest that diet-associated plastic recombination is variable across the genome, as is the case for other conditions associated with plastic recombination such as temperature and *Wolbachia* infection (Grell 1978; Singh 2019). Outside of the present study, no recent

investigations have been made into how starvation or diet affects recombination in flies, highlighting a need for additional research into the role diet may play in plastic recombination. Our study only uses three diet treatments, while a more rigorous investigation of the effects of varying levels of carbohydrates, proteins, and caloric content is needed to definitively assess the effect of diet on plastic recombination in flies.

Effect of *Wolbachia* Infection and Diet on Fecundity

Though our diet treatments did not affect recombination rate, there was an effect of diet on fecundity. We observed that the average number of offspring per vial was significantly different between diet treatments, with yeast-fed flies displaying the highest average fecundity (Figure 1.2). The influence of diet on lifespan and fecundity in *D. melanogaster* has been well-characterized, especially regarding sucrose and yeast content (Drummond-Barbosa and Spradling 2001; Bass *et al*. 2007). Specifically concerning fecundity, yeast-enriched diets greatly increase female fecundity, while sucrose-enriched diets decrease female fecundity (Bass *et al*. 2007).

Wolbachia are often associated with increased fecundity in host fly species (Weeks *et al*. 2007; Mazzetto *et al*. 2015; Singh 2019), yet we found no significant effect of *Wolbachia* infection on fecundity. However, this may reflect a strain-specific response, rather than the effect of *Wolbachia* infection on *D. melanogaster* as a whole. Differences in fly fecundity depend on *Wolbachia* genotype (Gruntenko *et al*. 2019), host genotype (Fry *et al*. 2004), and bacterial-host interactions (Singh 2019). For instance, the strain used in this experiment, RAL306, was also used in a study which reported an overall effect of *Wolbachia* infection on fecundity across multiple strains (Singh 2019). However, when examined individually, *Wolbachia*-infected RAL306 flies had a lower mean fecundity than uninfected RAL306 flies (Singh 2019). This suggests that *Wolbachia* broadly impacts fecundity, but this effect may vary with host genotype.

Effect of *Wolbachia* Titer on Recombination Rate

By using host diet to manipulate *Wolbachia* titer, we tested the effect of titer on the magnitude of *Wolbachia*-associated plastic recombination. We measured *wsp* gene expression relative to host genes to measure *Wolbachia* titer in infected fly ovaries for each diet treatment group. Our results agree with other studies which find that yeast-enriched diets decrease *Wolbachia* titer and sucrose-enriched diets increase *Wolbachia* titer in fly ovaries (Table 1.2) (Serbus *et al.* 2015; Christensen *et al.* 2019). Our yeast diet treatment had a much stronger effect on *Wolbachia* titer than our sucrose diet treatment, resulting in a 23% decrease in titer compared to control flies, while flies on a sucrose diet showed a 3% increase in titer compared to control flies.

Combined with the recombination analysis which found no effect of infection by diet interactions (Table 1.3), these results suggest that changes in *Wolbachia* titer did not induce a continuous response in plastic recombination. This is, perhaps, not surprising in the case of the sucrose diet treatment, since a small increase in *Wolbachia* titer may not be enough to significantly affect the host fly's biological processes. However, reanalysis of the data using only the control and yeast diet treatment groups still finds that *Wolbachia* infection significantly impacted recombination rate, but *Wolbachia* titer did not. So, while the yeast diet treatment significantly decreased *Wolbachia* titer, this decrease did not lower recombination rate relative to *Wolbachia*-infected control flies, yet still resulted in an increase in recombination rate relative to uninfected flies. These results provide us with several new pieces of information about *Wolbachia*-associated plastic recombination, which are discussed in more detail below.

Though *Wolbachia* titer did not affect the magnitude of recombination, it did influence recombination rate variance. *Wolbachia*-infected flies fed a sucrose-enriched diet, to promote

high *Wolbachia* titer, had significantly greater variance than *Wolbachia*-infected flies on either a control or yeast-enriched diet. This finding suggests that increased *Wolbachia* titer may increase recombination rate variation, rather than increase the average rate of recombination beyond that caused by standard *Wolbachia* infection. Changes in variance have not previously been reported for other inducers of plastic recombination in *D. melanogaster*, nor for other *Wolbachia*-associated host phenotypes, suggesting that this may be a unique feature of *Wolbachia*-associated plastic recombination. This finding inspires multiple questions for future research, including why low *Wolbachia* titer did not result in decreased variance and whether this phenomenon is robust in response to other modifiers of *Wolbachia* titer**.**

Discrete Phenotypic Responses

Based on our results, there are several new pieces of information we can conclude about *Wolbachia*-associated plastic recombination. First, the phenotype must require relatively large changes in bacterial titer to elicit a corresponding change in response. Neither the sucrose diet treatment group nor the yeast diet treatment group significantly affected recombination rate relative to controls. This suggests that changes in titer need to be more dramatic than what we observed (3-23%) to potentially affect recombination rates. It is possible that these changes in *Wolbachia* titer caused small, non-significant changes in recombination rate; if so, these changes are smaller than the effect of *Wolbachia* infection alone. This suggests that this phenotype displays discrete rather than continuous responses, where large changes in *Wolbachia* titer are required to cause the magnitude of plastic recombination to increase. It is also interesting that the yeast diet treatment decreased *Wolbachia* titer, but not enough to eliminate the *Wolbachia*-associated plastic recombination phenotype. Logic would suggest that there must be some minimum threshold of bacteria below which plastic recombination would not be induced in flies,

but we did not reach that minimum in this experiment. Future work exploring even lower ranges in *Wolbachia* titer may be able to locate this threshold level.

If *Wolbachia*-associated plastic recombination displays discrete phenotypic responses, this follows the same trend as male-killing, another *Wolbachia*-driven trait in *Drosophila.* In *D. bifasciata*, *Wolbachia* infection causes increased mortality of male offspring, leading to modified sex ratios (Hurst *et al*. 2000). However, *Wolbachia* titer decreases in flies exposed to elevated temperatures, which causes male mortality rates to decrease and offspring sex ratios to return to normal (Hurst *et al*. 2000). These findings suggested that this phenotype requires a threshold level of *Wolbachia* to be expressed and displays discrete responses at low titers and continuous responses at high titers. The same may be true for *Wolbachia*-associated plastic recombination, where recombination is modified in discrete amounts in infected flies. It may also be true that *Wolbachia*-associated plastic recombination is continuous and dose-dependent, but only at titer levels more extreme than could be achieved through manipulations in host diet.

Another study looked at the effect of bacterial titer on plastic recombination using different strains of *Wolbachia* (Bryant and Newton 2020). They find that *D. melanogaster* infected with the *Wolbachia* strain *w*MelPop display a higher recombination rate across the *yellow*-*vermillion* interval of the X chromosome when compared to flies infected with a different *Wolbachia* strain, *w*Mel (Bryant and Newton 2020). The *w*MelPop strain maintains a much higher titer in flies, which could suggest that the magnitude of recombination corresponded with *Wolbachia* titer and indicates a dose-dependent relationship. Yet, as noted above, this study cannot separate the effect of titer from *Wolbachia* strain since two different strains were used in the experiment. Though *w*Mel is the native *Wolbachia* strain in *D. melanogaster*, *w*MelPop is considered pathogenic because it maintains a high titer and significantly decreases host lifespan

(Strunov *et al*. 2013; Chrostek and Teixeira 2015). Other pathogenic bacteria have been shown to plastically increase recombination rate in *D. melanogaster* (Singh *et al*. 2015), making it difficult to say whether an increase in recombination rate in *w*MelPop-infected flies is due to bacterial titer, its pathogenic nature, additional genetic differences between the two bacterial strains, or a combination of factors.

