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Molecular Basis of Adaptation in Experimentally Evolved Drosophila

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILISOPHY

In Biological Sciences

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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ABSTRACT OF DISSERTATION

Molecular Basis of Adaptation in Experimentally Evolved Drosophila

By

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Doctor of Philosophy in Biological Sciences

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Professor Laurence D. Mueller, Chair

The molecular mechanisms underlying adaptation have eluded evolutionary biologists even with the advent of new sequencing technology. Many attempts to address this issue have focused on using knockout or knock-down genes in inbred populations, but have failed to fully characterize how molecular changes ultimately effect phenotypic changes. These systems likely fall short because inbred populations give variable and inconsistent results, while knockout and knock-down genes have unknown pleiotropic effects.

Experimental evolution in *Drosophila* features replicated outbred populations with easy access to both phenotypic and molecular characterization. Experimentally evolved *Drosophila* populations are outbred and offer ample replication which is necessary when using statistical learning tools to search for the molecular basis for phenotypic change. My work chiefly focuses on 20 experimentally evolved *Drosophila* populations, ten selected for short life-cycles and ten selected for long life-cycles. Using these 20 populations, I find that phenotypic divergence from an ancestral population occurs rapidly, within dozens of generations, regardless of evolutionary history and similarly, populations sharing a selection treatment converge on common phenotypes in the same time frame (Chapter 1). From the same 20 populations, I find that traits are heavily

influenced by selection regime when the trait is fitness-related; conversely, when the trait is not fitness-related, evolutionary history takes precedent (Chapter 2). Next, I sequenced the transcriptome from these 20 populations and found evidence for convergence within each group of populations undergoing the same selection regime, and moderate differentiation between the two groups of populations (Chapter 3). Lastly, I applied statistical learning tools to genomic, transcriptomic, and phenotypic data obtained from these 20 populations. I found that (a) the transcriptome is static in adult *Drosophila*, (b) both genome and transcriptome can be good predictors for phenotypic characters, and (c) gene expression is influenced by genomic sites found all across the genome (Chapter 4).

INTRODUCTION

Evolutionary biology has long sought to uncover how molecular mechanisms shape phenotypic characters. The overall pathway of these molecular mechanisms is known in general terms, but how specific changes in the genome or the transcriptome affect specific phenotypic traits remains unknown. Many attempts to address this question have used knockout gene studies or even knockdown gene studies (Alberts et al. 2002; Hall et al. 2010). These studies tend to focus on using inbred lines in order to properly set up the knockout gene. These inbred lines, of course, have little to no genetic variation and whatever results may be concluded from these experiments seem to be case-specific. In other words, if the same genes were to be knocked out in another inbred line, the results are likely to vary (Eisener-Dorman et al. 2009).

Contrary to using inbred lines and working with knockout genes, experimental evolution has become an alternative means of addressing the basic mechanisms of evolution. Experimental evolution has been key in furthering our understanding of evolutionary mechanisms in microbial ecology (Chao et al. 1997; Turner and Chao 1999; Crill et al. 2000; Kaltz and Bell 2002), life history evolution (Mueller and Ayala 1981; Luckinbill et al. 1984; Rose 1984; Service et al. 1988; Chippendale et al. 1997), and evolutionary physiology (Graves et al. 1992; Rose et al. 1992; Gibbs et al. 1997; Djawdan et al. 1998; Swallow et al. 1998; Swallow et al. 1999; Roff et al. 1999). In addition, experimental evolution has become a great tool for understanding the genetic foundations of adaptation when paired with next-generation sequencing for both genomics and transcriptomics (Long et al. 2015; Schlötterer et al. 2015). Experimental evolution of outbred sexual populations has clear advantages over the use of inbred lines and knockouts, in that it maintains a moderate level of genetic variation and allows for a high level of replication. These two strengths together allow the use of high powered statistical tools, which further allow

tests for interactions between the genome, transcriptome, and phenotypic traits on a scale that has never been achieved before.

The goal of my thesis is two-fold. First, show the consistency and repeatability of experimental evolution when using outbred sexually reproducing populations with moderate replication. Unlike inbred or asexual populations, sexually outbred populations have the capacity to adapt to novel selection regime in a parallel fashion due to their maintenance of genetic variation, at least when they are not inbred. Although both inbred and asexual populations have their advantages in simpler laboratory maintenance, these populations do not accurately simulate how adaptation functions in nature for many organisms. Experimentally evolved outbred populations may also not be a perfect simulation for adaptation in nature, but will almost always be a closer emulation than inbred or asexual populations. For the second part of my thesis, I use genomic, transcriptomic, and phenotypic data from these 20 outbred sexual populations to determine how these three components interact with one another.

Numerous attempts have been made to tackle the overarching issue of how molecular changes ultimately affect phenotypic traits in inbred or asexual populations, but this has not been the case for outbred sexual populations. Experimentally evolved, outbred, sexual populations offer statistical power that results from extreme phenotypic differentiation (Garland and Rose 2009). The statistical power gained by using these populations is critical in dissecting the interplay between the three levels of genome, transcriptome, and phenotype.

My scientific material consists of experimentally evolved outbred sexual populations of *Drosophila melanogaster* maintained in the Rose Lab at the University of California, Irvine. Specifically, I have studied two sets of 10 replicate populations maintained under different

selection regimes. Within both sets of 10 replicate populations, five are considered long-standing in that they have been maintained in their current selection regime for over 300 generations. The remaining five are considered newly-derived, in that they have only been maintained in the current selection regime for less than 250 generations. Using these populations, I measure how rapidly and repeatedly these populations have converged within selection regime and diverged between selection regimes for mortality and fecundity (Chapter 1). After seeing how rapid and repeatedly these populations have converged and diverged for mortality and fecundity, I sought to see if the same trend remains for other phenotypic characters such as development time and egg hatching time (Chapter 2). Next, I characterize the transcriptomes of the same 20 populations featured in Chapter 1 and 2, and again measure the level of convergence within selection regime and divergence between the two selection regimes (Chapter 3). Lastly, I use genomic, transcriptomic, and phenotypic data together in conjunction with statistical learning modeling to see how these three levels of biological machinery interact with each other (Chapter 4).

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Chapter 1

Rapid Divergence and Convergence of Adult Mortality and Fecundity in Experimentally Evolved *Drosophila melanogaster*

ABSTRACT

Laboratory selection experiments are alluring in their simplicity, power, and ability to inform us about how evolution works. A longstanding challenge facing evolution experiments with metazoans is that significant generational turnover takes a long time. In this work, we present data from a unique system of experimentally evolved laboratory populations of *Drosophila melanogaster* that have experienced three distinct life-history selection regimes. The goal of our study was to determine how quickly populations of a certain selection regime diverge phenotypically from their ancestors, and how quickly they converge with independently derived populations that share a selection regime. Our results indicate that phenotypic divergence from an ancestral population occurs rapidly, within dozens of generations, regardless of that population's evolutionary history. Similarly, populations sharing a selection treatment converge on common phenotypes in this same time frame, regardless of selection pressures those populations may have experienced in the past. These patterns of convergence and divergence emerged much faster than expected, suggesting that intermediate evolutionary history has transient effects in this system. The results we draw from this system are applicable to other experimental evolution projects and suggest that many relevant questions can be sufficiently tested on shorter timescales than previously thought.

INTRODUCTION

Evolutionary biologists have long used laboratory selection to explore hypotheses about adaptation. Such experimental evolution can quickly, dramatically, and reproducibly shape phenotypes in model species (Garland and Rose 2009). When adequately replicated, experimentally evolved populations can be used to test general theories about evolution in well-defined settings, albeit ones which may be significantly difference from any that actually exist in the wild (cf. Garland and Rose 2009; Rose et al. 2001; Barrick and Lenski 2013).

But there is an important bifurcation in the experimental evolution literature, that between (i) studies of rarely recombining microbes, like *Escherichia coli* (e.g. Lenski et al. 1991), and (ii) studies of outbreeding species that recombine sexually and maintain standing genetic variation, such as *Drosophila melanogaster* (e.g. Luckinbill et al. 1984). Recombination and standing genetic variation are either absent or rare in many paradigms for microbial experimental evolution (e.g. Tenaillon et al. 2012), though genetic variation can arise from mutator substitutions in some cases after many generations of clonal evolution (e.g. Barrick et al 2009). The lack of recombination in such clonal evolution experiments gives rise to very different patterns of adaptation from those observed in experiments with outbreeding sexual species. Clonal evolution features selective sweeps, clonal interference, and whole-genome hitchhiking, all of which both purge genetic variation and slow the genome-wide response to selection (reviewed by Burke 2012; Kawecki et al. 2012). By contrast, it has been found that experimental evolution in outbred *Drosophila* populations involves abundant standing genetic variation, many genomic sites that respond to selection, and rapid immediate responses to selection (Burke et al. 2010; Orozco-terWengel et al. 2012; Burke 2012; Rose et al. 2015). While

the differences between these two types of experimental evolution are considerable, ideas from one type of system can serve as useful sources of hypotheses for the other.

For example, an issue of great interest in the microbial evolution literature has been the extent to which multiple replicated lines converge on similar phenotypic and genetic outcomes, the so-called "chance versus necessity" or "history versus selection" issue (e.g. Woods et al. 2006; Tenaillon et al. 2012). Here we study experimental evolution in 30 populations of *Drosophila melanogaster*, with a view to addressing the repeatability of phenotypic evolution in outbreeding, sexually-reproducing populations, which for clarity we hereafter term 'Mendelian' populations.

Research on the Mendelian experimental evolution paradigm has already produced some useful initial findings concerning divergence, convergence, and the repeatability of evolution. First, it is clear that phenotypic divergence occurs rapidly in Mendelian populations subject to new types of selection. Phenotypes of newly-selected populations differentiate from their ancestors in tens of generations; this has been shown in multiple *Drosophila* experiments (e.g. Luckinbill et al. 1984; Rose et al. 1992; Chippindale et al. 1997; Zhou et al. 2007; Turner et al. 2011; Turner and Miller 2012), as well as in other insects (e.g. Roff et al. 1999; Beldade et al. 2002; Zera 2005; Michalczyk et al. 2011), mice (Swallow et al. 1999; Chan et al. 2012), domesticated birds (Johansson et al. 2010; Stringham et al. 2012), and foxes (Trut et al. 2004). Second, independent replicate populations experiencing identical selection pressures quickly converge on common phenotypes (Teotonio and Rose 2000; Simões et al. 2008; Fox et al. 2011; Fragata et al. 2014). However, studies that assess the rapidity of convergence and divergence simultaneously, in order to assess the importance of evolutionary history, are lacking. Here, we

carry out simultaneous comparisons of convergence and divergence to determine the degree to which selection might erase or preserve the signature of history for specific fitness traits.

We present life-history data from two sets of populations: 15 long-standing populations and 15 recently-derived populations. Five populations from each set are subject to one of three regimes of experimental evolution: (1) selection for accelerated larval development, (2) the ancestral laboratory selection regime of two-week life cycles, and (3) selection for postponed reproduction. Put another way, we present the results from three tests of evolutionary convergence and divergence involving ten populations each, with large-scale parallel assays of life-history characters. Our results reveal significant detail about how Mendelian experimental evolution produces both phenotypic divergence and convergence, on time scales vastly compressed compared to those of clonal evolution.

MATERIALS AND METHODS

Experimental evolution regimes

This study uses large, deliberately outbred, lab populations of *Drosophila melanogaster* selected for different patterns of age-specific reproduction. All the lines used in the current study originate from an ancestral "IV" population first collected from South Amherst, MA in 1975 by Phillip Ives (vid. Rose 1984), and then cultured in the lab using two-week discrete generations. These ancestral IV flies were subsequently used in February 1980 to create five "O" (old) replicate lines, using females of increasing ages over successive generations until these flies were maintained on a 10-week generation cycle (Rose 1984). The IV flies were also used to found five additional "B" lines in February 1980, lines which have since been cultured using the same

protocol as the IV populations from which they were derived. Detailed descriptions of the subsequent history and culture methods for these lines can be found in Rose et al. (2004).

The populations of the O selection treatment are the ancestors of 5 additional experimental treatments that along with the B selection treatment make up the 6 focal treatments of this study. These treatments belong to one of two temporal designations ("longstanding" or "recent") and one of three selection types ("A", "B", or "C", described below). Thus, these 6 treatments provide opportunities to study the differences between populations that have the same selection regime but were established long ago versus recently, and also to study the differences between populations that diverged from the same ancestor recently but experience selection for different life histories. See Figure 1.1 for an overview of the experimental evolution design of the present study.

We call these 6 selection treatments ACO, AO, B, BO, CO, and nCO, with each letter referencing a selection regime and evolutionary history. The CO populations were derived from the original O treatment after 57 generations of O-type selection in 1989. The "C" in CO indicates a 28-day selection regime while the O represents the CO treatment's most recent common ancestor. The ACO lines are the only populations that did not directly originate from the O treatment, but were instead derived from the CO populations after 27 generations of C-type selection in 1992. The AO, BO, and nCO lines ("n" stands for *new* CO treatment) were derived from the O treatment around 2007 after 153, 150, and 159 generations of O selection, respectively. Thus, these three treatments were derived relatively recently, while the CO, ACO, and B treatments are longstanding.

This system of 30 populations is now maintained using three distinct selection regimes: A, B, and C. *A selection regime*: the ten ACO and AO populations spend the first 9 days of life

in 8-dram glass vials, and at day 10 adults are transferred to a Plexiglass "cage" in which they are given fresh food and allowed to oviposit for 24 hours. *B selection regime*: the ten B and BO populations spend 14 days in 8-dram vials, and are then allowed 1-2 hours in fresh vials to oviposit before adults are discarded. *C selection regime:* the ten CO and nCO populations develop in vials for 14 days prior to being transferred to Plexiglass cages. C flies are then given 48 hours to oviposit before eggs are collected on day 28. All populations are supplied with food made from cooked bananas, barley malt, yeast (3.6% w/v), corn syrup, and agar. The populations that spend time in cages are also supplied with a 5% live yeast paste on the food surface to promote oviposition 24-48 hours prior to egg collection. Lastly, all populations are kept at 23° C and left in a 24-hour light cycle room. See Figure S1.1.

Experimental comparisons

Two experimental comparisons were performed: (1) Common-garden comparison of initial fecundity of flies from the AO, ACO, B, BO, CO, and nCO treatments; and (2) Parallel simultaneous cohort comparisons of adult fecundity and survivorship of flies from the AO, ACO, CO, and nCO treatments. The goal of these two comparisons was to determine the degree to which divergence has occurred among the three different selection treatments, as well as the degree to which convergence has occurred between the longstanding and recently-derived treatments in adult phenotypes. For these two experiments, all replicate populations assayed were reared in parallel on a B-type culture schedule for two generations before each experiment to minimize maternal effects.

Experiment 1: Initial fecundity of newly eclosed adults

Early-life fecundity measures were collected from the newly eclosed adults in all replicate populations of the ACO, AO, B, BO, CO, and nCO treatments. Newly eclosed flies were collected every 12 hours, sorted into 40 mating pairs, and then placed into vials to mate and lay eggs. Every 12 hours until day 14 of age from egg, the mating pair was given a vial cap containing fresh food to lay eggs. The eggs laid on the old vial caps were placed on a flatbed scanner to create a digital image for egg counting purposes.

Experiment 2: Adult mortality and fecundity from day 14 onward

For this experiment, adult mortality was measured in all replicate populations of the ACO, AO, CO, and nCO selection treatments. After two generations of standardized rearing, ~1200 adult flies were emptied into Plexiglass cages. Cages were supplied with fresh food daily. Dead flies were counted and eggs collected from cages at the same time every day until all flies in the experiment died.

Mortality data were obtained from the 30 experimental populations over all adult ages. Each assayed cohort began as four cages containing ~1200 flies each, where the volume of a cage was 13.2H x 18.5 W x 22.4L cm^3. We redistributed and combined flies periodically to maintain this 1200 flies/cage density as the number of individuals in the cohort declined. When a cohort fell to 600 individuals (50% cage density), flies were transferred to a half cage, at 300 individuals, the cohort was transferred to a quarter-cage, and at 100 individuals, the cohort was transferred to a single 8-dram vial. Flies were briefly anesthetized using carbon dioxide during these consolidations. The number of dead flies in each cage or vial was recorded over daily intervals.

A fecundity measure was made in concert with the mortality assay. After the daily mortality count, flies were given a new plate of food. Eggs that had been laid on the surface of the old food plate were collected through a filtration process onto a membrane using a modified Buchner funnel. A digital image of the membrane was then taken and the number of eggs laid were counted using ImageJ software. Carbon dioxide can make females eject eggs in greater than normal numbers. To compensate for this problem, the number of eggs laid during the 24 hours after exposure to carbon dioxide was removed from the fecundity analysis.

Statistical methods

Experiment 1

We tested for convergence between paired selection treatments (i.e., CO vs. nCO, AO vs. ACO, and B vs. BO) for effects of selection on fecundity over 3-4 consecutive ages. The observations consisted of fecundity at a particular age (t) but within a small age interval (k = 1,2,…,m). These age intervals were chosen to span the ages, such that all comparison populations had live flies. Within each interval, fecundity rates were modeled by a straight line and allow selection regime (j = 1 (ACO or CO or B) j = 2 (AO or nCO or BO)) to affect the intercept of that line but not the slope. However, slopes were allowed to vary between intervals. Populations (i = 1,…,10) were assumed to contribute random variation to these measures. With this notation, the fecundity at age-t, interval-k, selection regime-j, and population-i, is y_{ijkt} and is described by,

$$y_{ijkt} = \alpha + \beta_k + \delta_j\gamma_j + (\omega + \pi_k\delta_k)t + \delta_k\delta_j\mu_{jk} + c_i + \varepsilon_{ijkt} \quad (1)$$

where $\delta_s = 0$ if $s = 1$ and 1 otherwise, and c_i and ε_{ijkt} are independent standard normal random variables with variance σ^2_c and σ^2_ε respectively. The effects of selection on the intercept are assessed by considering the magnitude and variance of both γ_j and μ_{jk}.

To test for divergence, the six selection regimes were reclassified to three difference categories: AO and ACO to A; CO and nCO to C; B and BO to B. Equation (1) was then used to assess the effects of the three selection treatments. Parameters of equation (1) were estimated by the restricted maximum likelihood techniques implemented by the *lme* function in R (R Core team 2014). For both the convergence and divergence analysis, we used the Bonferroni correction to adjust the significant level for each pair-wise comparison made by dividing the significance level by the number of age intervals used in the analysis (0.05/n, where n is the number of age intervals used).

Experiment 2

The same basic analysis as that of Experiment 1 was used to test for convergence and divergence of age-specific fecundity and mortality across the adult portion of the life-cycle. However, this experiment did not contain all six selection treatments, instead it only contained four (ACO, AO, CO and nCO). For convergence the same pairing was used (i.e., ACO vs. AO and CO vs. nCO) and for divergence we used the same classification used in Experiment 1.

RESULTS

Early fecundity results from Experiment 1:

We observe similar early-life fecundity trajectories in the matched long-standing and recently derived A, B, and C-type selection treatments. Populations that share the same selection

regime recently (e.g. ACO and AO) are markedly different from those that do not. Qualitatively, the A, B, and C treatment groups of populations are clearly divergent from each other, although this is most obvious in the earliest ages assayed. Statistically comparisons made between treatments of the same type return with no difference, with the exception of ACO vs AO at ages 12, 13, and 14 (bold values in Table 1.1; hours 276-336, $p<0.0125$). In contrast, comparisons made between selection types return with statistically significant differences, with the exception of B-type vs C-type after 11 days of age (bold values in table 1.2, hours 276-336, $p>0.0166$). Our significance thresholds vary between experiments and sometimes between treatment comparisons within an experiment due to the number of tests involved in each comparison.

Adult life-history results from Experiment 2:

Figures 1.3 and 1.4 reiterate the convergence and divergence patterns observed in experiment 1. Within A and C-type treatments, long-standing and newly derived populations are not significantly different from one another (Table 1.3). On the other hand, populations that do not share the same selection regime are highly divergent from one another. A notable exception from this trend is evident in middle-period fecundity, between ages 17 and 25 days from egg, when there is no detectable difference in fecundity among those flies that are still alive in A and C cohorts. But in keeping with the results from experiment 1, fecundity prior to age 16 is significantly different among selection treatments (Table 1.4, $p<0.005$), and furthermore, fecundity after age 25 is significantly different (Table 1.4, $p<0.005$), as A flies die in large numbers after this age (Fig. 1.3).

DISCUSSION

Overview of results: rapid divergence and convergence

First, when comparing the degree of phenotypic *divergence* among populations of the three recently derived treatments (AO, BO, and nCO) with those of the long-standing treatments (ACO, B, and CO), it is apparent that adult life-history differentiation is remarkably similar when the comparison is made between these two sets of treatments. In effect, hundreds of additional generations of A, B, and C-type selection seem to have yielded at most minor increases in adult life-history differentiation.