Our data are consistent with the plastic recombinational response to *Wolbachia* infection being discrete, with even low bacterial titers inducing the response. It is certainly possible that larger changes in *Wolbachia* titer can induce different magnitudes of plastic recombination in the host. Future experiments which test a large range of *Wolbachia* titers are necessary to fully understand the nature of titer effects on *Wolbachia*-associated plastic recombination.

The *Drosophila* Microbiome

It may also be true that *Wolbachia*-associated plastic recombination is continuous and dose-dependent, but that this effect is masked in our study due to complex interactions between diet, host, and the microbiome. Diet is known to have a significant impact on microbiome composition in several species (Turnbaugh *et al*. 2008; Read and Holmes 2017; Erkosar *et al*. 2018). In *D. melanogaster*, diets rich in either yeast or sucrose caused significant changes in abundance of certain members of the gut microbiome (Chandler *et al*. 2011). These changes in microbiome composition can have drastic impacts on host biology including hormone production, metabolism, and nutrient acquisition (Leulier *et al*. 2017). Specific members of the *D. melanogaster* microbiome have been shown to support larval feeding under starvation conditions (Consuegra *et al*. 2020), suggesting that diet-induced changes in the microbiome can significantly impact host development. Though our results suggest that diet had no significant effect on recombination rate, as uninfected flies showed similar mean recombination rate for

each diet treatment (Figure 1.3), it is difficult to rule out without directly measuring changes in microbiome composition.

In addition to the gut microbiome, which comes in direct contact with nutritional elements, host diet also affects *Wolbachia*. One finding our study takes advantage of is that increased sucrose or yeast in *D. melanogaster* diets can manipulate *Wolbachia* titer (Serbus *et al.* 2015). *Wolbachia* rely on their host to acquire nutrients, so changes in diet can affect microbe behavior and replication and may ultimately impact host biology. Yeast diets have been shown to affect *Wolbachia* cell physiology, which could influence the growth and behavior of the bacteria (Serbus *et al.* 2015). However, *Wolbachia* in flies fed the yeast diet treatment still produced the same increase in recombination rate as control flies, suggesting that changes in cell physiology did not impact plastic recombination. Additionally, *Wolbachia*-infected *D. melanogaster* have been shown to alter behavior and diet preference, potentially as a strategy to reduce negative effects on lifespan and fecundity (Ponton *et al.* 2014; Truitt *et al.* 2018). Though flies may alter their behavior under these conditions, *Wolbachia* have been shown to have no effect on emergence time or host nutrition under starvation conditions (Harcombe and Hoffmann 2004). We saw no significant differences in fecundity or viability related to infection status in this study, but it is unclear whether *Wolbachia*-infected flies fed experimental diets experienced changes in behavior that may have impacted recombination estimates.

Finally, the gut microbiome and *Wolbachia* have been shown to influence one another. *Wolbachia* infection can alter relative abundances of members of the gut microbiome compared to uninfected flies (Simhadri *et al.* 2017). Conversely, ingestion of certain species of gut bacteria has been shown to influence *Wolbachia* abundance (Rudman *et al.* 2019). Taken together, these findings present a complex web of interactions between host, diet, the gut microbiome, and

Wolbachia. Though it is difficult to estimate the impact of these interactions on our results, it remains clear that *Wolbachia*-associated plastic recombination is robust in response to both measured changes in diet and unmeasured changes in microbiome composition. Future work may focus on studying *Wolbachia*-only experimental flies, where germ-free flies are reinfected with *Wolbachia*, to remove potentially confounding variables caused by these complex interactions. However, there is also value in studying these systems in their natural state in order to gain a more complete understanding of native host-microbe associations.

Conclusions

Our current inability to transgenically modify *Wolbachia* makes it impossible to assess the effect of titer alone on *Wolbachia*-associated phenotypes. Though differences in titer can be assessed through manipulation of host diet (Serbus *et al*. 2015), temperature (Hurst *et al*. 2000; Moghadam *et al*. 2018), or *Wolbachia* strain (Chrostek and Teixeira 2015), these methods include confounding variables which make it difficult to definitively assign *Wolbachia* titer as the causative agent in phenotypes of interest. Our present study controls for host and microbe genotype and finds that *Wolbachia*-associated plastic recombination is a phenotype with discrete responses, while acknowledging the ways in which changes in host diet may influence that finding. Future advances toward making genetic manipulation possible in *Wolbachia* would allow the role of titer to be more definitively tested without confounding effects.

Bridge

The results from this chapter demonstrate that *Wolbachia* titer does not affect the magnitude of plastic recombination in *D. melanogaster*. To investigate other ways that

Wolbachia may influence its host in order to alter recombination rate, the next chapter details my work looking at transposable element expression during *Wolbachia* infection.

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CHAPTER III

PATHOGEN INFECTION ALTERS THE GENE EXPRESSION LANDSCAPE OF TRANSPOSABLE ELEMENTS IN *DROSOPHILA MELANOGASTER*

The experimental question and design for this project was developed by myself and Dr. Nadia Singh. I identified a majority of the relevant datasets, researched and assembled the necessary programs and code for the analysis pipeline, and wrote the manuscript for this project. Dr. Nadia Singh also aided with identifying relevant datasets and providing editorial comments on the manuscript.

Introduction

Transposable elements (TEs), or transposons, were first discovered by Barbara McClintock as genetic elements capable of moving around the genome and affecting kernel coloration in *Zea mays* (McClintock 1950). TEs are believed to have originated from ancient viral DNA that became integrated into a host's genome during a viral infection and make up anywhere from 5-90% of eukaryotic genomes (Guio and González 2019). Though the field originally adhered to the belief that TEs were selfish elements that needed to be transcriptionally silenced, we are now beginning to understand the crucial roles TEs play in genome evolution, gene regulation, and host development for almost all eukaryotes (for review, see Cosby, Chang, and Feschotte 2019).

Today, TEs are organized via several levels of classification. The highest level is class and relates to the method of TE transposition. The most common classes are Class I or DNA

transposons, which include TEs that use transposase to invade host genomes, and Class II or RNA transposons, which use reverse transcriptase to replicate throughout a host genome via an RNA intermediate. Within classes, TEs are further classified into families based on sequence similarity and evolutionary history. Finally, at the individual level, different TEs vary in their copy number and locations across host genomes.

Over time, TEs are thought to have changed in one of three ways: (1) hosts developed ways to suppress disruptive TEs, (2) TEs mutated and adapted to become an integral part of a host's gene expression networks, or (3) TEs accumulated mutations and deletions to eventually become silent and obsolete (for review, see Cosby *et al.* 2019). TEs that still retain the ability to transpose themselves throughout the genome are generally considered disruptive for their potential to insert themselves into gene regions and interrupt proper gene transposition. To combat the negative effects associated with these disruptive TEs, hosts have developed methods for silencing their expression or preventing their free transposition. One of the most widespread methods is small RNA-mediated silencing of TEs. An example of an sRNA method is the piRNA pathway in *Drosophila melanogaster,* which uses degraded copies of TEs to form piRNA clusters that regulate TE expression in the germline (Kelleher *et al*. 2020). Additionally, ectopic recombination between homologous TEs can result in negative selection against TEs with high copy numbers and transposition into highly active regions of the genome (Kelleher *et al*. 2020). Due to these methods of TE control, most TEs in *D. melanogaster* are found in areas with low recombination and few genes.

However, some TEs have instead evolved to become integrated into host gene networks, fostering a more adaptive relationship. In many cases, TEs have taken on the role of promoter, enhancer, or insulator and impact the expression of nearby genes. As an example, in *D.*

melanogaster TEs from the *Ty3* (*gypsy*) family can act as promoters or insulators for various genes depending on the genomic location of the TE insertion (Moschetti *et al*. 2020). Sometimes TEs impact gene expression via changes in methylation, as seen in *Arabidopsis thaliana* (Stuart 2016). Additionally, TEs can take on larger roles in cellular process beyond promoters. A well-known example of this phenomenon in *D. melanogaster* are the elements *TART* and *HeT-A*, which are major components of the telomere elongation system in flies (Moschetti 2020).

These systems of regulation and suppression of TEs can become disrupted when the host experiences novel environmental conditions. As early as 1984, McClintock hypothesized that TE activation could occur in response to genome challenges (McClintock 1984). Evidence to support this hypothesis can be found in several systems. For example, in *Caenorhabditis elegans,* heat shock and aging can increase the expression of some TEs (Li *et al*. 2021; Kurhanewicz *et al*. 2020). Temperature stress, including both heat shock and low temperature exposure, impacts TE expression in both *D. simulans* and *D. melanogaster* (Vasilyeva *et al*. 1999; Viera and Biémont 1996; Giraud and Capy 1996; Ratner *et al*. 1992). Additionally, nutrient deficiencies can also lead to TE activation in *Escherichia coli* (Hall 2000) and the wheat pathogen *Zymoseptoria tritici* (Fouché *et al*. 2020).

The effect of infection on TE expression is particularly interesting, given the nature of how TEs became integrated into host genomes initially. Prior to discovering the roles TEs play in host gene networks, many in the field initially theorized that hosts repressed all TEs, but that infection could reawaken those elements. This can be true in some cases, but the hypothesized mechanism is via deregulation, rather than reactivation. For example, in both *Drosophila* and *Rattus* species, the piRNA regulatory pathway can become saturated with pathogenic RNA during a viral infection, resulting in de-repression of native TEs (Durdevic *et al.* 2013; van

Gestel *et al*. 2014; Roy *et al*. 2020). However, not every pathogen, host, or TE react the same, and the field has more recently seen TEs play a crucial role in immune system function during pathogen infection. In this case, TEs are upregulated early in viral infection as part of the immune response in both humans and mice (Macchietto *et al*. 2020), as well as in *Drosophila* (Roy *et al*. 2020; Tassetto *et al*. 2017).

Despite the many ways TEs are affected by and can affect the response of their host to infections, efforts to understand these interactions struggle to capture the broader dynamics of the system. Although many studies have investigated the response of TEs to infection, these studies have been generally limited to one host and one infection, and sometimes even a limited number of specific TEs. In particular, though studies of TE expression during viral infection are numerous, investigations using other types of pathogens are scarce. Additionally, studies often analyze different host species, genotypes, sexes, or tissue samples, as well as different pathogen species or strains. These differences make it difficult to understand the broader patterns of TE activation outside of a few, very specific circumstances, even in our model organisms. One potential solution to this problem would be to conduct a large-scale study which directly measures the changes in TE expression within a single model organism while altering the type of infection (e.g. viral versus bacterial), host genotypes, and other variables of interest. However, the time, expense, skills, and facilities required for such a study can present significant barriers. An alternative, and more feasible, solution would be to compare numerous studies using the same model organism, allowing us to begin untangling the impact of these host and pathogen variables on TE expression. The fruit fly, *D. melanogaster*, is an ideal candidate for investigating these dynamics because of the number of tools available for TE annotation and the amount of gene expression datasets available.

Here, we investigate broad-scale patterns in TE expression during infection of the model organism, *D. melanogaster*. We gathered RNAseq samples from published datasets of *D. melanogaster* infected with a broad range of bacterial, fungal, and viral pathogens. We measured TE expression between control and infected samples and compared patterns both within and between pathogen groups to assess the effect of pathogen species, host genotype, host tissue sample, and host sex on TE expression. Our results show some shared patterns of TE activation across different infections, with a strong preference for RNA retrotransposons and members of the *copia* and *Ty3* families, as well as patterns unique to each pathogen group. We also find the effect of pathogen to be much greater than the effect of host variables on changes in TE expression. These findings provide critical insight into how host and pathogen variables can impact TE activity during infection in *D. melanogaster*.

Results

In this study, we analyzed changes in transposable element (TE) expression between control and infected *Drosophila melanogaster* using RNA-seq samples from 14 published datasets (Table 2.1). Together, these datasets include 31 different fly genotypes and 19 species of individual and multi-species infections of bacterial, fungal, and viral pathogens. Our analyses resulted in 231 differentially expressed transposable elements (DETEs), 21 of which were unclassified with an unknown class and family. Though these unknown TEs represented approximately 10% of the DETEs in our study, their unclassified status makes it impossible to comment on their influence on host biology and we have excluded them from the rest of our analyses. Future studies may reveal more details about these TEs and their role in host gene networks, but is outside the focus of the work presented here.

Table 2.1. The RNA-seq datasets used in this study. Uninfected flies with a conventional microbiome are represented by "CV", germ-free flies are represented by "GF", single-species infections are represented by "SS", and multi-species infections are represented by "MS."

Paper	Bio Project	Pathogen Group	Microbe Species	Control	Infection	Host Genotypes	Host Tissue	Host Sex
Dobson, *et al*. 2016	PRJNA347655	Bacteria	*Acetobacter pomorum (DmCS_004)* and *A. tropicalis (DmCS_006)* and *Lactobacillus brevis (DmCS_003)* and *L. fructivorans (DmCS_002)* and *L. plantarum (DmCS_001)*	GF	MS	1	Whole Body	Male
Guo, *et al*. 2014	PRJNA232924	Bacteria	Conventional microbiome	GF	CV	1	Intestine	Female
Troha, *et al*. 2018	PRJNA428174	Bacteria	*Enterococcus faecalis, Erwinia carotovora (Ecc15), Escherichia coli, Micrococcus luteus, Providencia rettgeri, P. sneebia, Pseudomonas entomophila, Serratia marcescens (Db11), S. marcescens (Type), Staphylococcus aureus*	CV	SS	1	Whole Body	Male
Elya, *et al*. 2018	PRJNA435715	Fungi	*Entomophthora muscae*	CV	SS	1	Brain, Carcass	Female
Moskalev, *et al*. 2015	PRJNA295562	Fungi	*Beauvaria bassiana*	CV	SS	1	Whole Body	Male
Paparazzo, *et al*. 2015	PRJNA279177	Fungi	*Beauvaria bassiana*	CV	SS	4	Whole Body	Male
Ramírez-Camejo and Bayman. 2020	PRJNA377735	Fungi	*Aspergillus flavus*	CV	SS	1	Whole Body	Female

Table 2.1. (continued).

Paper	Bio Project	Pathogen Group	Microbe Species	Control	Infection	Host Genotypes	Host Tissue	Host Sex
Harsh, *et al*. 2020	PRJNA533975	Virus	*ZIKV (MR766)*	CV	SS	1	Whole body	Female
Roy, *et al*. 2020	PRJNA540249	Virus	*Sindbis virus (SINV)*	CV	SS	2	Carcass, Ovary	Female
Lindsey, *et al*. 2021	PRJNA682591	Virus, Wolbachia	*Sindbis virus (SINV), Wolbachia pipientis (wMel2)*	CV	SS, MS	1	Whole body	Female
Detcharoen, *et al*. 2021	PRJNA602188	Wolbachia	*Wolbachia pipientis* (wMel)	CV	SS	1	Whole body	Female
Frantz, *et al*. In press.	N/A	Wolbachia	*Wolbachia pipientis*	CV	SS	4	Ovary	Female
Grobler, *et al*. 2018	PRJNA483452	Wolbachia	*Wolbachia pipientis*	CV	SS	1	Cell culture	Unknown
He, *et al*. 2019	PRJNA439370	Wolbachia	*Wolbachia pipientis*	CV	SS	1	Ovary	Female

Exclusion of unclassified TEs resulted in a total of 210 DETEs that we used to assess the effect of pathogen and host variables on TE expression. A subset of DETEs shared across multiple datasets and/or with a large change in expression are presented in Figure 2.1. Of all DETEs, 18 came from fungal infection datasets, 47 came from viral infection datasets, and 145 came from bacterial infection datasets, of which 116 were from *Wolbachia pipientis* infections and 29 from non-*Wolbachia* infections. Due to these differences in DETEs between the datasets, we tested whether there was a correlation between sample size and the number of significant TEs. We used a linear model to test for this potential relationship and found no correlation between dataset sample size and the number of significant TEs (R^2 = -0.0016, p-value = 0.34).