Second, and conversely, there is a high degree of phenotypic *convergence* within each of the three sets of A, B, and C-type populations. There are some exceptions to this general pattern. For example, (i) early fecundity over age 12, 13 and 14 days was significantly different between the ACO and AO populations, and (ii) early fecundity over age 12 and 13 days was significantly different between the CO and nCO populations.

We find that the phenotypes of our newly derived populations usually converged with those of longstanding populations sharing the same selection regime within 200 generations. We also find that these newly derived populations, all initiated from a common ancestor, significantly diverge from one another within this time frame. The rapidity of convergence and divergence suggests that in this particular set of 30 populations, recent evolutionary history is highly predictive of phenotype. We do not presume that phenotypic divergence and convergence in these experiments necessarily involves the same underlying genetic mechanisms. In Mendelian evolution experiments, selection treatments sometime produce common phenotypes in independent replicates that are the result of different genetic "solutions" (e.g. Garland et al. 2002; Kawecki and Mery 2006). However, recent work by Graves et al. (2017) on these same

thirty populations reveals the same parallel genomic convergence as I find for life-history convergence. Specifically, they find genomic convergence in the frequencies of single-nucleotide polymorphisms, transposable elements, insertions, and structural variants. Likewise, they found similar patterns of divergence.

Given that Mendelian populations maintain a considerable amount of standing genetic variation which is reshuffled every generation by recombination, it is certainly reasonable to expect selection histories to be erased quickly even in moderately sized populations. Whether or not this occurs appears to depend on the details of the experiment in question. Populations of a bean weevil collected from different geographic origins continued to differ in host preference (Kawecki and Mery 2003), and in a number of life-history traits (Bieri and Kawecki 2003), despite 120 generations of adaptation to a common laboratory environment. Populations of *Drosophila* collected from different locations and reared in a uniform laboratory environment have previously been shown to converge for some phenotypes but not others (Cohan and Hoffman, 1989; Griffiths et al. 2005; Simões et al. 2007; Simões et al. 2008; Santos et al. 2010). This could be attributable to stochastic effects during the initial founding phase, insufficient sampling of natural variation or both.

It is also conceivable that the speed of convergence among populations with different lab evolutionary histories is trait-specific. One version of this hypothesis is that history should play a greater role in the convergence of traits less directly related to fitness; that is to say, fitness traits should converge faster and more consistently than less traits less obviously associated with fitness as determined by a particular selection regime. This is a classic result of evolution experiment with asexual populations (e.g. Lenski and Travisano 1994; Travisano et al. 1995), but remains ambiguous in experiments with Mendelian populations (e.g. Joshi et al. 2003; Matos et

al. 2002; Fragata et al. 2014; Simões et al. 2019). Our finding that early fecundity was the same between the long-established ACO and newly derived AO populations prior to age 13, but diverged after age 13, is potentially consistent with this idea. Early fecundity is the primary fitness trait in the A-type selection regime, as eggs are collected within 10 days from hatching in a single generation (Fig. S1.1). Thus, fecundity after this age should be effectively decoupled from fitness.

Another aspect of the hypothesis that the effect of history on convergence is trait specific is that past selection might continue to affect the adaptation of populations to new selection pressure if the past selection gave rise to particular patterns of genotype by environment (GxE) interactions. We have invoked this explanation before in a study of reverse evolution with some of the same populations as in the current study (Teotonio and Rose 2000). Notably, that study showed somewhat less phenotypic convergence for some characters and populations than we have generally found here. Recent work by Fragata et al. (2014) reports that strong initial differentiation among populations of *D. subobscura* is diminished within 22 generations of a common selection environment, both for traits expected to be correlated with fitness and those that were not. Overall, our results are consistent with this result and support the view that past evolutionary history generally has transient effects in the face of strong selection and ongoing recombination.

Novel aging result: early-adult mortality plateaus

The second major finding that emerged from this study was the virtual absence of aging between ages 14 and 28 days, from egg, in the age-specific mortalities and fecundities of the two C cohorts assayed in Experiment 2. These cohorts were derived from populations that had been

cultured for about 350 (CO) and 200 (nCO) generations without reproduction during, or of course before, this period of adult life. Thus, there has been full-intensity selection for continued survival to least up to the age of 28 throughout these hundreds of generations. From this standpoint, then, it is perhaps unsurprising that we find little statistically detectable aging during this period of adult life, despite the reproductive maturity of these fruit flies (Rose et al. 2007).

But prior studies of ours did not reveal this pattern; for example, previous experiments with CO lines do not reveal an absence of aging during the same period (Rose et al. 2002). Rather, aging appears to start soon after the age of 16 or 18 days from egg in the cohorts assayed at that time, when the CO populations were well over 150 generations of selection for survival until at least 28 days, bearing in mind their history of O selection prior to their derivation as CO stocks in 1989 (vid. Rose et al. 1992; Fig. 1.4). This disparity relative to the present data is particularly obvious for the female cohort data from the 2002 study of Rose et al., which shows a pattern of increasing mortality between ages 14 and 28 days from egg, unlike the data found in our present comparison of A and C type mortality rates.

At present, our interpretation is that this disparity was due to our earlier use of vial assays of age-specific adult life-history. The A, O, and C type populations are cultured using adults laying eggs in cages, with C populations living in cages for the two weeks between ages 14 and 28 from egg. Thus, we suggest, the present assay was performed under conditions more representative of the conditions that selection was actually focused on over the last 200 or 35 generations of the culture of nCO and CO populations, respectively. Conversely, our earlier assays were conducted under conditions that did *not* closely reflect the circumstances of C-type selection. Our conclusion is that, if we had used the same type of cage assay as that employed in

the present study before, we would have previously detected the virtual absence of aging during the 14-28 day life-history period.

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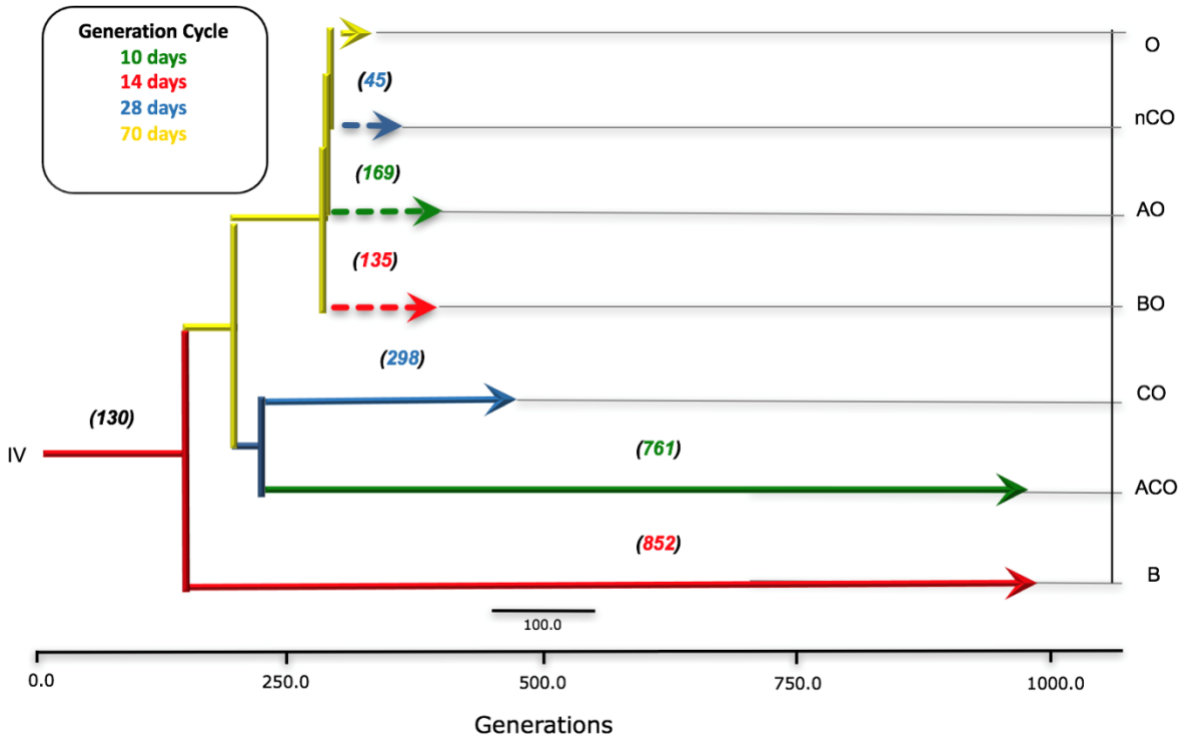

Figure 1.1. Schematic of population selection history. Abbreviated illustration of the evolutionary relationships between the focal selection treatments of this study. All treatments share ancestry, some more recently than others. The first capitalized letter of a treatment name indicates the type of life-history regime for which it has sustained selection: A (10-day cycle), B (14-day cycle), or C (28-day cycle).

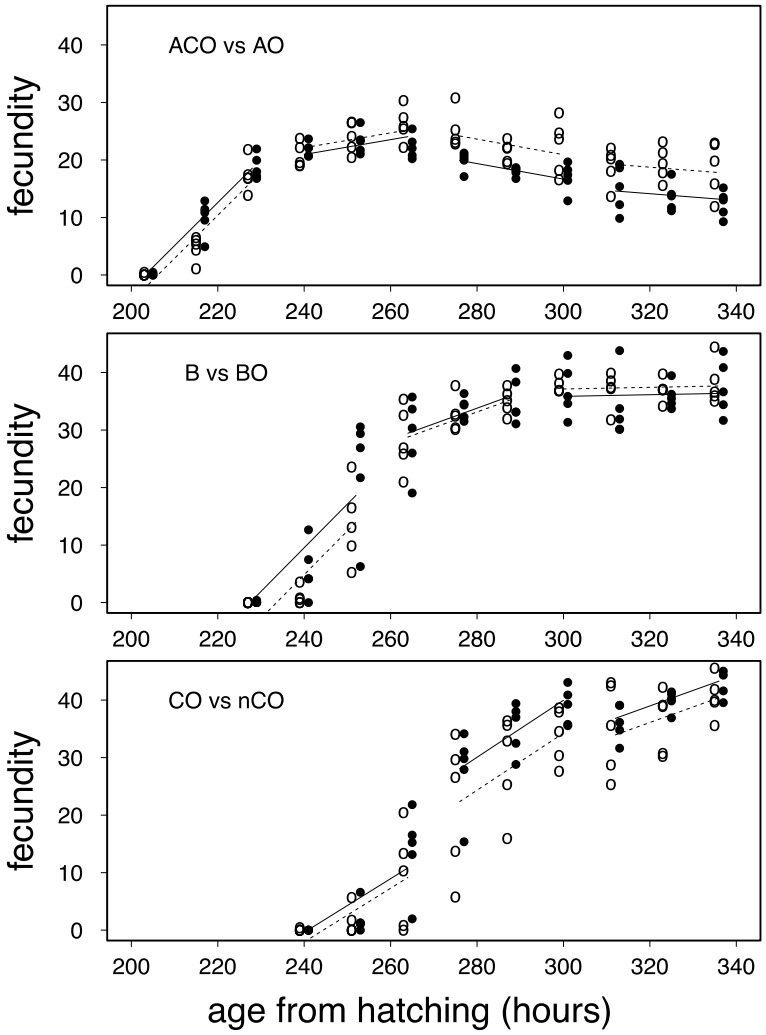

Figure 1.2. Early-life fecundity comparison in all six contemporary selection treatments. Initial age-specific fecundity prior to day 14 from 30 cohort of our six laboratory selection treatments. Open circles and dashed lines represent average eggs laid per female per day as a function of age in the five replicated longstanding populations (ACO in the top panel, B in the middle panel, CO in the bottom panel), and solid circles and solid lines represent the five replicated newer derived populations (AO in the top panel, BO in the middle panel, nCO in the bottom panel).

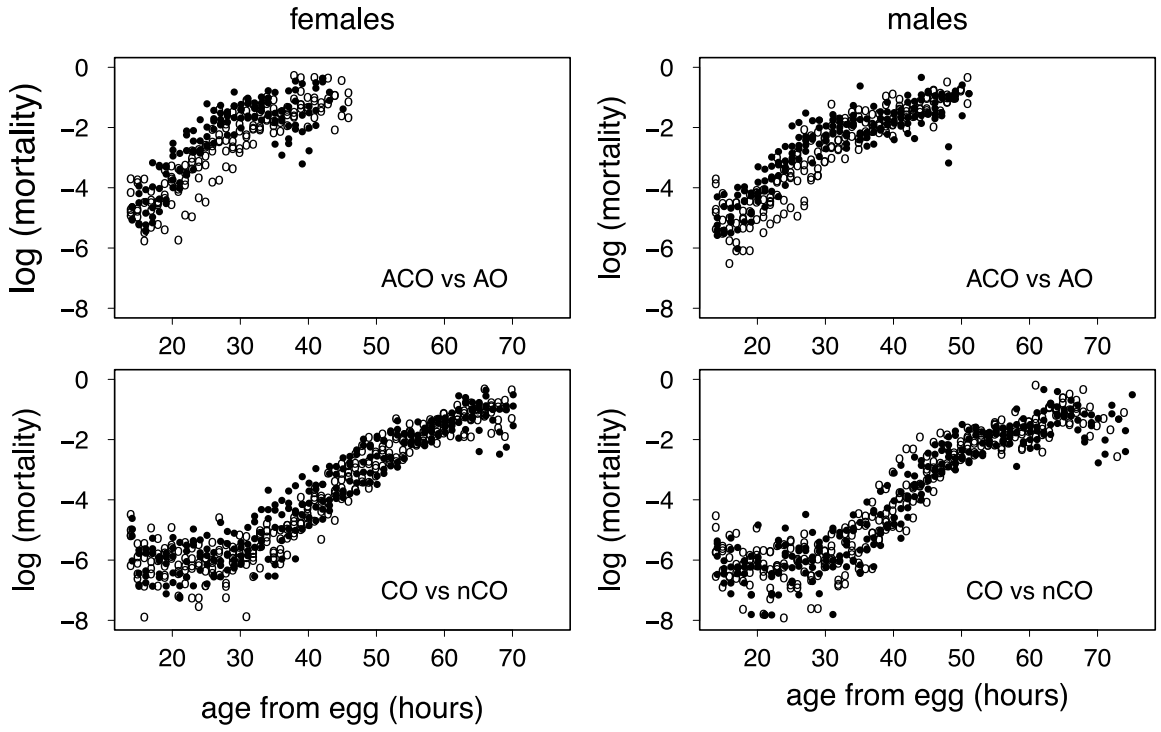

Figure 1.3. Adult age-specific mortality among males and females from A-type and C-type selection treatments. Points represent log-transformed mortality per population in each selection treatment, and are presented separately for females (left panels) and males (right panels). Open circles represent longstanding populations (ACO in the top panels and CO in the bottom panels), and solid circles represent newer derived populations (AO in the top panels and nCO in the bottom panels).

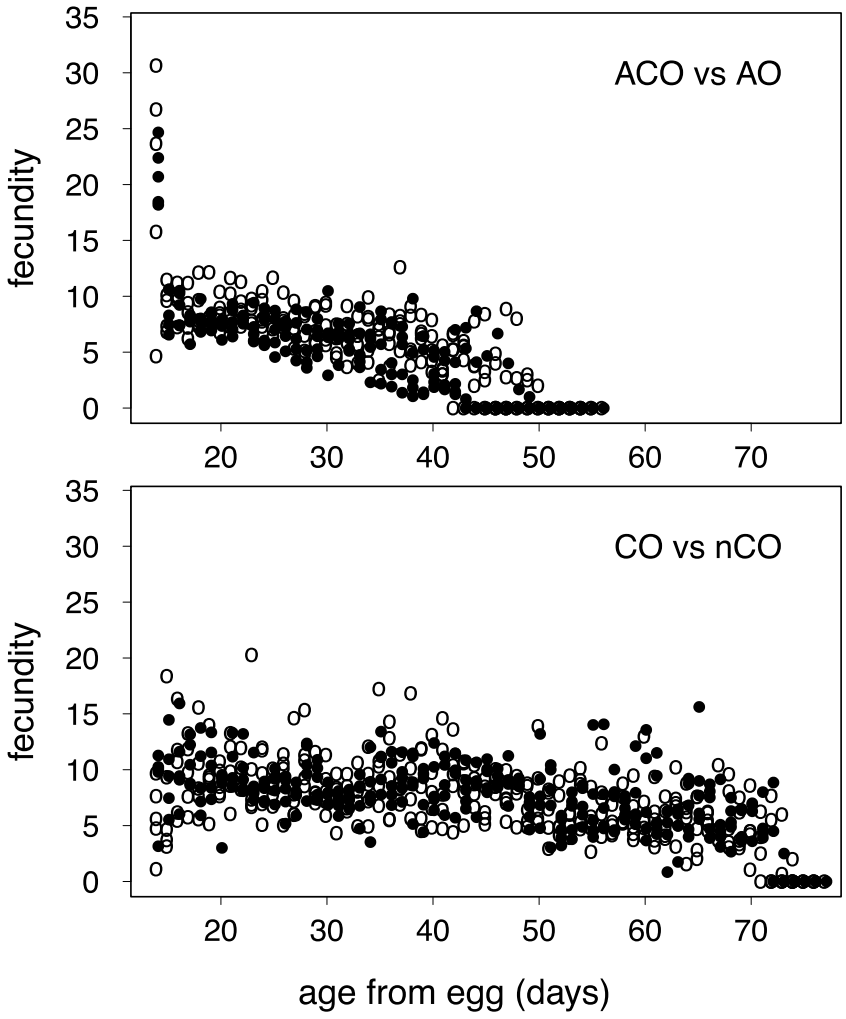

Figure 1.4. Adult age-specific fecundity from A-type and C-type selection treatments. Points represent average number of eggs laid per female per day as a function of age. Open circles represent longstanding populations (ACO in the top panel, CO in the bottom panel), where closed circles represent newer derived populations (AO in the top panel, nCO in the bottom panel).

Table 1.1. Convergence of early-life fecundity. Calculated *p*-values for the linear mixed effects model of convergence on initial fecundity between populations (Experiment 1). Fecundity estimates are compared between selection treatments within the same age intervals, although these intervals vary slightly by comparison. Bold values indicate significant non-convergence.

Age range (hours)	*ACO vs. AO*	*Age range (hours)*	*B vs. BO*	*Age range (hours)*	*CO vs. nCO*
204-228	0.025				
240-264	0.381	228-252	0.04	240-264	0.568
276-300	**0.011**	264-288	0.764	276-300	0.090
312-336	**0.008**	300-336	0.561	312-336	0.362

Table 1.2. Divergence of early-life fecundity. Calculated *p*-values for the linear mixed effects model of divergence on initial fecundity between selection regimes for Experiment 1. Bold values indicate non-significant divergence.

Age range (hours)	*A-type vs. B-type*	*A-type vs. C-type*	*B-type vs. C-type*
240-264	3.26×10^{-4}	2.57×10^{-12}	1.73×10^{-8}
276-300	4.74×10^{-9}	1.90×10^{-6}	**0.013**
312-336	5.29×10^{-12}	6.46×10^{-13}	**0.216**

Table 1.3. Convergence of A-type and C-type female fecundity. Calculated *p*-values for the linear mixed effects model of convergence on adult age-specific fecundity in Experiment 2 between ACO vs. AO and CO vs. nCO.

Age range (days)	*ACO vs. AO*	*CO vs. nCO*
14-16	0.889	0.137
17-19	0.637	0.801
20-22	0.528	0.91
23-25	0.45	0.713
26-28	0.573	0.667
29-31	0.758	0.942
32-34	0.639	0.899
35-37	0.357	0.982
38-40	0.272	0.89
41-43	0.502	0.97
44-46	0.574	0.654
47-49	0.384	0.732
50-52	0.989	0.95
53-55	0.717	0.865
56-58		0.742
59-61		0.519
62-64		0.759
65-67		0.467
68-70		0.895
71-73		0.907
74-76		0.762

Table 1.4. Divergence of A-type and C-type female fecundity. Calculated *p*-values for the linear mixed effects model of divergence on female age-specific fecundity in Experiment 2 between A-type selection and C-type selection. Bold values indicate non-significant divergence.