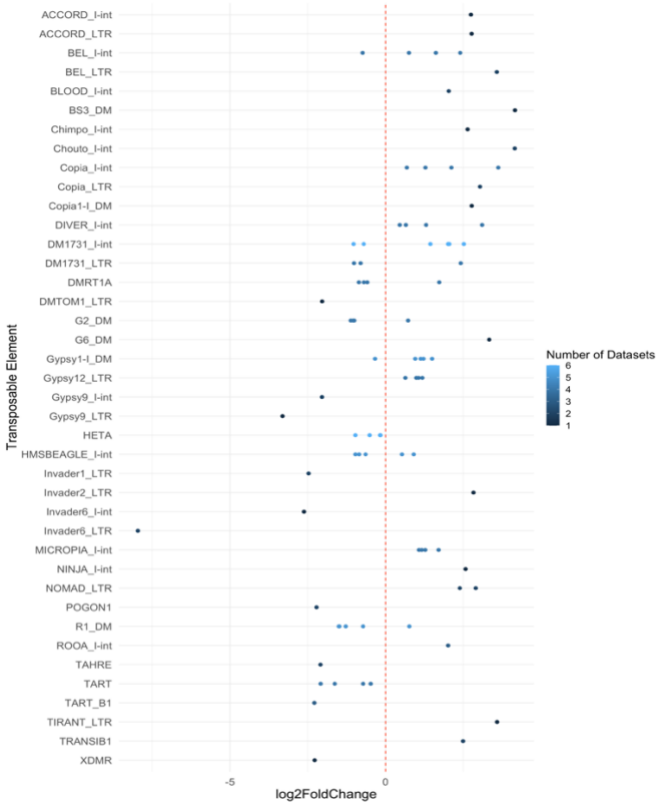

Figure 2.1. Transposable elements differentially expressed during infection in D. melanogaster. The TEs presented in this figure are those that were expressed across multiple infection datasets, and/or that had a log2-fold change greater than 2 or less than -2. Each dot represents differential expression in a single dataset. Dots are colored based on the number of datasets each TE was present in.

Comparisons of Infection by Different Pathogens

To understand how infection affects TE activity, we compared TE expression between flies infected with different pathogen groups, including bacterial, fungal, and viral infections. We analyzed DETEs at the level of class and family classification and the direction of expression in order to identify larger patterns in TE activity. Due to the large number of datasets with *Wolbachia pipientis*, and because *Wolbachia* is generally not considered pathogenic in *D. melanogaster* (Fry *et al*. 2004), we considered *Wolbachia* infection separately from other bacterial infections.

First, we tested for differences in the types and expression of TEs affected by infection between the pathogen groups. Bacterial infection significantly affected different classes and families of TEs compared to all other infection types (Table 2.2). Fungal, viral, and *Wolbachia* infections affected similar TE classes and families, and comparisons between these groups were nonsignificant (Table 2.2). Pathogen groups also significantly differed in the proportion of TEs with increased or decreased expression during infection (χ-squared = 61.36, df = 3, p-value = 3.01e-13), with bacterial and *Wolbachia* infections more likely to decrease TE expression and fungal and viral infections more likely to increase TE expression.

Table 2.2. Results from chi-square comparisons of class and family TE proportions between pathogen groups.

	Class			Family		
	Fungi	Virus	*Wolbachia*	Fungi	Virus	*Wolbachia*
Bacteria	6.1e-3	1.3e-3	1.6e-4	0.019	< 2.2e-16	0.019
Fungi		0.60	0.69		0.31	0.98
Virus			0.77			0.79

Genomic Distribution of TEs During Infection

Next, we analyzed patterns in genomic location of DETEs. Across all infections, DETEs were generally located in centromeric and telomeric regions of chromosomes 2, 3, and X, but were more evenly distributed across the 4th and Y chromosomes. To determine if different pathogen groups showed a location preference for activated TEs, we compared chromosomal proportions of expressed TEs (Figure 2). Pathogen group had a significant effect on the genomic location of DETEs (χ-squared = 171.60, df = 18, p-value < 2.2e-16), with a strong bias in bacterial infections for TE expression on the Y chromosome (Chi-square, residual = 8.50), and more TE expression on chromosome 4 in *Wolbachia* infections than in other types of infections (Chi-square, residual = 6.47).

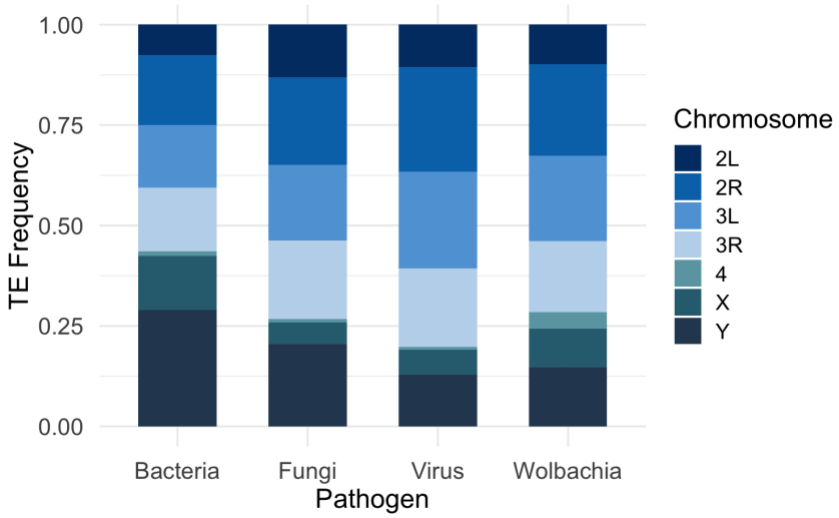

Figure 2.2. Genomic location of activated transposable elements during infection varies by infection type. Each bar represents the chromosomal proportions of TEs activated during infection with bacterial, fungal, viral, or *Wolbachia* infections.

Effect of Fungal Infections on TE Expression

Next, we investigated TE patterns within each pathogen group. Our analyses of fungal infections included four datasets of single-species infections, including *Aspergillus flavus*, *Beauvaria bassiana*, and *Entomophthora muscae* (Table 2.1). Comparison of control and infected samples resulted in 18 DETEs, 75% of which increased in expression during infection. Of the 18 DETEs, 89% belonged to the LTR class (Figure 2.3A), and the most common family was *Ty3* (Figure 2.3B). To test whether the proportions of DETEs, classified by TE class and family, differed significantly from the proportions present in the genome, we conducted a chi-square goodness of fit test. At the class level, DETEs were not significantly different from the class proportions of TEs present in the *D. melanogaster* genome (χ-squared = 10.99, df=8, p-value=0.20). This was also true at the family level, where DETEs did not differ significantly from expected family proportions (χ-squared = 15.98, df = 30, p-value = 0.98).