Age range (days)	*Female fecundity*
14-16	2.03×10^{-5}
17-19	**0.193**
20-22	**0.195**
23-25	**0.089**
26-28	0.004
29-31	0.002
32-34	4.45×10^{-4}
35-37	1.11×10^{-5}
38-40	2.71×10^{-5}
41-43	2.48×10^{-5}
44-46	8.94×10^{-5}

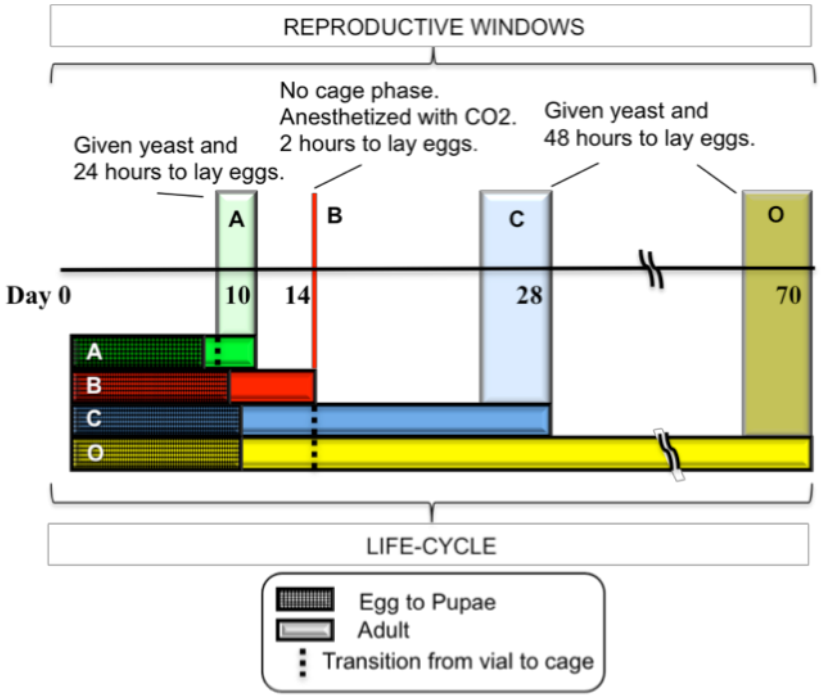

Figure S1.1. Detailed schematic for A, B, and C culture protocols.

Chapter 2

Divergence and Convergence of Egg Hatching and Larval Development in Experimentally Evolved *Drosophila melanogaster*

ABSTRACT

Laboratory selection experiments are characteristically simple, yet powerful enough to allow insights into the mechanisms of evolution. But a longstanding challenge facing evolution experiments with metazoans is the sheer amount of time required to see significant changes due to selection. In this work, we present data from a unique system of experimentally evolved laboratory populations of *Drosophila melanogaster* that have experienced three distinct life-history selection regimes. The goal of our study was to determine how quickly populations undergoing selection diverge phenotypically from their ancestors, and how quickly they converge with independently derived populations that share a selection regime. Our results indicate that if phenotypic divergence from an ancestral population occurs rapidly when a trait is related to fitness under a particular selection regime. Similarly, populations sharing a selection regime converge phenotypically for the same traits over similar time frames. In both instances, evolutionary history appears to have little to no influence on the evolution of fitness-related phenotypes. Conversely, the absence of phenotypic response to a particular selection regime suggests that a trait is not related to fitness in that selection regime. In addition, populations that fail to show convergence for the same traits. For these fitness-unrelated traits, evolutionary history had a larger influence on predicting phenotypes than recent selection regime. Ultimately, these results demonstrate that selection regime can have varying impact on numerous traits depending on their relation to fitness.

INTRODUCTION

Laboratory selection experiments have been often used to explore hypotheses concerning adaptation. Experimental evolution can quickly, dramatically, and most importantly reproducibly shape phenotypes in model species (Garland and Rose 2009). Experimentally evolved populations, when adequately replicated, can be used to test general theories about evolution in well-defined settings (cf. Garland and Rose 2009; Rose et al. 2001; Barrick and Lenski 2013).

Although these experimentally evolved populations are prime tools for testing hypotheses concerning adaptation, the general patterns of phenotypic responses to selection remain inadequately characterized. In some cases, populations exposed to the same selection regime do not show signs of convergence for several life-history traits even after numerous generations (Kawecki and Mery 2003; Bieri and Kawecki 2003). In other cases, populations from numerous backgrounds have shown convergence for some, but not all, phenotypes when reared in a uniform laboratory environment (Cohan and Hoffman, 1989; Joshi et al. 2003; Griffiths et al. 2005; Simões et al. 2007; Simões et al. 2008; Santos et al. 2010). Lastly, we have seen rapid convergence within populations experiencing the same selection regime and rapid divergence between populations of differing selection regime for adult mortality and fecundity (Chapter 1). These varying results could be attributed to insufficient sampling of natural variation, the founding populations being too different, or both.

It's possible that the impact of selection on convergence and divergence is trait-specific. Traits that are more closely related to fitness under a particular selection regime are probably affected by such selection more so than traits that are less related to fitness. In the same vein, traits not related to fitness may be impacted by evolutionary history more than selection. This has

been shown in asexual populations (e.g. Lenski and Travisano 1994; Travisano et al. 1995), but not in sexual populations, with the possible exception of some of the research of Matos et al. (multiple references). Previously, we have shown in Chapter 1, here, that adult mortality and fecundity, two traits that are heavily related to our three selection regimes, have converged and diverged rapidly, in keeping with this notion (Chapter 1). Here we examine the effects of selection on three additional traits that are conceivably related to our selection regimes (egg hatching time, larval development, and pupal development), in 30 experimentally evolved populations of *Drosophila melanogaster*.

Our 30 experimentally evolved populations are broken into two sets of populations: 15 long-standing populations and 15 recently-derived populations. Five populations from each set are subject to one of three regimes of experimental evolution: (1) selection for accelerated larval development, (2) the ancestral laboratory selection regime of two-week life cycles, and (3) selection for postponed reproduction. Due to the nature of the selection regimes, it is reasonable to believe that development as a whole will be impacted by these varying selection regimes. Specifically, the populations selected for accelerated larval development will likely show a change in development, but populations that are not directly selected for development may nevertheless show signs of change in their development due to pleiotropic effects.

Similarly to Chapter 1, we aim to study the extent to which multiple replicated lines converge on similar phenotypic and genetic outcomes. We use 30 experimentally evolved populations of *Drosophila melanogaster* to address the repeatability of phenotypic evolution in outbreeding, sexually-reproducing populations. Here, we test for rapid convergence and divergence between and within treatment types for egg hatching times, larval development, and

pupal development to determine the degree to which selection might erase or preserve the signature of evolutionary history in these traits.

MATERIALS AND METHODS

Experimental evolution regimes

This study uses large, deliberately outbred, lab populations of *Drosophila melanogaster* selected for different patterns of age-specific reproduction. All the lines used in the current study originate from an ancestral "IV" population first collected from South Amherst, MA in 1975 by Phillip Ives (vid. Rose 1984), and then cultured in the lab using two-week discrete generations. These ancestral IV flies were subsequently used in February 1980 to create five "O" (old) replicate lines, using females of increasing ages over successive generations until these flies were maintained on a 10-week generation cycle (Rose 1984). The IV flies were also used to found five additional "B" lines in February 1980, lines which have since been cultured using the same protocol as the IV populations from which they were derived. Detailed descriptions of the subsequent history and culture methods for these lines can be found in Rose et al. (2004).

The populations of the O selection treatment are the ancestors of 5 additional experimental treatments that along with the B selection treatment make up the 6 focal treatments of this study. These treatments belong to one of two temporal designations ("longstanding" or "recent") and one of three selection types ("A", "B", or "C", described below). Thus, these 6 treatments provide opportunities to study the differences between populations that have the same selection regime but were subjected to them long ago versus recently, and also to study the differences between populations that diverged from the same ancestor recently, but experience

selection for different life histories. See Figure 2.1 for an overview of the experimental evolution design of the present study.

We call these 6 selection treatments ACO, AO, B, BO, CO, and nCO, with each letter referencing a selection regime and evolutionary history. The CO populations were derived from the original O treatment after 57 generations of O-type selection in 1989. The "C" in CO indicates a 28-day selection regime while the "O" represents the CO treatment's previous selection regime. The ACO lines are the only populations that did not directly originate from the O treatment, but were instead derived from the CO populations after 27 generations of C-type selection in 1992. The AO, BO, and nCO lines ("n" stands for *new* CO treatment) were derived from the O treatment around 2007 after 153, 150, and 159 generations of O selection, respectively. Thus, these three treatments were derived relatively recently, while the CO, ACO, and B treatments are longstanding.

This system of 30 populations is now maintained using three distinct selection regimes: A, B, and C. *A selection regime*: the ten ACO and AO populations spend the first 9 days of life in 8-dram glass vials, and at day 10 adults are transferred to a Plexiglass "cage" in which they are given fresh food and allowed to oviposit for 24 hours. *B selection regime*: the ten B and BO populations spend 14 days in 8-dram vials, and are then allowed 1-2 hours in fresh vials to oviposit before adults are discarded. *C selection regime:* the ten CO and nCO populations develop in vials for 14 days prior to being transferred to Plexiglass cages. On day 26 C flies are given 48 hours to oviposit before eggs are collected. All populations are supplied with food made from cooked bananas, barley malt, yeast (3.6% w/v), corn syrup, and agar. The populations that spend time in cages are also supplied with a 5% live yeast paste on the food

surface to promote oviposition 24-48 hours prior to egg collection. Lastly, all populations are kept at 23° C and left in a 24-hour light cycle room. See Figure S2.1.

Experimental comparisons

Two experimental comparisons were performed: (1) Common-garden comparison of egg hatching time from the ACO, AO, B, BO, CO, and nCO treatments; and (2) Common-garden comparison of development times from (a) hatching to the start of pupariation and (b) hatching to adult eclosion from pupae, assayed in parallel among flies from the ACO, AO, B, BO, CO, and nCO treatments. The goal of these two comparisons was to determine the degree to which divergence has occurred among the three different selection treatments, as well as the degree to which convergence has occurred between the longstanding and recently-derived treatments in adult phenotypes. For these two experiments, all replicate populations assayed were reared in parallel on a B-type culture schedule for two generations before each experiment to minimize maternal effects.

Experiment 1: Comparison of egg hatching time for 30 populations

Egg hatching time measures were collected from freshly laid eggs in all replicate populations of ACO, AO, B, BO, CO, and nCO treatments. After the two generations of standardized rearing, females from each replicate were provided with ample yeast to promote egg laying days before the collect. Prior to the collect, fresh food was provided to allow for additional egg laying to minimize the effects of slightly ovoviviparous flies. Then, the females were given one hour with a charcoal infused food plate supplied with yeast in the center for oviposition. For the subsequent 30 hours, plates were monitored every hour for the presence of

newly hatched larvae. Newly-hatched larvae were noted and recorded and removed from the plate.

Experiment 2: Parallel assays of two developmental stages in 30 populations

For this experiment, two phases of metamorphosis were measured, from hatching to the onset of pupariation, and from hatching to adult eclosion. After the two generations of standardized rearing, each population from the ACO, AO, B, BO, CO, and nCO treatments laid eggs on a food-free agar plate. From each such plate, 30 first-instar larvae were individually transferred to a food vial, three vials per population. For the subsequent 14 days, vials were monitored every four hours for the presence of newly formed pupal casings, as well as newly eclosed adult flies. Newly formed pupae were noted and the time recorded, while newly eclosed flies were collected, sexed, and counted.

Statistical methods

Experiment 1

In experiment 1, we tested for convergence between paired selection treatments (i.e., ACO vs. AO, B vs. BO, CO vs. nCO) for effects of selection on egg hatching time. The observations consisted of the hatching time of individuals (y_{ikm}) from selection treatments (i = 1 (ACO or B or CO), 2 (AO or BO or nCO)), populations-k (k = 1,…,10), and plate m (m = 1,…,3), and are assumed to be described by,

$$y_{ikm} = \mu + \alpha\delta_i + b_k + c_{km} + \varepsilon_{ikm} \quad (1)$$

where $\delta_s = 0$, if $s = 1$ and 1 otherwise, and b_k, c_{km}, and ε_{ikm} are independent standard normal random variables with zero means and variance σ_b^2, σ_c^2, and σ_ε^2, respectively. Statistically testing for a significant effect of selection regime on egg hatching time corresponds to determining if α is significantly different from 0. Parameters of equation (1) were estimated by the restricted maximum likelihood techniques implemented by the *lme* function to R (R Core team 2014).

To test for divergence, we used the same basic analysis for convergence, except the treatments were not separated in a pairwise fashion. Instead all treatments were included at once (i = 1 (ACO), 2 (AO), 3 (B), 4 (BO), 5 (CO), and 6 (nCO)). Since the degree of divergence varied between treatments we then compared all treatments in a pairwise fashion using lsmeans (Lenth 2016).

Experiment 2

The same basic analysis for convergence as that of Experiment 1 was used for pupal and adult development time. To test for divergence, the six selection regimes were reclassified to three different categories: ACO and AO to A; B and BO to B; CO and nCO to C. The effects of selection regime were then evaluated with equation (1).

RESULTS

Egg hatching results from Experiment 1:

Figure 2.2 illustrates a pattern of the older derived populations hatching earlier than the newly derived populations disregarding the current selection regime. With the matched A, B, and C-type populations, it appears that the long-standing and recently derived populations have not

converged in their egg hatching patterns. In addition, populations undergoing entirely different selection regimes share similar egg hatching patterns (e.g. AO and CO).

Comparisons made between treatments of the same type showed significant differences (see Table 2.1) except for ACO and AO. In contrast, comparisons made between the old derived populations, B v. BO and CO v. nCO, show no statistically significant differences, whereas AO is significantly different than that of its newly derived counterpart, ACO.

Development time results from Experiment 2:

Figure 2.3 illustrates patterns of convergence and divergence observed for the developmental characters measured in Experiment 2. First, measures of pupariation and eclosion taken within matched A, B, and C-type treatments, both long-standing and recently derived, are very similar. Thus, both metrics of development show strong convergence, regardless of a population's specific evolutionary history. Second, populations that share the same selection regime recently (e.g., ACO and AO) are markedly different from those that do not; that is to say, A-type development is more rapid than B-type development, which is more rapid than C-type development. Thus, both the time from hatching to pupariation and the time from hatching to eclosion are development characters that have unambiguously diverged in the A, B, and C treatment groups of contemporary populations.

Adult and pupal development times show no significant differences when populations subjected to the same selection regimes are compared, despite the differences in duration of the shared selection regime (Table 2A). For pupal development time, these tests could have detected differences at 7% of the mean and for adult development times about 4% of the mean (Table 2A), thus these are not insensitive tests. However, when populations subjected to *different*

selection regimes are compared we see significant development time differences, with A-type selection resulting in the fastest development time followed by B-type selection and then C-type selection (Table 2B).

DISCUSSION

Similar to the results of Chapter 1, when comparing the degree of phenotypic divergence among populations of the three recently derived treatments (AO, BO, and nCO) with those of the long-standing treatments (ACO, B, and CO), it is apparent that larval and pupal development differentiation is remarkably similar between the recently derived treatments and the long-standing treatments. In effect, hundreds of additional generations of A, B, and C-type selection seem to have yielded at most minor increases in larval and pupal development differentiation. To add to this, there is a high degree of phenotypic convergence within each of the sets of A, B, and C-type populations for larval and pupal development.

Similar to the phenotypes examined in Chapter 1, we find that the larval and pupal development of our newly derived populations usually converged with those of the longstanding populations sharing the same selection regime within 200 generations. In addition, these newly derived populations, all initiated from a common ancestor, significantly diverged from one another within this time frame. These results suggest that recent evolutionary history is highly predictive of phenotype. Although the phenotypes have converged, we do not assume that the genetic mechanisms underlying these phenotypes are the same between the two groups of populations. Selection treatments sometimes produce common phenotypes in independent replicates that are the result of different genetic "solutions" (e.g., Garland et al. 2002; Kawecki and Mery 2006). However, recent work by Graves et al. (2017) on these same thirty populations

reveals the same parallel genomic convergence as I find for life-history convergence. Specifically, they find genomic convergence in the frequencies of single-nucleotide polymorphisms, transposable elements, insertions, and structural variants. Likewise, they found similar patterns of divergence.

Although the trend of rapid convergence and divergence held consistent for larval and pupal development, this was not the case for egg hatching time. First, there is varying phenotypic convergence within each of the three sets of A, B, and C-type populations. The A-type populations were the only treatment type to show evidence of convergence for egg hatching, where the two sets of B and C-type populations show no signs of convergence. Second, there is very little phenotypic divergence within populations of the three recently derived treatments and within populations of the three long-standing treatments. The only exception is the AO populations which have diverged from the remaining two recently derived treatments.

The contrasting results of convergence and divergence between the egg hatching times and larval and pupal development in our 30 populations suggest that the speed of convergence and divergence is trait-specific and selection-specific. In addition, this would imply that the impact of evolutionary history and current selection regime on a given phenotype are also trait and selection specific.

Both the B and C-type populations showed evidence of convergence within treatment type and divergence between treatment type for larval and pupal development, but not for egg hatching time. Due to the nature of the B and C-type selection regime, in that the selection focuses on later life, it's conceivable that only certain aspects of development were indirectly affected by selection via antagonistic pleiotropy. In other words, egg hatching time may not be considered a fitness-related trait for the B and C-type selection regimes, therefore there is no

evidence of convergence or divergence. For larval and pupal development, recent selection regime played a critical role in differentiating these populations, whereas evolutionary history appeared to have no significance. The opposite holds true for egg hatching, in that for it evolutionary history contributed more to the phenotype than current selection regime.

In contrast, the A-type populations, who are selected for accelerated larval development, do show convergence within treatment type and divergence between treatment type for all three developmental traits. Due to the A-type selection regime directly focusing on the developmental stages of the flies, it is reasonable that all three stages of development show rapid convergence within treatment and divergence between treatment. Similarly to Chapter 1, recent selection regime seems to be the only driving force on these developmental phenotypes, and previous evolutionary history seems to have been erased.

Recent selection regime and evolutionary history appear to be the two major forces contributing to any given phenotype, assuming the environment is held constant. These forces are inversely related to one another, in that, as one force is stronger the other force appears to diminish in effect. The strength of either force on a trait is entirely dependent on how relevant the trait in question is to fitness. Simply put, the more related to fitness the trait is, the more likely the recent selection regime will impact the trait's phenotype.

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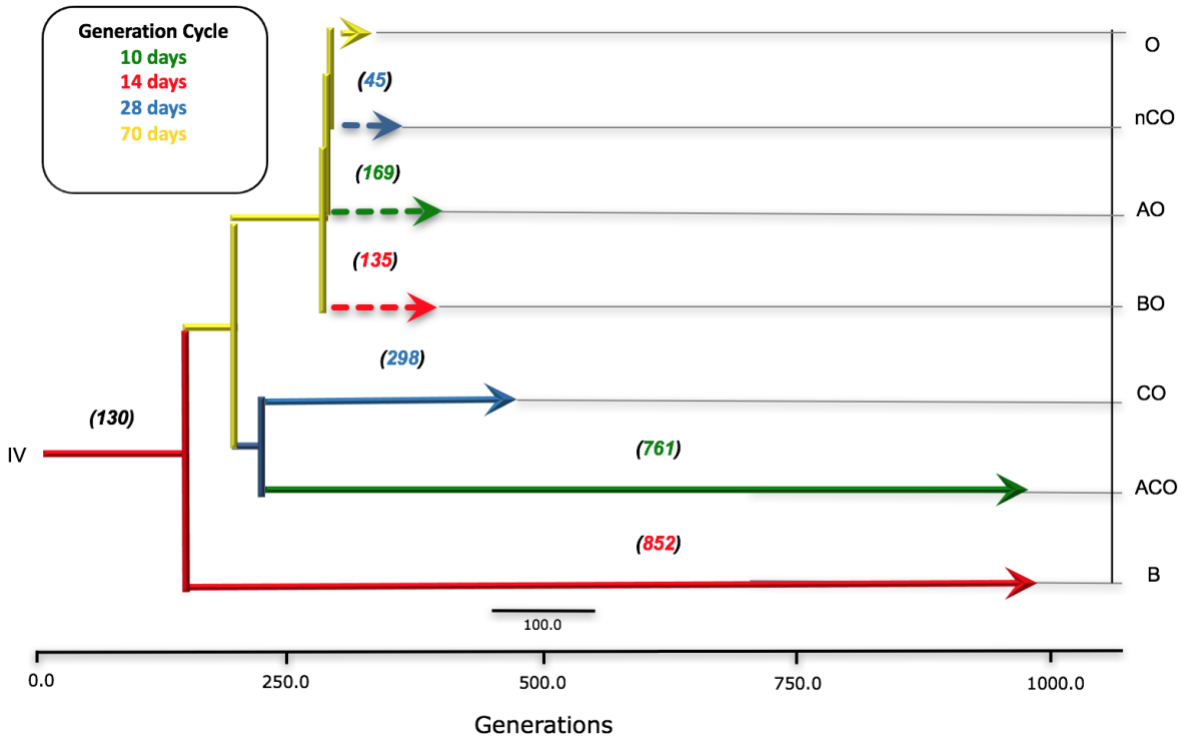

Figure 2.1. Schematic of population selection history. Abbreviated illustration of the evolutionary relationships between the focal selection treatments of this study. All treatments share ancestry, some more recently than others. The first capitalized letter of a treatment name indicates the type of life-history regime for which it has sustained selection: A (10-day cycle), B (14-day cycle), or C (28-day cycle).

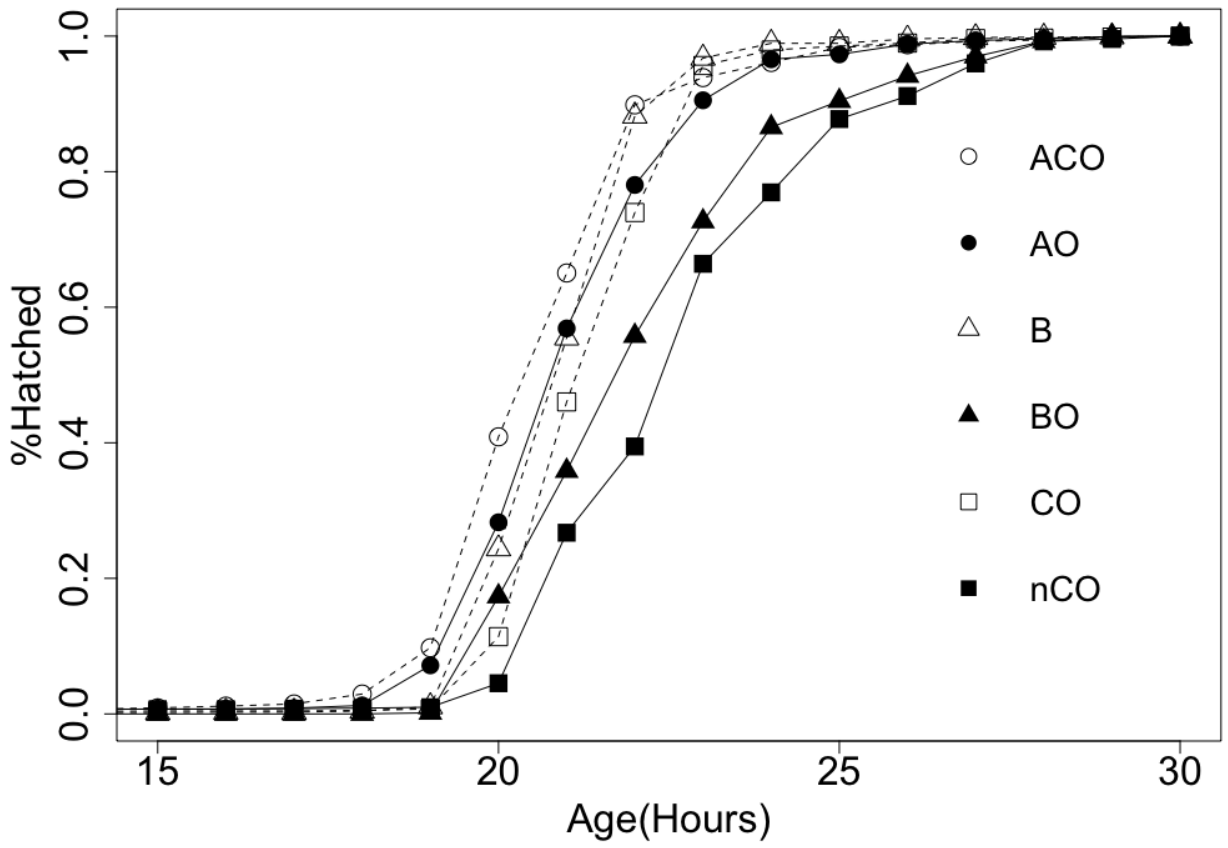

Figure 2.2. Comparison of egg hatching times in all 6 contemporary selection treatments. Time to egg hatching from 60 cohorts of our six laboratory populations averaged at the population level. Open circles and dashed lines represent older derived populations, where solid circles and solid lines represent newer derived populations. First 15 hours are not shown in figure due to there being very minimal hatching prior to hour 15.

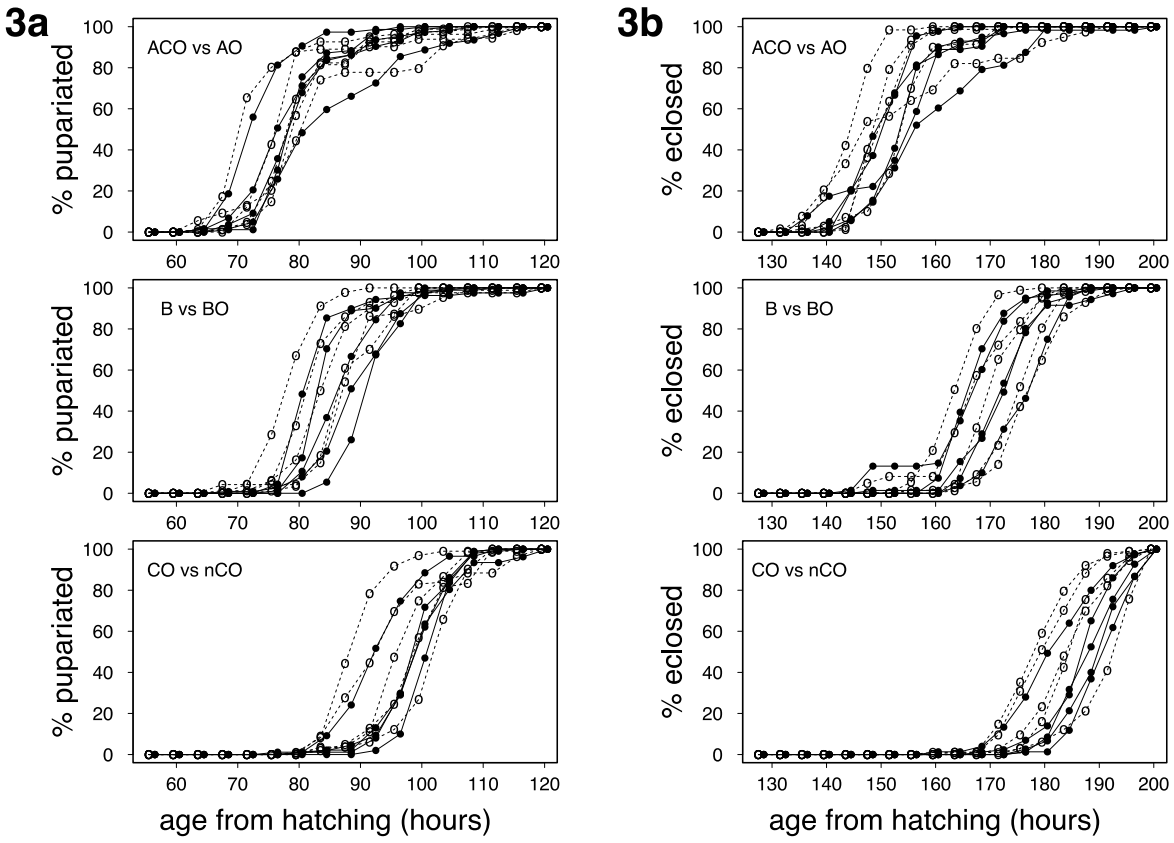

Figure 2.3. Comparison of larval and pupal development time in all 6 contemporary selection treatments. (a) Time to pupariation from thirty cohorts of our six laboratory populations. Open circles and dashed lines represent the percentage of cohort pupariated by each hour assayed in the five replicated longstanding populations (ACO in the top panel, B in the middle panel, CO in the bottom panel), and solid circles and solid lines represent percentage of cohort pupariated per hour assayed in the five replicated newer derived newer derived populations. (b) Time to eclosion from thirty cohorts of our six laboratory populations. The percentage of each cohort that reached eclosion by each hour assayed s expressed following the same conventions as 3a.

Table 2.1. Calculated p-values for the linear mixed effects model of convergence on egg hatching times between all 6 selection treatments.

Comparison	**p-value**
ACO vs AO	0.3898
ACO vs B	0.9192
ACO vs BO	**<0.0001**
ACO vs CO	0.2628
ACO vs nCO	**<0.0001**
AO vs B	0.9301
AO vs BO	**0.0122**
AO vs CO	0.9999
AO vs nCO	**<0.0001**
B vs BO	**0.0007**
B vs CO	0.8342
B vs nCO	**<0.0001**
BO vs CO	0.0252
BO vs nCO	0.1677
CO vs nCO	**<0.0001**

Table 2.2. Convergence and divergence in development time among contemporary populations. Egg-to-pupa and egg-to-adult development times (hours) in Experiment 2. 95% confidence intervals are computed for the development time differences using eq. 2. Newly-derived selection treatments of similar types (A) show no significant differences from long-established selection treatments of the same type. Conversely, selection regimes of the different types (B) are all significantly diverged from one another.

	Comparison	Pupae	Diff	95% c.i.	Adult	diff	95% c.i.
A	AO	80.5			155.0		
	ACO	81.3	(ACO-AO) 0.8	± 5.8	152.3	(AO-ACO) 2.7	± 5.4
	BO	88.1			172.1		
	B	86.4	(BO-B) 1.7	± 5.9	173.1	(B-BO) 1.0	± 7.4
	nCO	99.4			188.4		
	CO	98.2	(nCO-CO) 1.2	± 6.1	186.1	(nCO –CO) 2.3	± 6.6
B	A	80.9	(B-A) 6.4	± 3.6	153.6	(B-A) 19.0	± 4.0
	B	87.3	(C-B) 11.5	± 3.6	172.6	(C-B) 14.6	± 4.0
	C	98.8	(C-A) 17.9	± 3.6	187.2	(C-A) 33.6	± 4.0

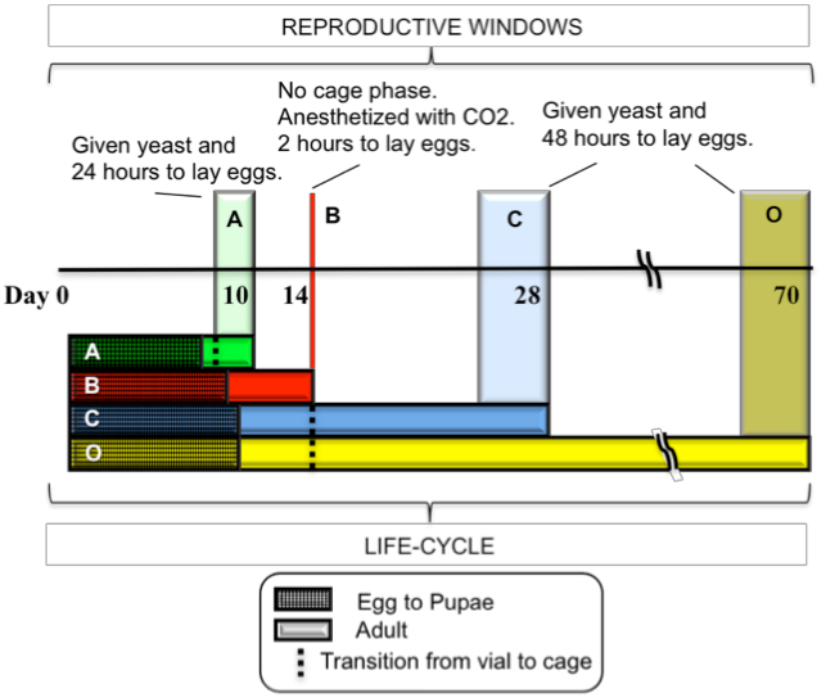

Figure S2.1. Detailed schematic for A, B, and C culture protocols.

CHAPTER 3

Drosophila Transcriptomics with and without Ageing

ABSTRACT

The genomic basis of ageing still remains unknown despite being a topic of study for many years. Here, we present data from 20 experimentally evolved laboratory populations of *Drosophila melanogaster* that have undergone two different life-history selection regimes. One set of 10 populations demonstrates early ageing whereas the other set of 10 populations shows postponed ageing. Additionally, both types of populations consist of five long standing populations and five recently derived populations. Our primary goal was to determine which genes exhibit changes in expression levels by comparing the female transcriptome of the two population sets at two different time points. Using three different sets of increasingly restrictive criteria, we found that 2.1-15.7% (82-629 genes) of the expressed genes are associated with differential ageing between population sets. Conversely, a comparison of recently derived populations to long-standing populations reveals little to no transcriptome differentiation, suggesting that the recent selection regime has had a larger impact on the transcriptome than its more distant evolutionary history. In addition, we found very little evidence for significant enrichment for functional attributes regardless of the set of criteria used. Relative to previous ageing studies, we find little overlap with other lists of aging related genes. The disparity between our results and previously published results is likely due to the high replication used in this study coupled with our use of highly differentiated populations. Our results reinforce the notion that the use of genomic, transcriptomic, and phenotypic data to uncover the genetic basis of a complex trait like ageing can benefit from experimental designs that use highly replicated, experimentally-evolved populations.

INTRODUCTION

The use of evolutionary genomics to study aging is still in its infancy (Braendle et al. 2011; Rose and Burke 2011; Hubley et al. 2016; Graves et al. 2017). Experimental evolution offers extreme phenotypic differentiation among traits in replicated populations (Garland and Rose 2009). Combining such populations with high-throughput omics can identify important genetic variants (Hubley et al. 2016; Bryant et al. 2017) and intermediate molecular phenotypes such as expression levels (Remolina et al. 2012; Mallard et al. 2018). This experimental and analytical framework has the potential to suggest causal genetic regions in the genome and unexpected molecular mechanisms responsible for the differentiation of ageing and characters related to it.

Despite ageing having been a subject of study for many decades, a precise understanding of its underlying physiological and molecular mechanisms remains elusive. One type of study has focused on knocking out candidate genes to examine its effect on longevity (Bray et al. 2016). Other types of studies have focused on changes at the genomic level between populations of varying ageing patterns (Remolina et al. 2012), with an extension of this approach to the analysis of transcriptome differentiation (Carlson et al. 2015; Sarup et al. 2011). Many of these latter studies focus on either a single population tracked over several life stages or on different populations exposed to different treatments that result in differing ageing patterns, in order to uncover the transcriptome changes that result from altered ageing.

A more extreme kind of comparison is possible when populations of the same chronological age differ with respect to whether they are ageing at all. Burke et al. (Burke et al. 2016) have demonstrated that two sets of ten *Drosophila melanogaster* populations, called "A" and "C", show distinctively different ageing patterns: The A populations begin demographic

ageing at least two weeks before the C populations. These twenty populations also show extensive and consistent differences in genome-wide patterns of single-nucleotide polymorphism (SNP), transposable element (TE), and structural variant (SV) frequencies (Burke et al. 2016; Graves et al. 2017).

We sequenced the transcriptomes of the twenty A and C populations of *D. melanogaster* populations. Expression profiling was performed on whole-body females collected at both day 14 and day 21 from each cohort's egg-stage. At these collection points, the individuals from the ten A populations had an adult age of 6 and 13 days respectively whereas those from the ten C populations had an adult age of 2 and 9 days. These time points were specifically chosen because A-type populations show demographic ageing at those times, unlike the C-type populations (Fig. 3.1). Given this extreme demographic contrast, we compiled three different lists of genes putatively associated with ageing in *D. melanogaster*.

MATERIALS AND METHODS

Experimental populations

The populations used here were experimentally evolved over numerous generations (Rose et al. 2004; Burke et al. 2016; Graves et al. 2017). These populations were subject to two selection regimes which differed in length of their discrete generations. Each selection regime was applied to two sets of five populations, each with known distinct evolutionary histories (Fig. S3.1). The ACO and AO sets are both A-type, whereas the CO and nCO populations are both C-type. Although the individuals of the two population-types differ in body size, there is no evidence of major allometric differences.

Fly husbandry

Each population is maintained over generations at a census size of ~2000 individuals in order to reduce the effects of genetic drift on genetic variation. Flies are kept in 8-dram plastic vials during development and placed in Plexiglass cages on day 10 (A-type) and day 14 (C-type). For the A-type treatment, once the flies are placed in cages, they are given a 24 hour oviposition window on fresh food. For the C-type treatment, the flies are left in cages until day 26, when they are given 48 hours to oviposit on fresh food. All populations are fed with fresh medium made with cooked bananas, corn syrup, yeast, barley malt, and agar (Rose et al. 2004). Fresh food is supplemented with 5% live yeast paste to enhance oviposition duration egg-laying. All populations are kept at about 23°C and exposed to a 24-hour light cycle.

RNA preparation and sequencing

For each population and time point combination, 150 whole-body females from the same cohort were submerged in TRIzol, snap-frozen in liquid nitrogen, and stored at -80C until extraction. Total RNA was purified using the RNeasy Mini Kit (Qiagen). RNA concentration, purity, and integrity were estimated using a NanoDrop 8000 Spectrophotometer and the RNA 6000 Nano Chip Kit (Agilent Technologies) with an Agilent 2100 Bioanalyzer. Ribodepleted, strand-specific paired-end libraries were prepared using the Ribo-Zero Gold Set A and the TruSeq Total RNA Library Prep kits from Illumina. Libraries were multiplexed and sequenced from both ends for 75 cycles over four lanes on an Illumina HiSeq2500 at the University California Irvine Genomics High Throughput Facility.

Gene expression analysis

Quality checks of each RNA paired-end sequencing output were performed with FastQC v0.11.5 (Andrews 2010). Subsequent sequence processing with Trimmomatic v0.35 (Bolger et al. 2014) included removal of adapter sequences, trimming of 3' nucleotide calls with Phred score lower than 30, and filtering out of sequencing reads with a final length lower than 36 bp or overall Phred score lower than 30. Paired-end reads were mapped to the *D. melanogaster* Release v6.18 reference genome assembly (dos Santos et al. 2015) using STAR (Dobin et al. 2013) –under default settings except for an adjustment to avoid the detection of novel junctions– as this tool shows enhanced accuracy mapping rates in relation to other alignment tools (Baruzzo et al. 2017). The average alignment rate for the 40 sequencing outputs was 89.5% (minimum=73.3%, maximum=97.8%). Alignment post-processing was performed with SAMtools v0.1.19 (Li et al. 2009). Read counting per gene and population was done using HTSeq v0.6.1p1 (Anders et al. 2013) at default settings. Genome coverage was estimated using the genomeCoverageBed utility from BEDTools v2.25.0 (Cridland et al. 2015). Summary statistics and NCBI SRA accession numbers are provided in Table S3.1.

For each sample, per gene read counts were normalized using the default DESeq2 settings (Love et al. 2014). Genes showing normalized count values greater than 4 in at least 8 out of 10 populations, within at least one of the treatment types, were included in downstream analyses. To see any relationships between populations, we conducted a principal component analysis with the normalized count data using prcomp and ggplot2 (Wickham 2016). To accommodate any block effect associated with different rounds of extraction and sequencing, the normalized count data for reproducibly expressed genes were analyzed using a linear mixed effects model (R Development Core Team 2016). In each population we have a measure of gene

expression, e_{ijk}, from selection treatment-*i* (*i*=1 (A) or 2(C)), block-*j* (*j*=1 (ACO and CO) or 2 (AO and nCO)), and population-*k* (*k*=1,..,20). We can then model expression with the mixed linear effects function,

$$e_{ijk} = \mu + \delta_i \alpha_i + b_j + \varepsilon_k,$$

where δ_i =0, if *i*=1 and 1 otherwise, *b* and ε are assumed to be independent random variables with a normal distribution with zero mean and variances σ_1^2 and σ_2^2 respectively. Significant treatment effects are determined by testing whether α_2 is significantly different from zero. Statistical significance for differential expression of any given gene was set at a 5% FDR for ~4000 tests, *i.e.* the number of expressed genes that passed filtering (Benjamini and Hochberg 1995).

Searches for biological patterns across GO and KEGG terms, and other genome features such as chromosome distribution, were performed using DAVID 6.8 (Huang da et al. 2009). The Benjamini-Hochberg correction for multiple tests was applied given that we were performing 50-600 tests, *i.e.* the number of differentially expressed genes inputted into DAVID, across different gene lists.

RESULTS

Evidence for transcriptional convergence within selection regimes

We employed double-stranded Illumina RNA-seq to sequence the transcriptome of whole-body females from the A and C populations to determine the magnitude and patterns of differentiation as a result of the selection regimes to which they have been exposed. Due to the recent increase in annotated noncoding RNA genes in *D. melanogaster* (Matthews et al. 2015), a fraction of which lacks poly(A) tail (Yang et al. 2011), we decided not to enrich for poly(A)

mRNAs. We made this choice in order to perform an unbiased search across all transcripts. For each population type by time point combination, 10 populations were profiled.