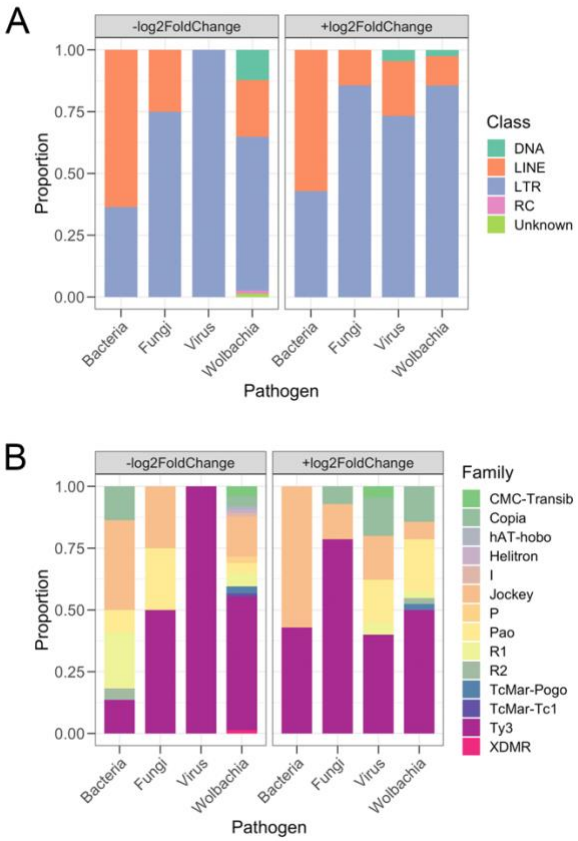

Figure 2.3. Different infections significantly impact types of TEs expressed during infection in D. melanogaster. Each bar represents the proportions of differentially expressed TEs, classified by (A) TE class and (B) TE family, within each pathogen group. TEs are also separated by whether they increased (+log2FoldChange) or decreased (-log2FoldChange) in expression during infection.

Effect of Viral Infections on TE Expression

To assess the effect of viral infection, we analyzed three RNAseq datasets which included infection with the Zika virus (ZIKV) and Sindbis virus (SINV) (Table 2.1). Across the datasets, there were 47 DETEs, 96% of which increased in expression during infection. The most common class and family were LTR and *Ty3*, respectively (Figure 2.3). At the level of class, DETEs differed significantly from expected genome proportions (χ-squared = 19.63, df = 8, p-value = 0.01), with residuals indicating that there were more TEs from the LTR class (Chi-square, residual = 2.72) and fewer TEs from the RC class (Chi-square, residual = -2.60) than expected.

The family classifications of DETEs also significantly differed from genome proportions (χ-squared = 52.62, df = 30, p-value = 0.0065), with residuals indicating that there were more TEs from the *copia* family (Chi-square, residual = 5.24) and fewer from the *Helitron* family (Chi-square, residual = -2.60) than expected.

Effect of Bacterial Infections on TE Expression

We analyzed one dataset comparing infection with ten different bacterial species in *D. melanogaster*, and two datasets with conventional and/or germ-free flies (Table 2.1). Between the two datasets of conventional and germ-free flies, there was only one DETE, while infection with pathogenic and non-pathogenic bacterial species resulted in 31 DETEs, 75% of which displayed decreased expression during infection. Most DETEs belonged to the LINE class (Figure 2.3A), and the most common family was *Jockey* (Figure 2.3B). The class proportions of DETEs in the bacterial infection datasets differed significantly from expected proportions based on the *D. melanogaster* genome (χ-squared = 35.48, df = 8, p-value = 2.18e-05), with more TEs from the LINE class (Chi-square, residual = 5.05) and fewer TEs from the RC class (Chi-square, residual = -2.04) than expected. Family proportions also differed significantly from expected proportions (χ-squared = 90.70, df = 30, p-value = 5.14e-08), with more TEs from the *copia* (Chi-square, residual = 2.59), *Jockey* (Chi-square, residual = 5.02), *R1* (Chi-square, residual = 3.92), and *R2* families (Chi-square, residual = 5.51) and fewer TEs from the *Helitron* family (Chi-square, residual = -2.04) than expected.

Within the ten bacterial species, there were seven gram-negative and three gram-positive species, so we tested whether there were differences within this dataset based on Gram staining. However, there were no significant differences between gram-negative and gram-positive bacteria species for TE class (χ-squared = 0.78, df = 2, p-value = 0.68) or TE family (χ-squared

= 10.71, df = 10, p-value = 0.38). In addition, gram-negative and gram-positive bacteria had nearly identical proportions of TEs with increased or decreased expression, with approximately 75% of TEs decreasing in expression during infection.

Effect of *Wolbachia* Infections on TE Expression

We analyzed five datasets of *Wolbachia* infection in *D. melanogaster*, including the wMel and wMel2 variants and one dataset of co-infection between *Wolbachia* and SINV (Table 2.1). Across the datasets of *Wolbachia*-only infections, there were 116 DETEs, the majority of which belonged to the LTR class (Figure 2.3A) and *Ty3* family (Figure 2.3B). Similar to other bacterial infections, *Wolbachia* infection caused a majority of TEs to decrease in expression. We found that class proportions of DETEs were significantly different from the proportions of TEs found in the *D. melanogaster* genome (χ-squared = 36.21, df = 8, p-value = 1.61e-05), with more TEs from the LTR class (Chi-square, residual = 3.69) and less from the RC (Chi-square, residual = -3.84) and Satellite classes (Chi-square, residual = -2.37) than expected. Family proportions also differed from expected genome proportions (χ-squared = 98.59, df = 30, p-value = 3.10e-09), specifically that there were more *copia* (Chi-square, residual = 3.45), *R2* (Chi-square, residual = 2.49), *TcMar-Pogo* (Chi-square, residual = 5.90), and *Ty3* TEs (Chi-square, residual = 3.13) and less *CR1* (Chi-square, residual = -2.11), *Helitron* (Chi-square, residual = -3.84), and *Satellite* TEs (Chi-square, residual = -2.37) than expected. Our analyses identified no DETEs in flies co-infected with *Wolbachia* and SINV.

Comparison of Host Variables during Infection

Across the datasets we analyzed, samples varied by host sex, genotype, and tissue. To assess the effect of these variables on TE expression during infection, we compared the expression of TEs during infection between males and females, different host genotypes, and

different tissue samples. Similar to our analyses of infection, we analyzed DETEs at the level of class and family classification and the direction of changes in expression between different host variables during infection.

Effect of Host Sex on TE Expression During Infection

Host sex significantly affected the proportions of each class (χ-squared = 29.53, df = 8, p-value = 0.00026) (Figure 2.4A) and family (χ-squared = 106.92, df = 28, p-value = 3.72e-11) of TEs that were differentially expressed during infection. Additionally, female samples accounted for 133 DETEs, compared to 30 DETEs in male samples and 47 DETEs in samples of unknown sex. A majority of TEs in female samples showed increased expression during infection, compared to male and unknown sex samples which had more TEs with reduced expression.

Unequal associations between pathogen groups and host sex in our datasets may have caused the effect of sex to be confounded by the effect of pathogen. To disentangle this association, we tested for the effect of sex within the fungal infection datasets, which had equal numbers of female and male fly datasets and found no significant effect of sex on DETE class identity (χ-squared = 0.11, df = 1, p-value = 0.74) or family identity (χ-squared = 6.19, df = 3, p-value = 0.10). Female samples still had higher counts of DETEs than male samples, consisting of 16 DETEs versus 2 DETEs in the respective sexes. Female samples within the fungal infection datasets also contained more TEs with increased expression.