The median number of uniquely mapped sequencing reads per population was 8.1 million, bringing the total to 332.7 million reads, which in total sequence length amounts to ~21 Gb. This represents ~272 fold coverage of the fraction of the genome that has been found to produce primary transcripts (Graveley et al. 2011). Out of 17,481 gene features (from protein-coding sequences to pseudogenes to non-coding RNA genes) annotated in the R6.18 of *D. melanogaster*, 3,994 were found to be reproducibly expressed at a significant level across a majority of at least one of the two population types, thereby excluding potential transcriptomic noise, and therefore were considered suitable for downstream analyses (Material and Methods).

After data normalization relative to the total transcript output, we performed a principal component analysis on the transcript level of each of the expressed genes. This led to a clear segregation of the samples by time point and selection regime (Fig. S3.2). Due to sequencing batch discrepancies at day 21, we employed a linear mixed-effects model to account for the effect of sequencing batch.

We then calculated the pairwise correlation coefficient between the expression levels of the 20 populations assayed. To study the relationships among all 20 populations studied, we used Pheatmap (Kolde 2015), a clustering program, that uses these correlation coefficients and generates a dendrogram. The resulting dendrogram perfectly separates the A- and C-population types (Fig. S3.3). It is remotely conceivable that these populations are grouped in the expected manner due to chance. To address whether or not this result occurred due to chance, we shuffled the expression values within each gene and recreated 1000 simulated dendrograms. We found that none of the simulated dendrograms showed the same topology as our experimental result

(P<0.001). This is a particularly remarkable result, because the populations within each of the A and C types feature two subsets that have only recently experienced parallel selection regimes. This result provides strong evidence of genome-wide transcriptional convergence of the two subtypes of populations within each selection regime, which is precisely in alignment with the convergence they exhibit for both life history traits and genome-wide variation (Burke et al. 2016; Graves et al. 2017).

Transcriptional differentiation between population types

At a 5% false discovery rate (FDR) per gene, we found 906 genes differentially expressed in the A and C populations: 277 at day 14 only, 366 at day 21 only, and 263 at both time points (Fig. 3.2). 24.6% (*i.e.* 133) of the differentially expressed genes at 14 days and 23.5% (*i.e.* 148) of the differentially expressed genes at 21 days were found to differ by two-fold or more, respectively (Fig. 3.3a-b). The highest fraction of differentially expressed genes, across all genes analyzed, is seen on day 21 (16.2% of expressed genes vs 14.7% on day 14). 24.9% (*i.e.* 225) of the genes that are significantly differentiated at one of the two time points are not significantly differentiated at the other time point. Of the 540 differentially expressed genes found at day 14, 229 (42.4%) genes were significantly overexpressed in the A-type populations in relation to the C-type populations whereas 311 genes showed the opposite pattern. At day 21, the proportion of significantly overexpressed genes in the A-type versus the C-type population is very similar, with 282 (44.8%) genes significantly overexpressed out of 629.

Gene ontology (GO), KEGG pathway, and chromosomal enrichment analyses found no biologically interpretable patterns of differentiation at either time point. Notably, when reviewing the gene-feature types among the differentially expressed genes, we found numerous

non-coding RNA genes (ncRNA). Specifically, we found significant differentiation for 402 and 482 protein-coding genes for day 14 and day 21, respectively. There were 136 and 246 ncRNA genes that were significantly differentiated for those same time points, respectively. One annotated pseudogene (CR18275) appeared also as differentially expressed at each time point, and one tRNA gene was also found differentially expressed on day 14. In light of the numerous ncRNA genes found to be differentially expressed, and the fact that there is little known about them at the functional level, we repeated the GO analyses without the inclusion of non-protein coding genes. However, this more selective analysis provided no evidence for significant enrichment with respect to any biologically interpretable pattern.

Transcriptional differentiation due to ageing

There are numerous ways of curating these data to obtain a list of genes differentially expressed due to ageing. We adopted three different approaches: I, II, and III. The relationships among these lists are highlighted in Fig. 3.4.

List for Approach I

First, we simply compiled the genes that were differentially expressed at day 21 between the A-type and C-type populations. In this case, we purposefully ignored day 14 because at day 14 both A-type and C-type populations were transferred from vials to cages shortly before being collected for sequencing, potentially influencing expression levels for some of the transcribed genes. In addition, day 21 is not affected by the females from the C-type populations not being fully sexually mature. More importantly, the degree of mortality-rate differentiation between A and C population types at day 21 is substantially higher than at day 14 (Fig. 3.1).

We found 629 differentially expressed genes between A-type and C-type populations at day 21. Of these genes, 282 were significantly more expressed in the A-type populations while 347 were more expressed in the C-type populations (Fig. 3.2b). Searching for biologically interpretable patterns within this list found no significant pattern. This result did not change after omitting 146 ncRNA genes and 1 pseudogene.

List for Approach II

Next, we adopted a more stringent approach and analyzed only genes that were differentially expressed at both day 14 and 21 between the A-type and C-type populations. The rationale behind this approach was that, at both time points, the A-type populations are ageing whereas the C-type populations have yet to age (Fig. 3.1). Essentially, we were interested in those genes that exhibit sustained interpopulation differences across 7 days. We found 263 differentially expressed genes between A-type and C-type populations at both day 14 and day 21. Of them, 94 genes exhibit significantly higher expression in the A-type populations whereas 169 do so in the C-type populations (Fig. 3.3a-b). Again, no biologically interpretable patterns were found, which did not change upon omitting 73 ncRNA genes.

List for Approach III

Lastly, we focused on genes that were differentially expressed in the ageing populations (A-type), but not in the non-ageing populations (C-type). More specifically, we were interested in the genes that were differentially expressed in the A-type populations between day 14 and day 21 but showed statistically similar expression in the C-type populations between day 14 and day 21. The A-type populations are clearly ageing between day 14 and day 21 (Fig. 3.1) and

therefore identifying genes that are differentially expressed between these two time points would capture potential ageing related genes. As the C-type populations are not ageing during this same time frame, the cross examination of the two sets of genes should eliminate any genes that are differentially expressed due to any random environmental artifacts at day 14. We ultimately found 82 genes that fit these criteria. Of these 82 genes, 38 had higher expression in the day 14 A-type populations and 44 had higher expression in the day 21 A-type populations (Fig. 3.3c-d). Further, the search for biologically interpretable patterns among these 82 genes revealed an enrichment for genes associated with the ABC transporter-like pathway (GO:0005524, GO:0016887: $P_{adj} = 0.021$). ABC transporter proteins are part of the ATP-Binding Cassette (ABC) superfamily and are widely used in the hydrolysis of ATP to energize many biological processes. These transporters are key for the import and export of many substrates, in particular toxins (Saurin et al. 1999). Similar to the previous analyses, there were numerous ncRNA genes within this list. Specifically, we found 61 protein coding genes, 19 ncRNA genes and 2 tRNA genes. Removing the 19 ncRNA genes due to their poor functional annotation led to the detection of no additional patterns.

The gene lists obtained upon applying these three sets of criteria not only differ in total number of genes included, but also in the degree of overlap (Fig. 3.4). List *II* is essentially a more restricted version of list *I*, as the transcriptome differentiation in day 14 is incorporated. List *III* differs substantially from list *I* and list *II*, with 49 (59.8%) of the genes being specific to it.

Genomic comparison

We compared our three lists of ageing-related genes with that previously identified from genomic analysis (Graves et al 2017), in order to determine what fraction of the differentially expressed genes between population types harbored significantly differentiated SVs, TE insertions, and SNPs. For SVs specifically, we were interested in in duplications and deletion events that could modify expression levels (Cardoso-Moreira et al. 2016). Next, we checked for local TE insertions that may also affect gene expression (Cridland et al. 2015). Lastly for SNPs, we were interested in finding differentiation in gene regions that can accommodate *cis*-regulatory motifs, *i.e.* the 5' and 3' UTRs and the promoter. The latter was taken to reside within 1 kb upstream of the transcriptional start site of the gene. To this purpose, we examined both coding and non-coding gene regions, including exons, introns, and the aforementioned untranslated regions, as well as the upstream region for each of the differentially expressed genes.

We identified 12, 8, and 2, in lists *I*, *II,* and *III* respectively, differentially expressed genes that harbor differentiated SVs. In contrast, only 3, 2, and 0, differentially expressed genes between the A-type and C-type populations contain differentiated TE. Further, we identified 48, 26, and 4 differentially expressed genes (Table 3.1) that harbored differentiated SNPs in lists I, II, and III respectively. Of these genes, 66.7% (32), 69.2% (18), and 75% (3), respectively, had such SNPs within exonic or intronic regions. More importantly, 60.4% (29), 65.4% (17), and 50% (2) of the genes, respectively, contained SNPs in at least one gene region where *cis*-regulatory motifs reside, potentially contributing to the detected differences in mRNA abundance. The remaining differentially expressed genes either have no differentiated SNPs or the SNPs are located in gene regions usually devoid of *cis*-regulatory motifs. Differences in gene expression for this latter gene set should be primarily influenced by trans-regulatory effects.

Comparison of ageing genes to previous lists of ageing-related genes

We compared our lists of candidate ageing genes to others obtained in similar studies. First, we considered the gene list at the repository for *D. melanogaster* at GenAge (de Magalhaes 2014). Of the 193 genes present in the GenAge database, only four were also part of List *I*. Lists *II* and *III* showed no overlap with the GenAge list. This lack of overlap may be due to the fact that GenAge specifically focuses on genes that have an ortholog associated with ageing in humans. In addition, GenAge focuses on genes that are only ageing-specific, and omits genes that may play roles in other biological processes.

Subsequently, and more in the context of transcriptomic changes associated with ageing, we compared our lists to that from a previously published expression profiling study in *D. melanogaster* (Carlson et al. 2015). That study found 1581 genes differentially expressed across 11 time points and 79 days, compared to a control sample consisting of 2-day old (after eclosion) females. We identified 51, 20, and 5 genes from their list that were also present in our *I*, *II* and *III* lists, respectively. Interestingly, this is lower than what we found by randomly selecting 1581 genes from the reference genome and compare it to our ageing lists. After 1000 simulations, we found that the probability of having at least 51, 20, or 5 overlapping genes from lists *I*, *II*, and *III* respectively was 0.824, 0.825, and 0.890. Thus random selections of genes have more overlap with our results than the genes identified by Carlson et al. (Carlson et al. 2015). Conversely, if we were to take a random sample from the reference genome using the length of our three lists individually and compared it to their list of 1581 genes, we found the probability of having at least the same number of overlapping genes as observed was 0.445, 0.574, and 0.759 for lists *I*, *II*, and *III* respectively. A major difference between our study and that of Carlson et al. (Carlson

et al. 2015) is how a gene was ultimately considered to be associated with ageing. The gene list in Carlson et al. (Carlson et al. 2015) was obtained by studying transcriptional changes in one large population over time, always relative to the first time point. By contrast, the lists generated in this study derive from comparing an ageing set of populations and a non-ageing set of populations under three different sets of assumptions. An additional difference is that the females collected in the study of Carlson et al. (Carlson et al. 2015) were exposed to males for only 24 hours before the beginning of the assay, whereas our samples contain females that were exposed to males the entire time up until their sampling point.

Power of replication

We evaluated how our level of replication might have impacted our inferences. The power to detect differentially expressed genes is negligible with only four or fewer replicates, which holds regardless of the set of criteria used to deem a gene as ageing-related (Fig. 3.5). Therefore, it is surprising that studies with this low level of replication find even more differentially expressed genes (Carlson et al. 2015; Hsu et al. 2019). Combining multiple inbred or isofemale lines is commonplace in *Drosophila* studies, but they might in fact contribute to the large number of differentially expressed genes documented. Increased linkage disequilibrium, which is a typical consequence of combining multiple lines, and having insufficient replication are likely to result in unforeseen effects in gene expression. Therefore, our results strongly suggest that, given the phenotypic plasticity of expression levels (Scheiner et al. 2012; Dayan et al. 2015), using properly maintained evolved populations that derived from an outbred population plus high replication levels are crucial to reduce both type I errors and type II errors when generating a portrait of differences in gene expression.

DISCUSSION

Having two clearly defined sets of *D. melanogaster* populations has enabled us to dissect what is ageing-related in the transcriptome. Specifically, one set of ten populations (A-type) is demographically ageing between day 14 and day 21 and the other set of ten populations (C-type) is not demographically ageing (Fig. 3.1). This extreme contrast of ageing vs non-ageing populations is particularly useful, because we can assay them at the same chronological ages, day 14 and day 21. In addition, these populations are not exposed to any metabolic arrest or other manipulation (*e.g.* nematodes, (Ayyadevara et al. 2008)); their differences are genetic. Lastly, these two sets of populations are closely related, despite the stark difference in their ageing patterns between day 14 and day 21.

Furthermore, these two sets of ten populations have been extensively characterized for genomic differentiation (Graves et al. 2017), development differentiation (Burke et al. 2016), and physiological differentiation (Rose et al. 2004; Kezos et al. 2019). Developmentally, the two sets of ten populations show SNP, TE and SV differentiation. Phenotypically, the 10 A-type populations develop from egg to pupation and pupation to eclosion significantly faster than the 10 C-type populations. Physiologically, the A-type populations have a shorter time to starvation than the C-type populations and the same is true for desiccation. Considering the stark difference between the two sets of populations at the genomic and phenotypic levels, it is not surprisingly that we find here a substantial amount of transcriptomic differentiation.

Unlike the statistical results testing for differentiation between A-type and C-type populations, within A-type comparisons of populations and within C-type comparisons show little to no differentiation. That is, the transcriptomic results are parallel to those previously

found for within-type convergence with respect to life-history phenotypes (Burke et al. 2016) and genomics (Graves et al. 2017). As before, this convergence has arisen despite two very different selection histories underlying the differentiation of members of the A and C sets of populations. Specifically, the A group is made up of five populations that share long-standing A-type selection (the ACO), as well as five *other* populations that have undergone A-type selection for substantially fewer generations (the AO). Likewise, the C group is made up of five populations that share relatively long-standing C-type selection (the CO), as well as five other populations that have undergone C-type selection for substantially fewer generations (the nCO). The newly derived populations are much more closely-related phylogenetically than the longer-standing populations, with just 327 generations separating them versus 1171 generations separating the longer-standing populations. When comparing the newly derived populations with each other and with their selection regime counter parts (AO with ACO; nCO with CO), we find that the newly-derived populations show no evidence of less differentiation, suggesting that recent selection regime has a larger impact on the transcriptome than evolutionary history, as we have previously found both phenotypically and genomically (Burke et al. 2016; Graves et al. 2017). We believe this notion only stands true for populations that maintain moderate census size to combat the effects of inbreeding. With inbred populations, we would expect evolutionary history to have a larger impact on the transcriptome than recent selection regime (Sarup et al. 2011).

When reviewing the nature of each differentiated gene, there was a high proportion of non-coding RNA genes that were differentially expressed. These genes are currently poorly understood. But due to their abundance among systematically differentiated transcripts in our data, our findings support the hypothesis that they can play an important role in gene regulation

(Deveson et al. 2017). Another striking result was that only 4-6%, depending on which criteria used, of the differentially expressed genes between the A-type and O-type populations harbored SVs, TE insertions, or SNPs in *cis*-regulatory regions, suggesting that much of the transcriptome differentiation between population types stems primarily from trans-regulatory effects.

Next, when comparing our lists of ageing-related genes to that of previous ageing related work (Carlson et al. 2015; Hsu et al. 2019), we find very little overlap with previous findings. In addition, we find little to no enrichment for biological processes delimited in GO with our ageing-related lists of differentiated transcripts. These two results together suggest that our lists are novel relative to the current state of ageing transcriptomics in *D melanogaster*.

Here, we have presented two-time point comparisons using 10 replicates for each set of populations. But it is clear from Fig. 3.5 that even at 10 replicates we have not reached saturation for detection of differentially expressed genes, suggesting that still more replication would allow better detection of transcripts that differentiate with respect to ageing versus non-ageing. In addition to increased replication, more time point sampling should improve our understanding of how the transcriptome is affected by ageing. Lastly, integrating genomic analysis with still more powerful transcriptomics should further improve our understanding of how differentially expressed transcripts are regulated.

Currently, we have only sequenced the transcriptomes of our A-type and C-type populations, because of their clear difference in ageing. Adding additional types of populations to transcriptomic analysis should foster the parsing of transcriptomic differentiation with respect to whether or not any particular transcript difference is involved in the differentiation of ageing versus other types of phenotypic differentiation. In previous phenotypic and genomic work, we have used an intermediate set of 10 populations, the B-type, in addition to the current sets (Burke

et al. 2016; Graves et al. 2017; Mueller et al. 2018). The B-type populations have a generation length of 14 days, which falls between that of the A-type and C-type populations. The B-type populations would be therefore a prime candidate to add to bolster our ability to parse transcriptomic differentiation.

Although having a full suite of genomic, transcriptomic, and phenotypic data is powerful in itself, parsing all three bodies of data together is challenging. Due to the inherent complexity of ageing, complexity should be maintained and not sacrificed when analyzing all these data together. Modeling techniques commonly called "AI", but more properly defined as statistical learning (Hastie et al. 2009), allow us to address this challenge of parsing complexity. Currently, the Fused Lasso Additive Model or FLAM (Petersen et al. 2016; Mueller et al. 2018) shows promise in making sense of large data sets, such as those of genomic data. Ultimately, we propose that combining the omics of highly replicated experimentally evolved populations with statistical learning tools could prove promising for uncovering the foundations of any complex trait, those of ageing included.

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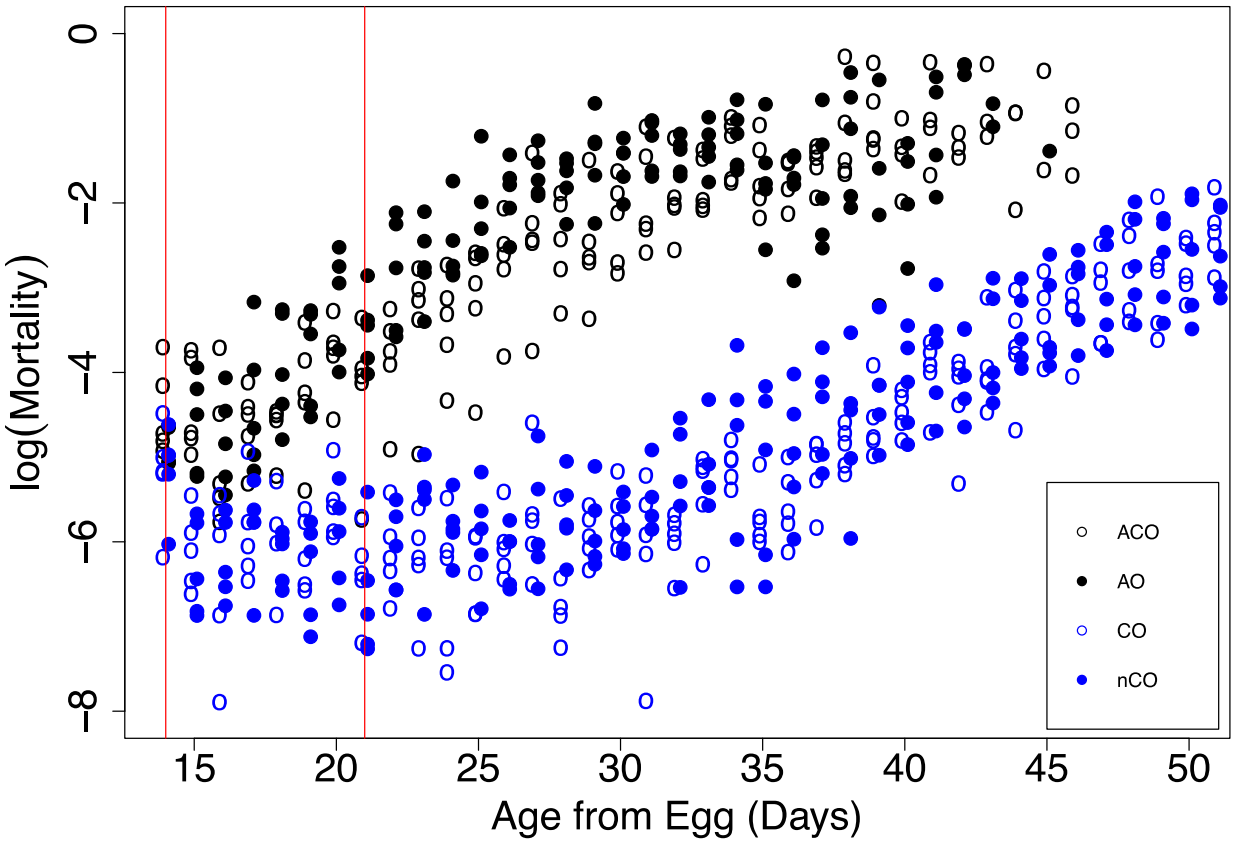

Figure 3.1. Age specific log mortality plotted for the 20 populations used. Mortality graphs were plotted for both A-type (black) and C-type (blue) populations. Both types of populations consist of five long standing populations (open circles) and five recently derived populations (closed circles). The red lines show when samples were collected for RNA-sequencing. More notable, for day 21, the A-type populations are within their ageing trajectory whereas the C-type populations are still in their pre-ageing phase.