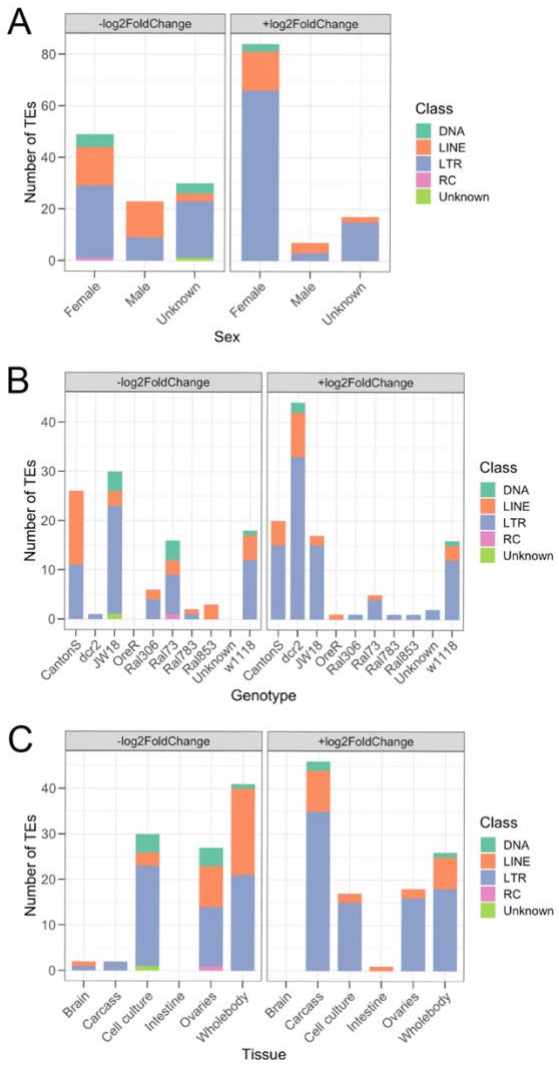

Figure 2.4. Host variables have a minimal effect on transposable element activity during infection. The number of TEs differentially expressed during infection are grouped by (A) host sex, (B) host genotype, and (C) sample tissue type. TEs are also separated by whether they increased (+log2FoldChange) or decreased (-log2FoldChange) in expression during infection and are colored based on their TE class.

Effect of Host Genotype on TE Expression During Infection

Additionally, the samples in our study included 31 different host genotypes (Table 2.1). However, due to the small sample size of the Dobson (2016) dataset, we were unable to include it in this particular analysis, reducing our final analysis to 17 different host genotypes. We found

that host genotype did not significantly affect class proportions (χ-squared = 45.54, df = 36, p-value = 0.13) (Figure 2.4B) or family proportions of DETEs (χ-squared = 92.71, df = 117, p-value = 0.95). There was a significant effect of host genotype on the direction of TE expression (χ-squared = 59.13, df = 9, p-value = 1.97e-09). However, this was again potentially due to correlations between host genotype and pathogen group, specifically in the *dcr2*$^{-/-}$ genotype, which was found in one viral infection dataset. When this data was reanalyzed after excluding samples with the *dcr2*$^{-/-}$ genotype, host sex did not significantly impact TE expression (χ-squared = 10.39, df = 8, p-value = 0.24).

Effect of Tissue Sample on TE Expression During Infection

We also tested whether DETEs varied significantly by host tissue and found that tissue did not significantly affect the class proportions (χ-squared = 25.36, df = 20, p-value = 0.19) (Figure 2.4C) or family proportions of TEs (χ-squared = 55.27, df = 65, p-value = 0.80). Host tissue did significantly affect the direction of foldchange expression of DETEs (χ-squared = 51.73, df = 5, p-value = 6.13e-10), specifically that 46 out of 48 DETEs in carcass samples had increased expression during infection (Figure 2.4C). TE expression was similar across cell culture, ovary, and whole-body samples, with approximately 63% of TEs displaying decreased expression during infection.

However, similar to our analyses of host sex, there were unequal associations between pathogen groups and host tissue, especially in the carcass samples which primarily came from viral infection datasets. Therefore, we reanalyzed the effect of host tissue after excluding carcass samples and found no significant difference in TE expression between tissues (χ-squared = 4.83, df = 4, p-value = 0.31).

Discussion

In this study, we investigated patterns of transposable element (TE) expression during infection in *D. melanogaster*. Other studies have investigated the effect of infection on TE expression in a variety of hosts and conditions, but experimental differences between these studies make it difficult to directly compare their findings and draw broader conclusions about TE dynamics during infection. By bringing together *D. melanogaster* datasets of differing host genotypes, tissue types, sex, and pathogens, we sought to uncover the influence of these variables on differentially expressed TEs (DETEs) and identify both unique and broad trends in TE expression during infection.

Impact of Infection on TE expression

To examine the effect of different infections on TE expression, we examined *D. melanogaster* samples infected with different species of fungal, viral, and bacterial pathogens. We observed that the expression, location, and classification of TEs were significantly different between different groups of pathogens.

First, we observed similarities and differences in expression and the types of TEs affected by infection. Samples from viral infections showed a strong bias for increased TE expression. Viral infections and TEs have been well studied, and consistently point to an upregulation of TEs during viral infection (for review, see Hale 2022). Our analyses also identified increased expression of TEs known to be active during viral infections. In 2 of the 3 viral datasets we analyzed, we specifically observed increased expression of the *Doc* element, which is known to play a role in virus resistance (Magwire *et al*. 2011; Barron *et al*. 2014). There were also striking differences between viral strains, where infection by SINV resulted in drastically more DETEs than infection by ZIKV. Both SINV and ZIKV infections are typically non-lethal in *D.*

melanogaster, and viral load is controlled by different host immune pathways between the two viral strains (Xu and Cherry 2014; Liu *et al*. 2018). These differences in immune gene response and our results suggest that viral strains may also differentially influence TE expression in *D. melanogaster*.

Similar to viral infections, we also observed more TEs with increased expression during infection with fungal pathogens. Fungal infections in *Nicotiana tabacum* can cause increased expression of the *Tnt1* retrotransposon (Grandbastien *et al*. 1997). TE insertions can also play a role in gene duplications that are associated with increased resistance to fungal infection (Tan *et al*. 2021), but there is currently less in the literature about how TEs in *D. melanogaster* respond to fungal infection directly. Our results suggest that, like in viral infections, fungal infections also lead to increased expression of TEs.

We observed the opposite pattern in bacterial infections, where a majority of TEs displayed decreased expression during infection with bacterial species, including *Wolbachia*. TE insertions are known to affect immune resistance to bacterial pathogens in humans (Bogdan *et al*. 2020) and *D. melanogaster* (Ullastres *et al*. 2021), but it is less clear if TE expression is directly related to bacterial infection resistance. Our results suggest that this may be true, based on our comparison between conventional microbiome and novel infection datasets. We analyzed two datasets comparing flies with and without the conventional gut microbiome and found only one significant TE associated with conventional microbe presence across both datasets. In contrast, novel bacterial infections resulted in several DETEs, suggesting that TE expression is significantly changed during infection and may play a role in the bacterial infection response, as seen in viral infections. Similar to Troha and colleagues (2018) who observed that bacterial

pathogenicity did not directly correlate with the number of host genes regulated during infection, we also found that the number of DETEs did not correlate with the severity of bacterial infection.

Following this trend, we observed that *Wolbachia* infections resulted in many DETEs, despite the fact that *Wolbachia* infections are native and non-pathogenic in *D. melanogaster*. *Wolbachia* is known to manipulate host gene expression (He *et al*. 2019; Frantz *et al*. In press), and we find that *Wolbachia* infection also alters the expression of host TEs. Another study also showed that *Wolbachia* can differentially affect expression of some TEs in *D. melanogaster*, both in increasing and decreasing TE expression depending on TE and host genotype (Eugénio *et al*. 2022).

Impact of Infection on TE Class and Family

In addition to the direction of expression, we also analyzed where and what types of TEs were differentially expressed during infection. In all infection types, we saw TE expression across all major chromosomes primarily in TE-rich regions like the centromere and telomeres, with large proportions of TEs coming from chromosomes 2R, 3L, and 3R. We did observe significant differences in the distribution of TE expression by infection type, where bacterial pathogens affected more TEs located on the Y chromosome, while *Wolbachia* infections more frequently activated TEs on the 4th chromosome than other infections. The chromosomes also differed in the types of TEs they contained, with higher proportions of LINE elements on the X and Y chromosomes than autosomes, and chromosome 4 containing the highest frequency of RC elements. This suggests a potential correlation between the chromosomal location and the types of TEs affected by different infection types, though it is unclear whether preference for TE location or class is the causative force behind these patterns.