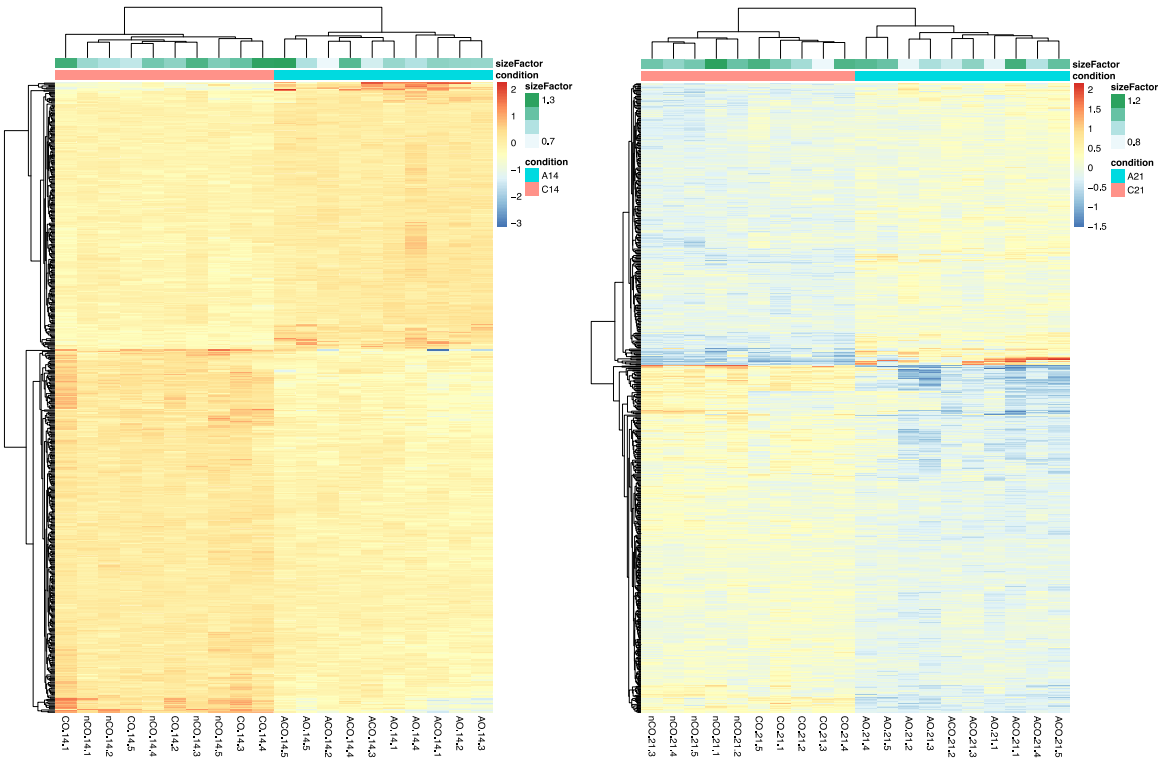

Figure 3.2. Clustering of differentially expressed genes in the female transcriptome of *D. melanogaster* between two population types. Heatmaps for the two time points assayed: 14 days (left), 492 genes; 21 days (right), 603 genes. Heatmaps were generated using hierarchical clustering of gene normalized mean expression levels. Ward's minimum variance was used as a distance metric. Gene clustering simultaneously results in a perfect grouping of populations based on the type of selection regime to which they were exposed. Blue, underexpression; red, overexpression. The identity of each population is shown at the bottom of each chart.

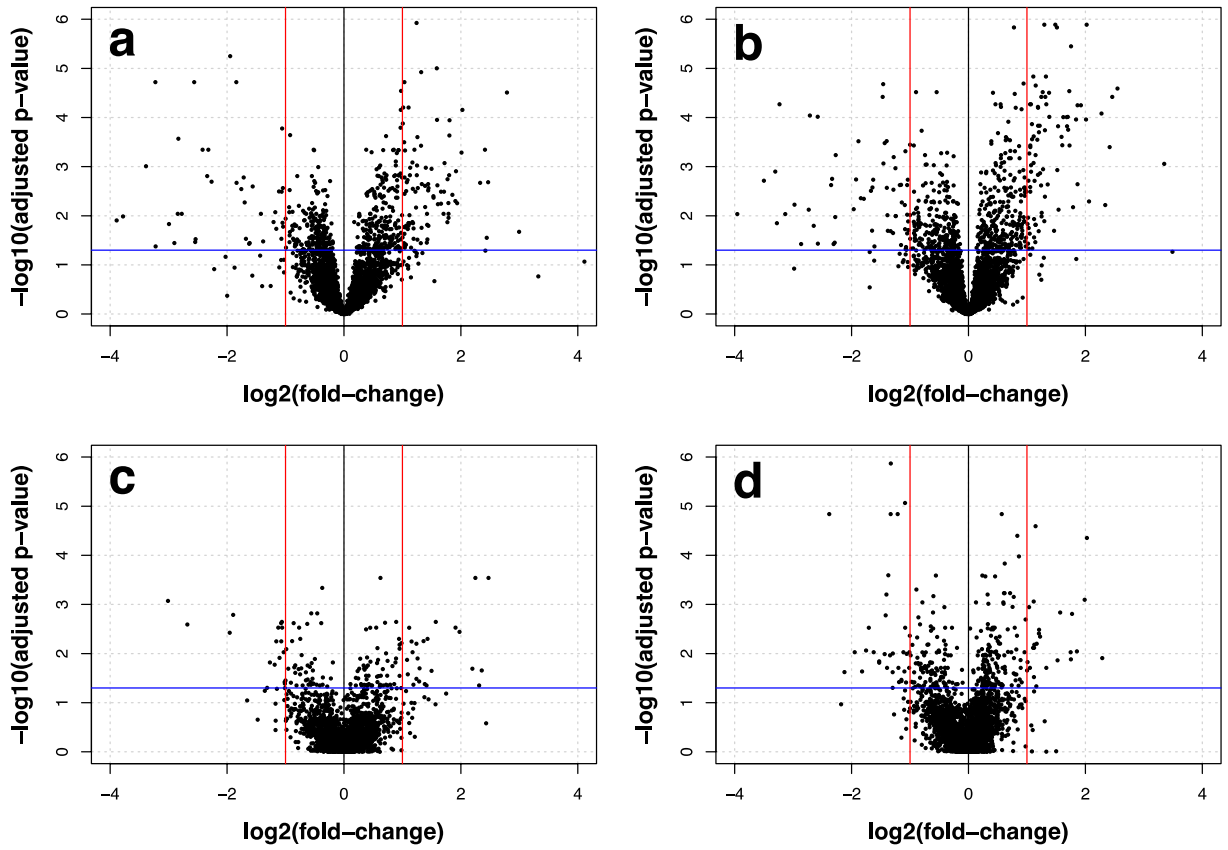

Figure 3.3. Differences in mRNA abundance in the female transcriptome between two population types and two time points. Volcano plots for day 14 and day 21 (a and b) time points show the differences in expression between the populations types A and C. Similar plots for A-type and C-type (c and d) populations showing differences in expression between the two time points. *x* axis, difference in normalized $\log_2$-transformed expression difference in mRNA abundance between day-21 and day-14; *y* axis, significance of the differences as $-\log_{10}(P_{adj}$ value). Statistically significant differences were determined using a linear mixed effect model. Blue dotted line, $P_{adj} = 0.05$.

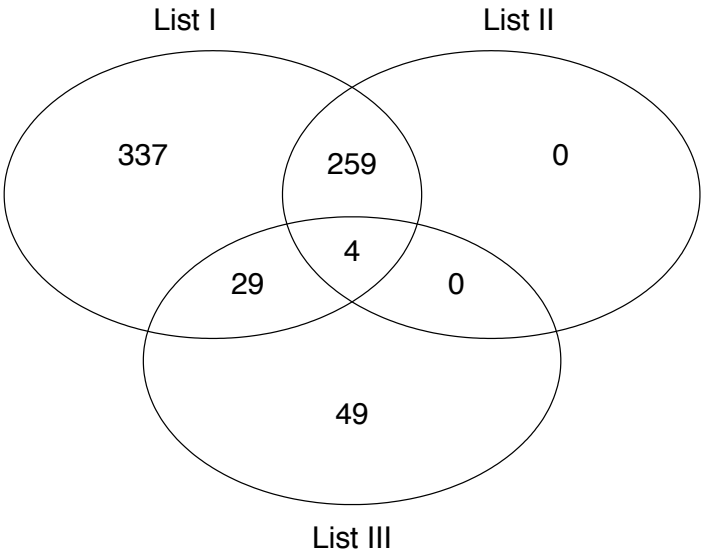

Figure 3.4. Venn diagram showing the overlap between the three different lists of ageing related genes. The three gene lists were curated using different outlooks on the same data set.

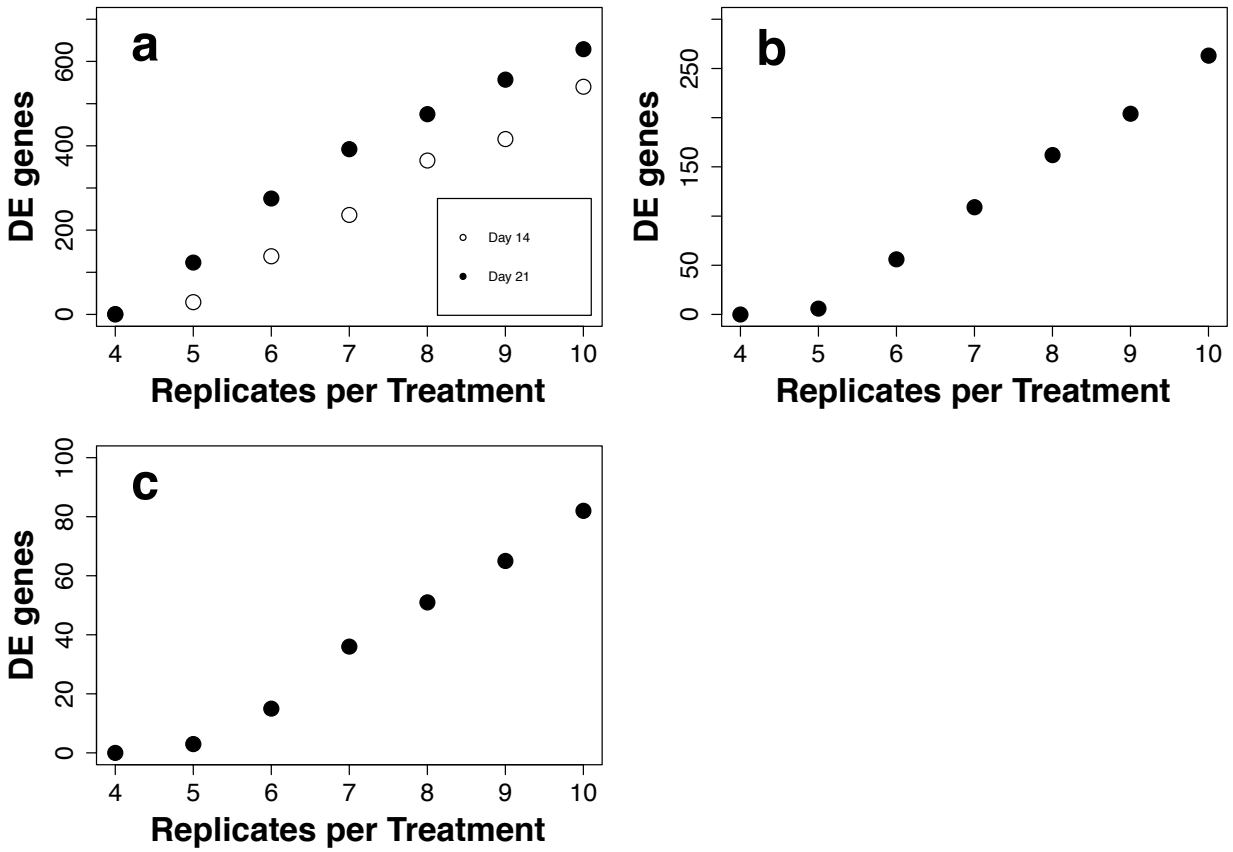

Figure 3.5. Differentially expressed genes versus number of replicates in each treatment. (a) Number of differentially expressed genes determined between the A-type and C-type for both day 14 and day 21 depending on the number of replicates included in the analysis. (b) number of differentially expressed genes between A-type and C-type populations sustained at both day 14 and day 21. (c) Number of differentially expressed genes deemed ageing-related between ageing populations and non-ageing populations.

Table 3.1. *Cis*-regulatory nature of population-differentiating-SNPs in ageing related differentially expressed genes.

	***Cis*-Regulatory Motifs**				**Non-*Cis*-Regulatory Motifs**			
	Promoter	**5'UTR**	**3'UTR**	**Combined ***	**Exons**	**Introns**	**Combined ***	**Total ***
List I	20	9	7	29	18	19	32	48
List II	13	4	4	17	12	14	18	26
List III	2	0	0	2	2	2	3	4

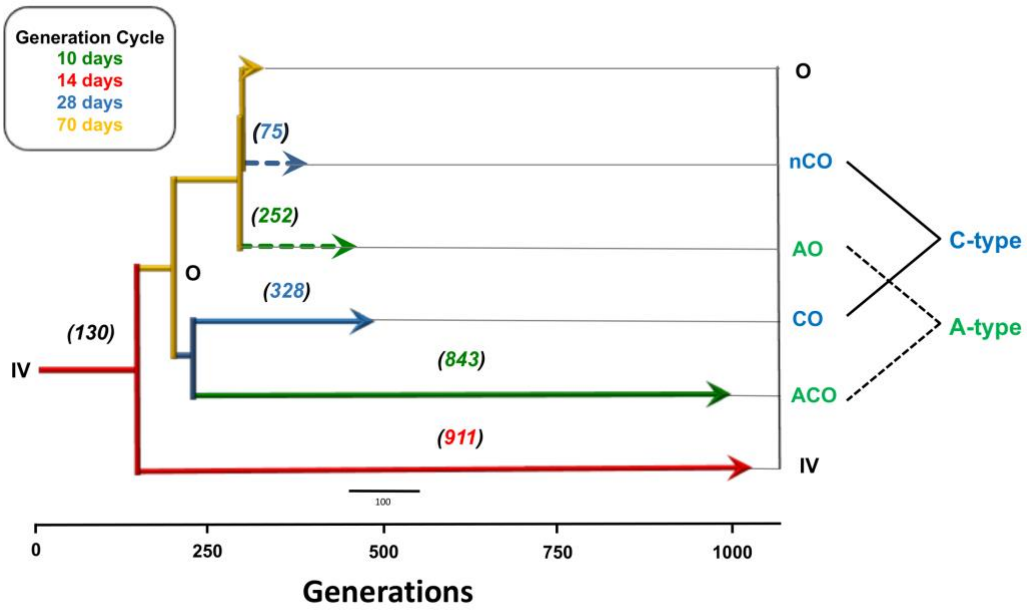

Figure S3.1. Timeline of the generation of the experimentally evolved populations of *D. melanogaster* used. Any terminal arrow denotes five populations. In parenthesis, number of generations elapsed of separated evolution for a particular subset of populations. All evolved populations analyzed derive ultimately from an outbred population, named IV, collected at South Amherst, MA (Rose 1984). More recently, all evolved populations are derived from an ancestral treatment "O", which is characterized by a generation length of 70 days. Initially, the "CO" populations were derived from the "O" populations by following the C-type selection regime, which entailed a 28-day generation length. From the "CO" lines, the "ACO" populations were generated by applying the A-type selection regime of accelerated development, shortening the generation length to 10 days. Lastly, the "nCO" and "AO" treatments derived from the original "O" populations undergoing again the mentioned C-type and A-type selection regimes, respectively.

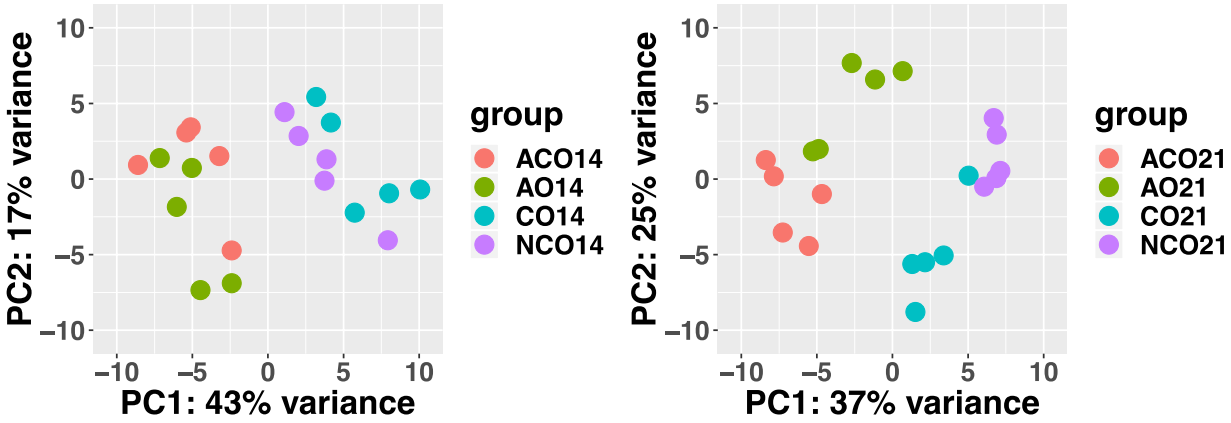

Figure S3.2. Principle component analysis (PCA) plots. PCA was done for 14- and 21-day time points (left and right, respectively) using normalized RNA-seq count data for ten early reproducing populations (five ACO and five AO) and ten late reproducing populations (five CO and five nCO). The proportion of variance explained by each component is indicated. For both time points, the grouping of the populations recapitulates the type of selection regime to which they were exposed.

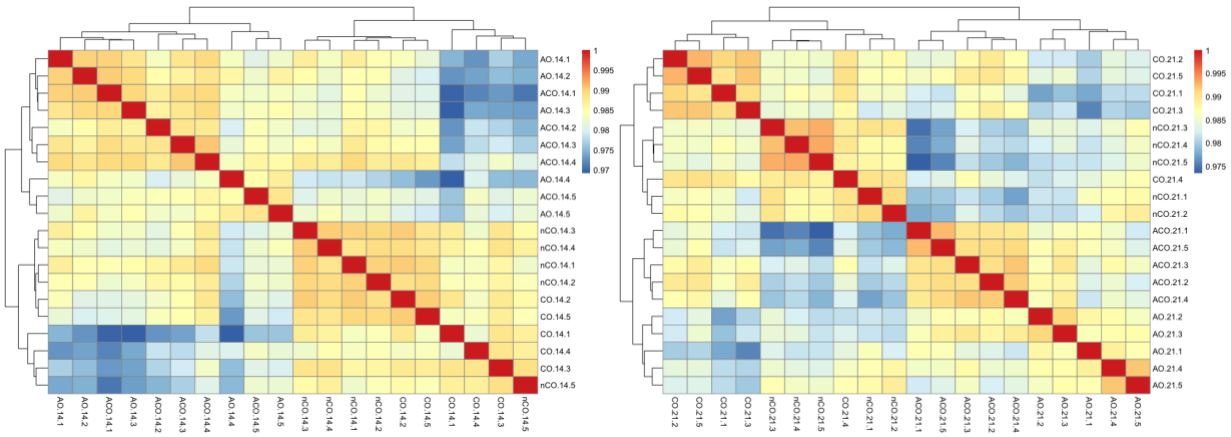

Figure S3.3. Correlation matrices among expression levels across populations. Correlation matrices were generated for 14- and 21-day time points (left and right, respectively) using normalized RNA-seq count data from each population. For both time points, the clustering of the populations precisely segregates to which of the two selection regimes the populations were individually exposed. Equivalent randomized expression data (per gene across populations) yielded no equivalent perfect segregation of populations based on their association with a particular treatment (P<0.0001; 1000 randomized datasets). The average correlation values were 0.987 and 0.987 day 14, and 0.986 and 0.987 day 21, for the A- and C-population types, respectively. The identity of each population is shown on the right and at the bottom of each chart.

Table S3.1. Salient features of the RNA sequencing datasets used.