Across all infections, most differentially expressed TEs were RNA retrotransposons, with a small number of DNA transposons differentially expressed in viral and *Wolbachia* infection datasets. Other studies have found that DNA transposons are the most active TEs in *Drosophila simulans* (Kofler *et al*. 2015), but our results agree with others that have found retrotransposons, specifically LTR elements, to be the most active elements in *D. melanogaster* (Kofler *et al*. 2015).

We also observed little or no Rolling Circle (RC) DNA transposons across all infections, and this was significantly less than expected in viral, bacterial, and *Wolbachia* infections. RC elements make up approximately 14% of TEs in the *D. melanogaster* genome, with the RC family *DINE-1* estimated to have the highest copy number of repeats of any TE family (Thomas *et al*. 2014). *DINE-1* elements are often involved in gene duplications, some of which have been linked to insecticide resistance in *Drosophila* (Carareto *et al*. 2014). Yet, despite their prevalence and involvement in insecticide resistance, we observed very few RC elements that were differentially expressed during infection in *D. melanogaster*.

Viral, fungal, and *Wolbachia* infections all affected TEs primarily from the LTR class, with *Ty3* being the most abundant TE family represented. However, LTR elements make up approximately 50% of TEs in the *D. melanogaster* genome, 35% of which are from the *Ty3* family. Therefore, we tested whether the class and family proportions of TEs differentially expressed during each infection were different from genome proportions.

The class and family identity of TEs differentially expressed during fungal infection did not significantly differ from genome proportions, suggesting no preference for the type of TE affected by fungal infections. However, we did find that both viral and *Wolbachia* infections significantly affected the class and family proportions of TEs expressed, specifically that there

was an overabundance of LTR elements and more TEs from the *copia* family than expected. *Copia* elements are known to regulate numerous host genes in *Drosophila* related to development (for review, see Moschetti *et al*. 2020). We observed relatively strong expression (> 2 $\log_2$-fold change) across multiple host genotypes and tissue types in both *Wolbachia* and viral infection datasets, suggesting a potential role for *copia* elements during the host response to infection.

Bacterial infections differed significantly from other types of infections by the types of TEs that were affected, where bacterial infections were more likely to affect LINE elements and TEs from the *Jockey* family. Across several datasets, *HeT-A* and *TART* elements decreased in expression during bacterial infection, which are known to affect telomere elongation and chromosome stability in flies (Frydrychova *et al*. 2008). This aligns with other studies which have found that flies modulate gene expression related to stress and cell homeostasis during bacterial infection (Troha *et al*. 2018). Bacterial infections also preferentially regulated *copia* elements, similar to viral and *Wolbachia* infections. Other notable TEs that were differentially expressed during bacterial infection include *invader*, *BURDOCK*, and *BS* elements, which have been linked to expression of immune-related genes that increase infection resistance in *D. melanogaster* (Ullastres *et al*. 2021).

Infection with *Wolbachia* was similar to other bacterial infections by generally decreasing TE expression during infection. These results agree with others that have found *Wolbachia* to decrease TE activity (Touret *et al*. 2014). We also observed several DETEs, such as *TART* and *invader* elements, that were shared with other bacterial infections. However, most of the types of TEs affected by *Wolbachia* infection differed significantly from other bacterial infections. Though other bacterial infections were more likely to affect LINE elements, infection

with *Wolbachia* led to more DETEs from the LTR and DNA classes. A large majority of DETEs in *Wolbachia* infection datasets belonged to the *Ty3* family, which act as promotors and insulators of host genes (Moschetti *et al*. 2020). *Wolbachia* infection can decrease expression of *Ty3* elements in *D. melanogaster* (Touret *et al*. 2014), though this effect may differ depending on host genotype (Eugénio *et al*. 2022). Though we did not find a significant effect of genotype, we observed that the effect of *Wolbachia* infection can differ across TEs and datasets, with *Wolbachia* infection increasing and decreasing the expression of various *Ty3* elements.

Wolbachia infection included the most DNA DETEs of any infection type and affected significantly more TEs from the *Tc1/mariner* superfamily than expected. This was of particular interest to us because of *Wolbachia*'s ability to induce plastic recombination in *D. melanogaster* (Singh 2019). DNA transposons are associated with increased recombination rate in the wood white butterfly (*Leptidea sinapis*) (Torres *et al*. 2022) and in *C. elegans* (Duret *et al*. 2000). Additionally, heat shock can cause increased gene expression of the *Tc1-mariner* retrotransposon in *C. elegans*, leading to increased DNA double-strand breaks (Kurhanewicz *et al*. 2020). The mechanism behind how *Wolbachia* alters recombination rate in *D. melanogaster* is currently unknown, but these results suggest a potential connection between increased expression of DNA transposons and increased recombination rate that is facilitated by *Wolbachia*.

Impact of Host Variables on TE Expression During Infection

We also examined how host variables affected TE expression. We analyzed differences between female and male fly samples, in addition to a cell culture of unknown sex, to determine whether host sex significantly changed the types of TEs expressed during infection. Sex is known to affect the susceptibility and intensity of infections in humans, with women generally less susceptible to infection due to more robust immune responses compared to men (Klein and

Flanagan 2016). Additionally, TEs are known to be involved in sexual development and other sex-specific forms of gene expression (for review, see Dechaud *et al*. 2019).

Our initial analysis showed that sex was associated with significant changes in class and family proportions of TEs, in addition to a larger number of DETEs coming from female samples compared to male samples. However, there was a nonrandom distribution of host sex in the datasets, where all but one of the viral and *Wolbachia* infection datasets used female flies and a majority of bacterial infection datasets used male flies. Therefore, we tested the effect of sex within the fungal infection datasets, which had equal proportions of male and female samples.

Sex did not significantly impact class proportions of TEs expressed during fungal infection, but female samples still had more DETEs and more TEs with increased expression. These findings suggest that host sex may influence the number and direction of TE expression during infection, but not necessarily the types of TEs affected. *Drosophila* is known to display sex-specific differences in immune response since a majority of innate immunity genes are located on the X chromosome (Taylor and Kimbrell 2007; Hill-Burns and Clark 2009). Our results suggest that these sex-specific differences in immune response also extend to TE expression during infection.

In addition to host sex, we also evaluated the effect of host genotype during infection. We analyzed a total of 17 genotypes and found no significant differences in TE class proportions or TE expression across host genotypes. Instead, we observed that the type of infection influenced TE dynamics more than host genotype, affecting both the type of TEs and the direction of expression. Similar to our analysis of sex, host genotype was significantly associated with differences in TE expression, although this was largely driven by one genotype, *dcr2-/-*, found in only viral infection datasets. Within these viral infection datasets, the *dcr2-/-* genotype had many

more DETEs than other host genotypes, even within the same dataset. These differences are likely due to a mutation in this genotype that impairs siRNA formation and leads to higher viral replication (Roy *et al*. 2020). Another host genotype, *CantonS*, was present in both bacterial and fungal infection datasets, but showed very few similarities within the genotype and was more similar to other fly genotypes within the same pathogen infection group. These results suggest that the influence of non-mutant host genotypes on TE expression is smaller than the influence of the pathogen.

Host genotype is known to influence TE copy number and insertion location between species and populations (Barron *et al*. 2014; Signor 2020). Therefore, it was expected that TE copy number and location would vary between the genotypes in our datasets. Indeed, we observed differences in base mean counts of the same TEs across different samples. TE insertions and copy number can affect TE expression (Lee and Langley 2012), but the piRNA pathway in *Drosophila* also employs copy-dependent silencing of TEs (Kelleher *et al*. 2020). By directly comparing uninfected and infected flies from the same dataset, our analyses assessed the relative TE expression within each genotype and found that host genotype does not significantly affect the change in TE expression during infection.