Population					
Type	Subtype	Time Point	Sample ID	Paired-End Reads *	Alignment-Rate
A	CO	14	ACO1-14	16025476	0.876808152
A	CO	21	ACO1-21	22176411	0.902874726
A	CO	14	ACO2-14	13943892	0.828386149
A	CO	21	ACO2-21	19202323	0.819104699
A	CO	14	ACO3-14	15683573	0.792458389
A	CO	21	ACO3-21	18643435	0.930756644
A	CO	14	ACO4-14	18485653	0.8895689
A	CO	21	ACO4-21	20590149	0.82164199
A	CO	14	ACO5-14	21404467	0.918499162
A	CO	21	ACO5-21	19896435	0.879402265
A	O	14	AO1-14	14056489	0.884199177
A	O	21	AO1-21	12519550	0.891047921
A	O	14	AO2-14	15437468	0.963942209
A	O	21	AO2-21	15069859	0.83153346
A	O	14	AO3-14	16755513	0.882576857
A	O	21	AO3-21	19414394	0.922414627
A	O	14	AO4-14	14028270	0.946618721
A	O	21	AO4-21	22313935	0.848291931
A	O	14	AO5-14	14046479	0.916425533
A	O	21	AO5-21	18176024	0.909287037
C	O	14	CO1-14	13367541	0.959978129
C	O	21	CO1-21	18687732	0.902321159
C	O	14	CO2-14	12568207	0.901285521
C	O	21	CO2-21	17717238	0.876936349
C	O	14	CO3-14	14347571	0.978301414
C	O	21	CO3-21	20501662	0.73301945
C	O	14	CO4-14	16408974	0.948465151
C	O	21	CO4-21	17343174	0.953346025
C	O	14	CO5-14	13202116	0.824949728
C	O	21	CO5-21	20626122	0.895099234
C	n	14	nCO1-14	15899729	0.86004252
C	n	21	nCO1-21	18663753	0.960601975
C	n	14	nCO2-14	13249715	0.914027811
C	n	21	nCO2-21	17073607	0.966421155
C	n	14	nCO3-14	14775698	0.94502703
C	n	21	nCO3-21	17917845	0.896108042
C	n	14	nCO4-14	14604651	0.896100359
C	n	21	nCO4-21	17559627	0.90201113
C	n	14	nCO5-14	14343972	0.937844552
C	n	21	nCO5-21	18494703	0.893432082

Chapter 4

Bridging the Gap between Genomics, Transcriptomics, and Phenotypes in Experimentally Evolved *Drosophila*

ABSTRACT

The molecular basis of adaptation has eluded biologists, even with the advent of new sequencing technology. Previous attempts to address this question have knocked out or knocked down genes, but these approaches are not applicable to populations with moderate to high genetic variation. By contrast, experimental evolution offers replicated outbred populations with well-defined selection regimes and known evolutionary histories. The goal of this chapter is to use experimentally evolved populations in conjunction with statistical learning tools to explore interactions between the genome, transcriptome, and phenotypes. Our results indicate that transcriptomic measures from adult female samples can predict phenotypic characters at many adult ages, suggesting that the adult transcriptome is relatively stable in *Drosophila*. In addition, when comparing the genome and transcriptome in predicting phenotypic characters, we find that both types of data are equally powerful. When using genome sites as predictors for the expression of the transcriptome, we find that gene expression is influenced by genomic regions across all large chromosome arms. Conversely, we found many genomic regions influencing the expression of numerous genes, suggesting that many genomic regions have widely pleiotropic effects. Our results also highlight the power of the combination of experimental evolution, next-generation sequencing, and statistical learning tools in exploring the molecular networks underlying adaptation.

INTRODUCTION

Despite some recent attempts (Graves et al. 2017; Barter et al. 2019), the study of how evolutionary mechanisms shape life history traits via genome-wide evolution is still in its infancy (Braendle et al. 2011). Experimental evolution establishes selection on populations in well-defined environments as a means to decipher the interplay between population genetics and selection (Bennett and Lenski 1999; Garland and Rose, 2009; Schlotterer et al. 2015). High-throughput omic methodologies can provide a precise catalog of the frequency spectrum of genetic variants in lab evolved populations, as well as a portrait of their expression levels (Remolina et al. 2012; Mallard et al. 2018). New statistical approaches have been developed that capitalize on large-scale omic data obtained from replicated experimental evolution (de Los Campos et al. 2013; Petersen et al. 2016; Mueller et al. 2018). The hope is that the combination of next-gen sequencing, statistical learning, and such massive data might help us to understand how genetic variation underpins evolution generally. Here, we study the interplay between genomics, transcriptomics, and life history traits from 20 experimentally evolved *Drosophila melanogaster* populations. Of these populations, 10 populations have been selected for accelerated development, and the remaining 10 have been selected for postponed reproduction. Previous work collected data on the genomics (Graves et al. 2017), transcriptomics (Barter et al. 2019), and life history traits (Burke et al. 2016) for all 20 populations. In this study we use genomic and transcriptomic data to infer which gene regions might be causally linked to the phenotypes of age-specific mortality and fecundity. These inferences are made using a statistical learning technique which computer simulations have suggested may work well with experimentally evolved population (Mueller et al., 2018).

MATERIALS AND METHODS

Experimental populations

The populations used in this study were subject to two selection regimes which differed with respect to age-at-reproduction (Rose et al. 2004; Burke et al. 2016; Graves et al. 2017). Each selection regime was applied to two sets of five populations, each with known distinct evolutionary histories (Fig. S4.1). The ACO and AO populations, collectively called A-type, were selected for accelerated development and have a generation length of 10 days. The CO and nCO populations, collectively called C-type, have a generation length of 28 days.

Phenotypic Data

We relied on age specific adult mortality and fecundity data for the ten A and ten C populations reported by Burke et al. (2016). [Also presented in Chapter 1 of this thesis, because those data were obtained by myself.] Burke et al. (2016) measured mortality and fecundity over the entire adult lifespan of the flies. In our analysis, phenotypes we focused on were the average mortality and fecundity measures over 3-day intervals.

Genomic Data

For our analyses, we used the genome-wide SNP data previously published in Graves et al. (2017). The details of extraction, sequencing, and read mapping are described in Graves et al. (2017).

We only considered biallelic sites and required each site to have coverages between 20x and 200x in each of the 20 populations. We also required each site to have a minimum minor allele frequency of 2% across all 20 populations. All sites failing to meet these criteria were

discarded. To test for SNP differentiation, we used the Cochran-Mantel-Haenzel (CMH) test as implemented in PoPoolation2 (Kofler et al. 2011). CMH tests were performed between the 10 A-type populations and 10 C-type populations at all sites meeting our SNP calling criteria. Populations were paired based on treatment and replicate number (e.g. ACO_1 was paired with CO_1, AO_1 with nCO_1, etc). To correct for multiple comparisons, genetic drift, and sampling, we used the permutation approach featured in Graves et al. (2017). Briefly, we randomly assigned each population to one of two groups, and then performed CMH tests at each polymorphic site in the shuffled data set to generate null distribution of *p*-values. We did this 1,000 times, and each time we recorded the smallest *p*-value generated. We then used the quantile function in R (R Core Team, 2018) to establish a significance threshold that defines the genome-wide false-positive rate, per site, at 5%. Using this significance threshold, we identified a total of 4,211 candidate SNPs that were differentiated between the A-type and C-type populations and spread out across the five major chromosome arms.

To minimize the effects of linkage disequilibrium on our analyses, we opted to establish candidate SNP regions around our list of 4,211 candidate SNPs rather than using each candidate SNP. We first divided each chromosome arm into 50 kb windows and discarded any windows that contained less than three candidate SNPs. For the remaining windows, we recorded the position in the window with the smallest *p*-value from the CMH tests. This resulted in a list of 194 positions that serve as representatives of the 50 kb genome regions that met our criteria. These positions and their associated SNP frequencies were then used as inputs in our analyses.

Transcriptomic Data

For our analyses, we used the genome-wide transcriptomic data published in Barter et al. (2019). The details of extraction, sequencing, and read mapping are highlighted in Barter et al. (2019). The two time points (day 14 and day 21 from egg) studied in Barter et al. (2019) are included in our analyses.

After the reads were mapped, alignment post-processing was performed with SAMtools v.0.1.19 (Li et al. 2009). Read counting per gene and population was done using HTSeq v0.6.1p1 (Anders et al. 2013) at default settings. For each sample, per gene read counts were normalized using the default DESeq2 settings (Love et al. 2014). Genes showing normalized count values greater than 4 in at least 8 out of 10 populations, within at least one of the treatment types, were kept and the rest were discarded. With these normalized gene count values, we used the linear mixed effects model featured in Barter et al. (2019) to determine which genes were differentially expressed between our two selection regimes. Put simply, our analysis took into account any block effects that may be associated with different rounds of extraction and sequencing. Statistical significance for differential expression of any given gene was set at a 5% false discovery rate (FDR) for ~4000 tests, *i.e.* the number of expressed genes that passed filtering (Benjamini and Hochberg 1995). The normalized gene count values for the differentially expressed genes were then used as inputs in our analyses.

FLAM Analyses

To find gene regions that might be causally related to the phenotypes we studied, we used a statistical learning method called the "fused lasso additive model" or "FLAM" (Petersen et al, 2016). This method has been shown to effectively identify loci which cause phenotypic variation

in experimentally evolved populations that exhibit large phenotypic differences (Mueller et al., 2018). In addition, FLAM has the ability to distinguish between these causal loci and those that show genetic differentiation between populations, but are *not* causally related to the phenotype of interest (Mueller et al., 2018). Mueller et al. (2018) describe a permutation procedure for expanding the list of causative loci. In this study, a total of 100 permutations of the columns of genetic data were done and the final list consisted of genes which occurred at a frequency of at least 50% of the most common gene in the list.

RESULTS

The plasticity of the transcriptome

Unlike the genome, the transcriptome is subject to change over the course of an organism's life, most notably in the developmental stages. This stands true in *Drosophila* for development, but once flies reach adulthood the transcriptome may shift to a more static state similar to the genome, due to minimal cellular changes during a fly's adulthood. [Few cells divide in the adult soma, and there is relatively little protein synthesis.] If the adult transcriptome is static, then assaying multiple time point analyses for adult *Drosophila* may be redundant.

Here we analyze how well the transcriptomic data, at both day 14 and day 21, predict age-specific mortality and fecundity for every adult age. We used the transcriptome data from the day 14 and day 21 differentially expressed genes as predictors of mortality at days 14-35. We used the differentially expressed genes from day 14 as predictors in our FLAM analysis for fecundity at days 14-35. From our analyses at day 14 and day 21, FLAM would report which genes were classified as viable predictors and these predictors were then labeled as focal

predictors. Our data was then fractured into two parts: 16 populations were used as a training set and the remaining 4 populations were used as a testing set. Using our training set of populations, we include all age-specific mortality and fecundity data as outcomes and the normalized gene count values for the focal predictors were used as inputs. By doing this, we restricted FLAM to using only these focal predictors determined from our analyses at day 14 or day 21. With the specific fit, we use the normalized gene count values from the test set to predict their phenotypes and compare it to the observed phenotypic values. The correlation between the predicted phenotype and observed phenotype was then determined (Figure 4.1).

We find that the transcriptomic data reasonably predicts mortality at all ages, even though the transcriptomic data was limited to the focal genes identified from either day 14 or day 21. However, day 21 transcriptomic data appear to be able to predict latter age-specific mortality marginally better. This marginal increase in predictability may originate from the fact that at day 14 the C populations may still have lingering developmental effects, since day 14 is shortly after the C populations have completed development.

The same trend is not seen for fecundity. FLAM could only create a good fit for day 14 data. There is no differentiation between the A and C population fecundity values at days 17-25. It appears that there is only a strong correlation between the actual phenotype and the predicted phenotype at the focal age, 14. This is plausible considering the fact that fecundity is significantly different at days 14-16, but not between days 17-25 (Fig. 4.2). Even though fecundity is significantly different for days after day 25, we still see only a weak correlation between the actual phenotype and the predicted phenotype. This is likely due to the fact that day 14 transcriptomic data does not reasonably predict fecundity at all ages.

Genomics vs transcriptomics on predicting phenotypes

It is imaginable that both the genome and transcriptome have the ability to predict a given phenotype, but here we ask whether the genome or transcriptome has more predictive power when using FLAM specifically. When compared against each other for the same phenotype, if one performs better in providing viable predictors, then it could suggest favoring one type of omic analysis over the other in further understanding the molecular basis of adaptation.

To address this, we did three different analyses. (i) We first used genomic data (SNP frequencies) to predict mortality at days 14 and 21, as well as early fecundity. (ii) We used transcriptomic (normalized gene expression counts) data as predictors. (iii) We used both genomic and transcriptomic data as potential predictors with the same phenotypic outcomes to see which type of –omic data is best at predicting these phenotypes.

The results vary depending on which phenotype is used as outcome (Fig. 4.3-5, Table S4.1-3). For day 14, it is evident that the genomic data appears to be better at predicting the age-specific mortality data, but the transcriptomic data appears to be better at predicting the age-specific fecundity data (Fig. 4.3-4, Table S4.1-2). For the most part, it appears that the genomic locations and the genes found as good predictors of phenotypes individually also show up in the combined list. It is clear that neither genomic or transcriptomic data are obviously better at day 14.

Day 21 genomic and transcriptomic data was only used to predict mortality, since there were no statistically significant differences in fecundity at ages greater than 21 days. When given both genomic and transcriptomic data for day 21, we find that both –omic data sets perform relatively well (Fig. 4.5, Table S4.3). The model does not appear to strongly favor one –omic data set over the other, the way we found for the day 14 data.

Due to the fact that both genomic and transcriptomic predictors are being selected as predictors suggests that using only one set of –omic data is ill-advised. The reason why one type of –omics may perform better for some phenotypes, but not for others remains unclear.

Unbiased approach on predicting transcriptomic expression using genomics

It's evident that the genome ultimately shapes the transcriptome, but how the genome is shaping the transcriptome remains largely unknown. The main focus of research on this topic has been around the issue of *cis*- versus trans- regulation of genes, in that there are specific regions in the genome that are either local or distant that might control the expression of a gene.

Although we do not focus on regulation specifically here, we use genomic data as predictors and each individual gene expression count data as outcomes. By doing this we are able to see which SNP regions are considered good predictors for the expression of each differentiated gene. All the SNP regions are included in this analysis to include all interactions between the genome and the transcriptome.

As shown in Figure 4.6, there are numerous interactions between the genome and the transcriptome. These interactions are not limited to the same chromosome, between the two sets of data, suggesting there is a lot of cross-chromosomal interactions. The average number of genomic sites that are considered good predictors for a given transcript is 5.7, with the lowest being 0 and the highest being 29. The average number of times a genomic site was chosen as a good predictor across all genes is 18.3, with the lowest being 0 and the highest being 77. The X chromosome contains the most predictors when compared to the other chromosome arms, but contained the largest portion of genomic inputs. Whereas, 3R had the least amount of predictors, and the trend remains the same in that 3R had the least amount of inputs.

Our results show evidence of pleiotropy in that a single genomic region is reliably contributing to the prediction of expression in numerous genes. In contrast, we see that the expression of a single gene is being predicted by numerous genomic regions, many that originate from a different chromosome. Although we see many interactions between the genome and the transcriptome, these interactions should not all be considered regulation due to the fact that the genomic regions are quite large and the true nature of how these regions are potentially affecting gene expression remains unknown.

DISCUSSION

Having two clearly defined sets of experimentally evolved *D. melanogaster* populations in conjunction with a full suite of genomic, transcriptomic, and phenotypic data for both sets of populations has enabled us to piece together how these three levels of biological machinery interact with one another. Specifically, the 10 A-type populations are clearly differentiated from the 10 C-type populations across the genome (Graves et al. 2017), across the transcriptome (Barter et al. 2019), and in age-specific mortality and fecundity (Burke et al. 2016). Conversely, there is little to no differentiation between the populations within a single set of 10 populations for all three sets of data. This level of differentiation between two sets of populations and level of convergence within each set has yet to be seen in sexually reproducing populations in other experiments. Lastly, these two sets of populations are closely related, despite their marked differentiation at all three levels, genomic, transcriptomic, and phenotypic.

When reviewing the plasticity of the transcriptome, we find that once the population has reached adult maturity, the transcriptome seems to be relatively static. Transcriptomic data at day 21 accurately predicts mortality for all ages after day 21. Since the transcriptome data did not

result in accurate predictions of fecundity after day 14 we are unable to make any inferences about the relative stability of transcriptome effects on fecundity at later ages.

Next, when comparing the genome and the transcriptome in predicting phenotypic outcomes, we find that there is no clear consensus on which –omic data set performs better. As it stands, both data sets are being used by the model to properly predict phenotypic outcomes.

We sought genomic regions that were good predictors for the expression of each differentiated gene. Although these regions may have predicted the expression of the differentiated gene, these regions should not be considered specific regulators for the gene. Some of regions may contribute to the regulation of the gene, but it is doubtful that that these regions have evolved solely for their effects on transcript regulation. In addition, the location of each predictive genomic region was not restricted to the cis-locale of the gene. It is very clear that gene expression is characteristically affected by many sites across the entirety of the genome. Lastly, it appears as if the X and 2L chromosome arms (Fig. 4.6, blue and red) are favored in the number of predictive genomic regions. However, this may have arisen because those two chromosome arms had the most candidate genomic regions. In other words, the number of predictors from a given chromosome arm is proportional to the number of sites in its sequence that are associated with evolutionary divergence between the "A" and "C" selection regimes.

Currently, we only have the full suite of genomics, transcriptomics, and phenotypic data for 20 populations. As shown in Mueller et al. (2018), a 20 population analysis is barely sufficient for detecting causal loci, and by no means will detect the full range of causally important sites in the genome. Ideally, the number of populations used in analyses of this kind should be toward 100 populations. Only at such a high level of replication is it plausible that this

experimental strategy will reveal a high proportion of the genomic sites that are involved in the response to selection.

Although having the full suite of all three types of data is ideal, just having genomics and phenotypic data in additional populations would allow a drastic increase in power for detecting causal loci. In recent phenotypic and genomic work, we have access to 30 additional populations. The first additional set of 10 populations were selected for reproduction in vials (Graves et al. 2017). The second set were selected for starvation resistance (Kezos et al. 2019), while the third additional set of 10 populations are on a standard 21-day generation cycle selection regime (Phillips et al. 2018). With the addition of the genomic and phenotypic data from these 30 populations, we can raise our population count to 50 and run similar analyses to those shown here for the genomic foundations of adaptation. Beyond those 50 populations, we have plans to sequence an additional 40 populations, allowing us to approach the level of hundred-fold replication, at which point thorough penetration of the genomic complexity of adaptation might be achievable.

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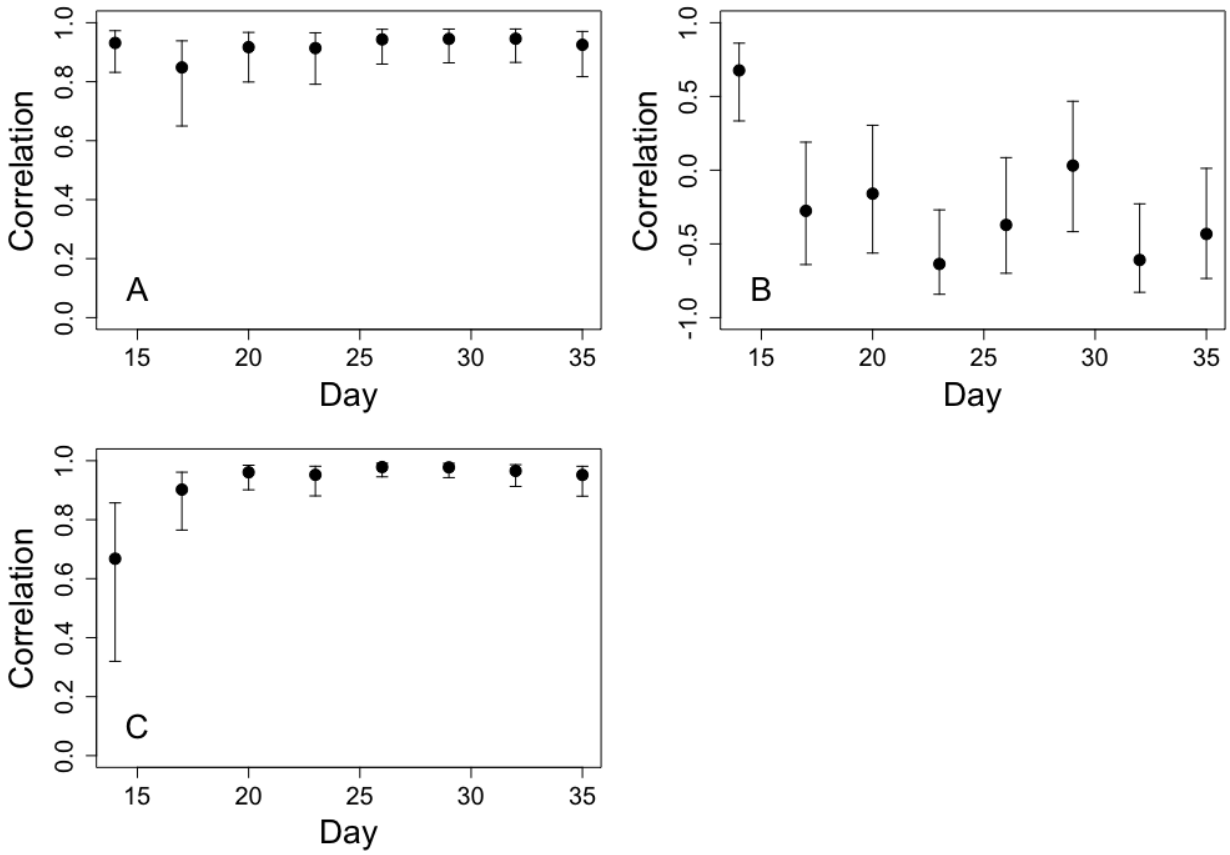

Figure 4.1. Correlation values when comparing predicted phenotypic values to true phenotypic values at every age. We compare how well focal transcriptomic predictors can predict phenotypes at non-focal ages. The values shown here are the comparison between the actual phenotypic values and the predicted phenotypic values from our test set. The predicted phenotypic values were generated using the model fit at each age from the focal transcriptomic predictors. (A) Day 14 transcriptomic data was used as predictors and age-specific mortality as outcomes. (B) Day 14 transcriptomic data was used as predictors and age-specific fecundity as outcomes. (C) Day 21 transcriptomic data was used as predictors and age-specific mortality as outcomes.