Sample tissue also varied across the datasets used in this study, including samples from brain, cell culture, carcass, intestine, ovary, and whole-body samples (Table 1). Host tissue was associated with significant differences in the direction of TE expression, but differences in TE class and family were nonsignificant. Carcass tissue samples shared similarities in TE expression across fungal and viral infection datasets, showing a strong preference for increased TE expression. The most common tissue samples were whole-body and ovary tissues, which were represented across multiple pathogen groups, but shared fewer similarities in expression patterns

overall. Outside of infection, hosts differentially regulate TE expression in different tissues, where somatic expression is generally repressed compared to germline expression (Haig 2016). However, one experiment found the opposite to be true during infection. In Roy *et al*. (2020) TE expression during viral infection was much higher in somatic tissues than in germline tissues, which was confirmed during our analysis of the same data.

Taken together, these results suggest a small role of host variables in influencing TE expression during infection. Although host sex and some tissue samples showed shared patterns in the direction of TE expression, other samples showed little consistency within groups and were more similar to samples from the same pathogen group. In contrast, samples from the same type of infection shared many more patterns in TE expression, suggesting that infection type had a stronger influence on TE expression than host variables. These findings differ from other studies analyzing gene expression during infection, where host genotype had a larger effect than pathogen in *D. melanogaster* (Frantz *et al.* In press) and humans (Idaghdour *et al*. 2012). These somewhat contradictory results may relate to differences in the ways that host genes and transposable elements are activated or suppressed during infection. A majority of TEs are repressed by host mechanisms which may become overwhelmed during infection, allowing previously silenced TEs to become active. This has been observed previously during infections in mammals (van Gestel *et al*. 2014) and flies (Roy *et al*. 2020). Therefore, the type of infection may impact which host defense mechanisms are activated and become unable to regulate TE expression.

Conclusions

Transposable elements can play crucial roles in the host's immune system response to infection, as has been demonstrated in humans, mice, flies, and more. However, our understanding of these interactions are limited by differences between pathogen and host variables in each study, limiting our ability to identify broader patterns in TE responses to infection. Our work presented here combines gene expression data from multiple infection studies in D. melanogaster to illuminate the influence of pathogen and host variables on TE activity during infection. We find that TE activity is strongly affected by differences in pathogen infection, while the effect of host variables is comparatively smaller. Future experimental work in flies, as well as additional comparative studies in other model organisms, would help to expand our understanding of TE activity during infection to even broader scales.

Materials and methods

Sequence Processing

The datasets used in this study were downloaded from the European Nucleotide Archive (ENA) at EMBL-EBI. Processing and analysis of sequence files was completed using the University of Oregon's high performance computing cluster, Talapas. Raw fastq files were merged and aligned to the *Drosophila melanogaster* reference genome (Release 6.41) as unsorted BAM files using the program STAR (v2.7.9a) (Dobin *et al*. 2013). To optimize the data for transposable element (TE) analysis, we used the recommended settings for STAR from Jin and Hammell (2018) by setting –outFilterMultimapNmax 100 and –winAnchorMultimapNmax 200, with default values for all other parameters.

Counting Transposable Elements

We used the program TEtranscripts to count TEs in our selected datasets (Jin *et al*. 2014). TEtranscripts requires two GTF files, one for gene sequences and one for TE sequences. We used the *D. melanogaster* TE GTF file provided on the Hammell lab website (labshare.cshl.edu/shares/mhammelllab/www-data/TEtranscripts/TE_GTF/dm6_BDGP_rmsk_TE.gtf) and the *D. melanogaster* genome Release 6.32 GTF file from Ensembl (ftp.ensembl.org/pub/release-107/gtf/drosophila_melanogaster/Drosophila_melanogaster.BDGP6.32.104.gtf.gz). After alignment, BAM files for control and infection groups from each dataset were analyzed using TEtranscripts, with –norm TC and default parameters, to produce TE gene counts.

When presenting our findings, we have also elected not to use the antiquated and problematic name for the *gypsy* TE family, instead opting for the alternate naming of *Ty3* as advocated for by others in the field (Wei *et al*. 2022).

Statistical Analyses

All statistical analyses and data visualization was completed in RStudio (v2021.09.0, "Ghost Orchid" release). Differentially expressed transposable elements were identified by calculating the log2-fold change between control and infection samples and assessing significance using DESeq2 (v1.32.0) (Love, Huber, and Anders 2014). We used a chi-squared goodness of fit test to examine whether observed patterns of differentially expressed TEs at class and family levels were significantly different from proportions of TEs present in the *D. melanogaster* genome, as well as to compare differences between and within pathogen groups. The proportions of TEs categorized by class and family in the genome were calculated from the *D. melanogaster* Release 6.32 Ensembl GTF annotation file.

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CHAPTER IV

CONCLUSION

This dissertation describes my work investigating *Wolbachia*-associated plastic recombination in *Drosophila melanogaster*. *Wolbachia pipientis* is an endosymbiont that infects the fruit fly, *D. melanogaster* and plastically increases recombination rate across areas of the fly's genome. Prior to my doctoral work, little was known about the dynamics of *Wolbachia*-associated plastic recombination aside from a small number of experiments which identified a few areas of the genome that displayed increased recombination rate during *Wolbachia* infection. Therefore, the goal of my dissertation was to investigate how host and microbe interactions influenced *Wolbachia*-associated plastic recombination and begin setting a foundation for future work in this field.

In my first chapter, I investigated the effect of bacterial titer on *Wolbachia*-associated plastic recombination. My findings were applicable to several areas of investigation, including the effects of diet and *Wolbachia* titer on recombination rate. I was able to reconfirm that *Wolbachia* infection increases recombination rate across the *yellow-vermillion* interval of the X chromosome, and that this effect was robust in response to changes in host diet. My results also demonstrated that host diet did not affect recombination rate in *D. melanogaster*, shining new light on a topic that has been severely understudied for several decades. Finally, though *Wolbachia* titer did not significantly affect the magnitude of recombination rate, I did find that *Wolbachia* titer was associated with significant changes in the variation of recombination rate in *D. melanogaster*. Recombination rate variance has not previously been reported in other studies of plastic recombination, suggesting a potentially novel and unique characteristic of *Wolbachia*-associated plastic recombination. Findings from this chapter can inform several areas of future

study, including whether larger changes in *Wolbachia* titer may influence recombination rate, or whether recombination rate variance appears in other systems and the evolutionary implications for that variation.

In my second chapter, I explored how *Wolbachia* infection changes transposable element (TE) expression in *D. melanogaster*, and how these changes may suggest a mechanism for *Wolbachia*-associated plastic recombination. My findings demonstrated that *Wolbachia* infection affects a substantial number of TEs, and that activation of some TEs is specific to certain types of infections. In particular, *Wolbachia* infection affected more DNA TEs than other pathogens, which are known to affect recombination in other organisms by inducing double-stranded breaks more frequently in DNA. These results are suggestive of a potential mechanism for *Wolbachia*-associated plastic recombination, where *Wolbachia* infection changes the activation of DNA TEs, resulting in downstream effects that alter the recombination landscape in *D. melanogaster*. Future experimental work can validate this hypothesis in one of several ways: by manipulating TE regulatory networks in *D. melanogaster* infected with *Wolbachia* to observe the corresponding change in recombination rate, or by investigating if *Wolbachia* infection in other species is also associated with changes in DNA TE expression.

At the start of my dissertation, few knew about or were interested in exploring *Wolbachia*-associated plastic recombination. Now, however, work by myself and others in the field has started to reveal aspects of this fascinating system that combines host-microbe associations and recombination. These findings allow us to better understand how *Wolbachia* influences its hosts and can alter the evolutionary future of species through recombination. My own contributions to the field have provided several findings that I hope can inspire new questions and further work in this system. Investigating *Wolbachia*-associated plastic

recombination can inform our understanding of how *Wolbachia* may affect other insect hosts relevant to humans, such as *Aedes egypti*, in addition to shining a light on potential mechanisms of plastic recombination more generally.