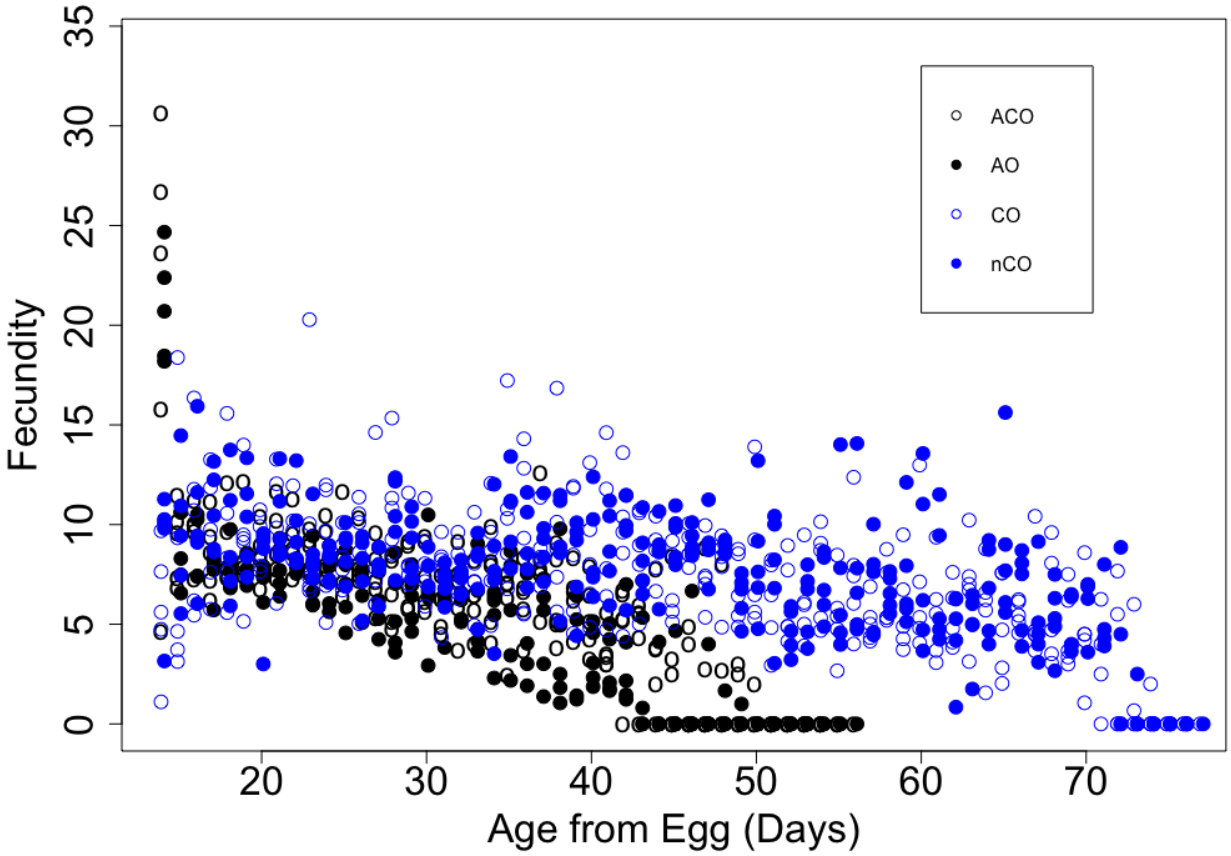

Figure 4.2. Adult age-specific fecundity from A-type and C-type selection treatments. Points represent average number of eggs laid per female per day as a function of age. Black signifies A-type populations and Blue signifies C-type populations. Open circles represent longstanding populations (ACO and CO), where closed circles represent newer derived populations (AO and nCO).

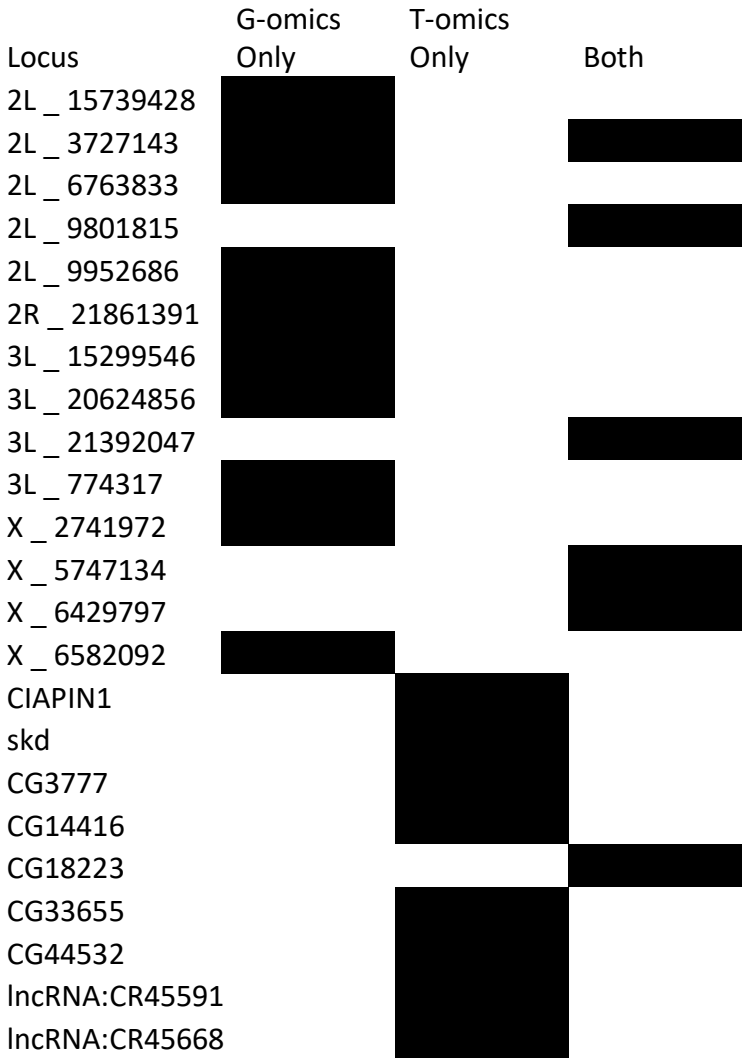

Figure 4.3. Genomics versus Transcriptomics in predicting mortality day 14. Predictive loci (black box) from genomics (G-omics) and transcriptomics (T-omics) in all three analyses and from which analyses they are considered predictive loci.

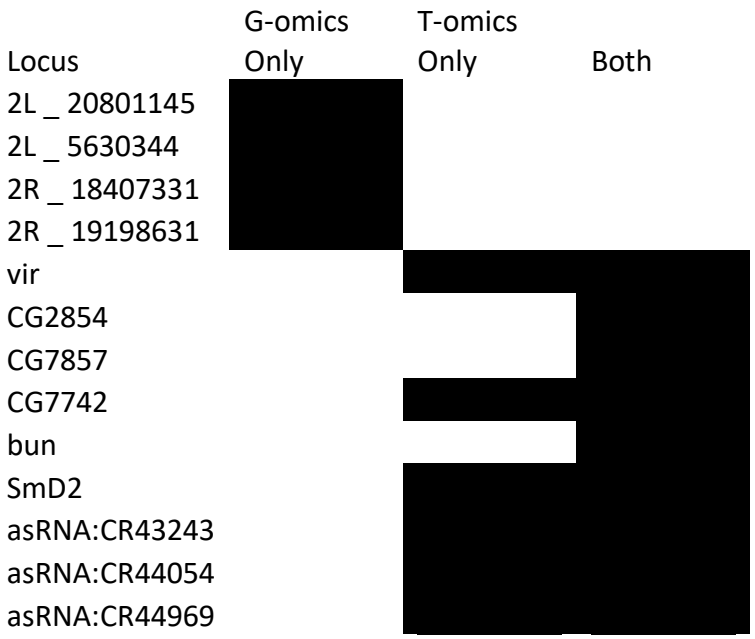

Figure 4.4. Genomics versus Transcriptomics in predicting fecundity at day 14. Predictive loci (black box) from genomics (G-omics) and transcriptomics (T-omics) in all three analyses and from which analyses they are considered predictive loci.

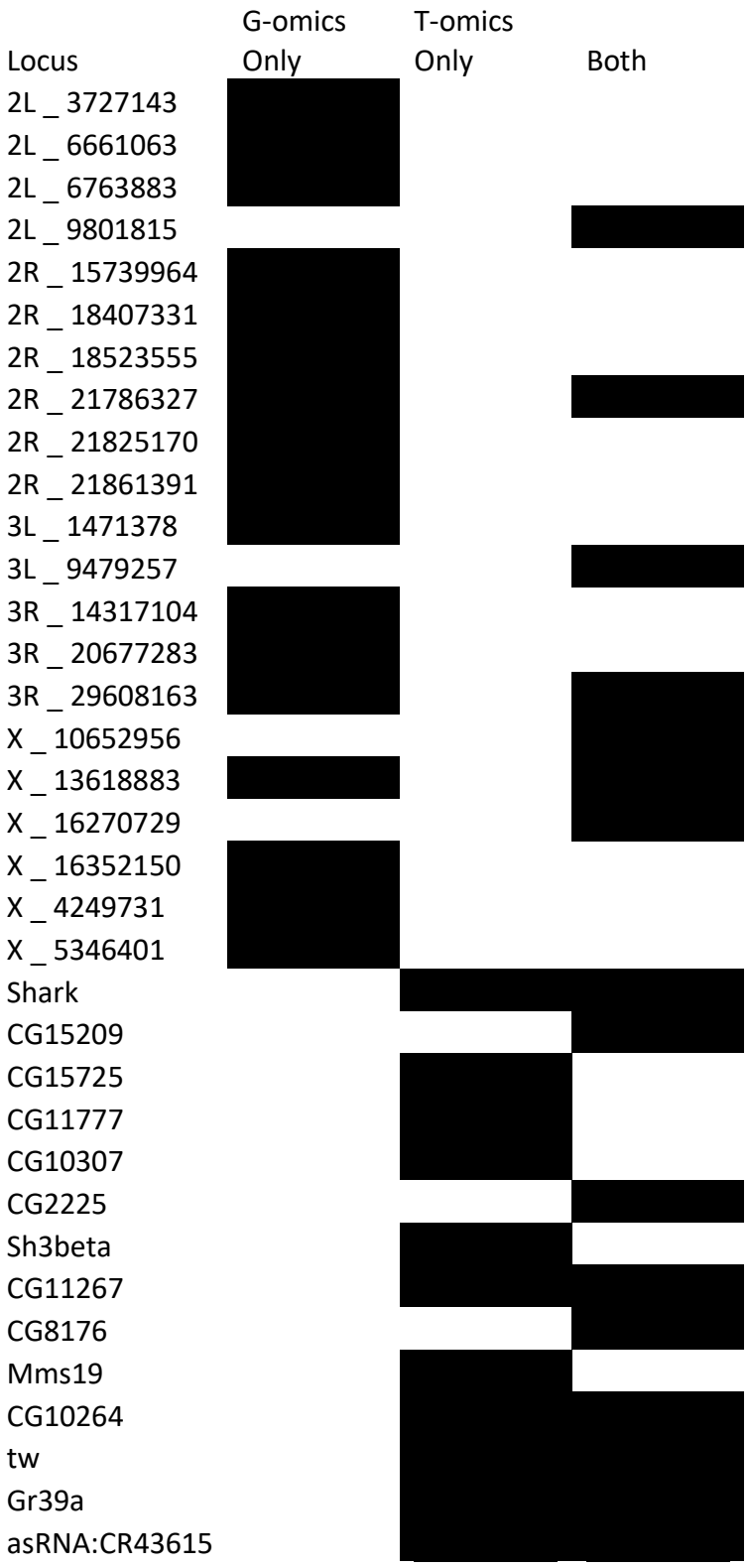

Figure 4.5. Genomics versus Transcriptomics in predicting mortality at day 21. Predictive loci (black box) from genomics (G-omics) and transcriptomics (T-omics) in all three analyses and from which analyses they are considered predictive loci.

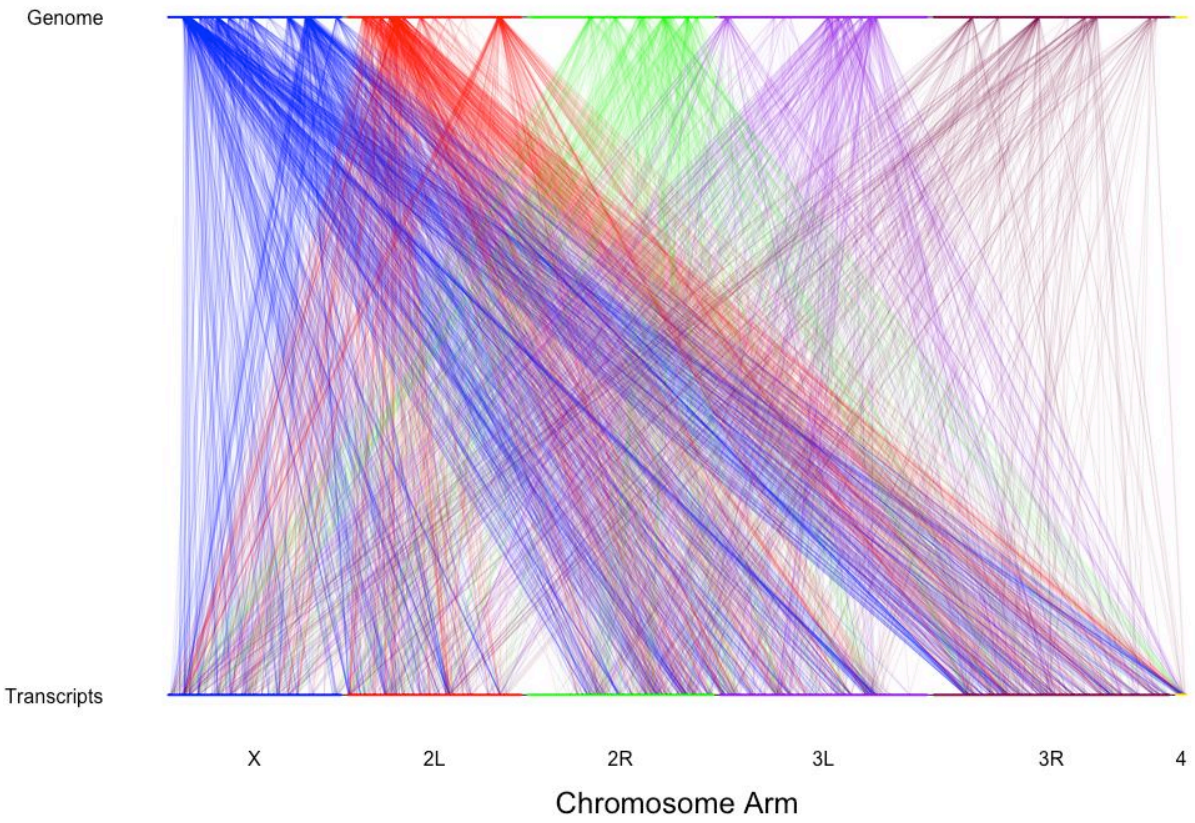

Figure 4.6. Interactions between predictive genomic regions and differentiated transcripts. Each line is an interaction between a predictive genomic region to a gene that the genomic region can accurately predict the expression. The color of the line denotes which chromosome arm the genomic region originates from. The genomic regions are classified as 50kb windows in which there contained at least 3 differentiated SNPs. The transcripts are classified as the expression of each differentially expressed gene.

Table S4.1. Genomics versus Transcriptomics in predicting mortality at day 14.

Genomics only	Transcriptomics only	Both
2L_15739428	CIAPIN1	2L_3727143
2L_3727143	skd	2L_9801815
2L_6763833	CG3777	3L_21392047
2L_9952686	CG14416	X_5747134
2R_21861391	CG33655	X_6429797
3L_15299546	CG44532	CG18223
3L_20624856	lncRNA:CR45591	
3L_774317	lncRNA:CR45668	
X_2741972		
X_6582092		

Table S4.2. Genomics versus Transcriptomics in predicting fecundity at day 14.

Genomics only	Transcriptomics only	Both
2L_20801145	vir	vir
2L_5630344	CG7742	CG7857
2R_18407331	SmD2	CG7742
2R_19198631	asRNA:CR43243	CG2854
	asRNA:CR44054	bun
	asRNA:CR44969	SmD2
		asRNA:CR43243
		asRNA:CR44054
		asRNA:CR44969

Table S4.3. Genomics versus Transcriptomics in predicting mortality at day 21.

Genomics only	Transcriptomics only	Both
2L_3727143	Shark	2L_9801815
2L_6661063	CG15725	2R_21786327
2L_6763833	CG11777	3L_9479257
2R_15739964	CG10307	3R_29608163
2R_18407331	Sh3beta	X_10652956
2R_18523555	CG11267	X_13618883
2R_21786327	Mms19	X_16270729
2R_21825170	CG10264	Shark
2R_21861391	tw	CG15209
3L_14741378	Gr39a	CG2225
3R_14317104	asRNA:CR43615	CG11267
3R_20677283		CG8176
3R_29608163		CG10264
X_13618883		tw
X_16352150		Gr39a
X_4249731		asRNA:CR46058
X_5346401		

CONCLUSIONS

Selection experiments using experimentally evolved populations have become quite popular and can be useful in determining the underlying mechanisms of evolution itself. Although the field of experimental evolution is quantitatively dominated by studies of asexual populations (Barrick et al. 2009; Tenaillon et al. 2012; Maddamsetti et al. 2015), there is much to gain in using sexual populations. For instance, asexual populations undergoing selection must wait for a beneficial *de novo* mutation before they can start to adapt, whereas adaptation in sexual populations is primarily driven by standing genetic variation (Burke 2012). Considering this, sexual populations with moderate standing genetic variation should adapt rapidly and in parallel with other populations, providing those populations have similar levels of genetic variation and undergo identical selection. Ultimately, the possibility of rapid repeated adaptation in sexual populations allows us to use statistical learning tools to better understand the interconnections between the genome, transcriptome and phenotype, as these tools require heavy replication.

This dissertation first sought to determine how quickly and repeatedly selection in experimentally evolved sexual populations resulted in these populations achieving similar phenotypes and gene expression levels. Secondly using the same experimentally evolved populations and a statistical learning model, the thesis research attempts to uncover interactions between the genome, transcriptome, and phenotypes.

Chapter 1 of my thesis measured adult life-history in 30 experimentally evolved *Drosophila* populations. Ten of these 30 populations were selected for accelerated development, ten were selected for standard laboratory selection in vials, and ten were selected for postponed reproduction. Within each group of 10 populations undergoing the same selection regime, there

were 5 populations that were considered long-standing while the remaining 5 were considered recently-derived. The long-standing populations each had over 200 generations in their current selection regime, whereas the recently-derived populations had as little as 45 generations under their current selection regime. I measured age-specific early fecundity (pre-day 14) for all 30 populations, as well as life-long mortality and fecundity (post-day 14) for 20 populations. First, I found that there was significant evidence for divergence between all three selection regimes. Second, I found there was significant evidence for convergence within each selection regime. This led to the conclusion that populations with moderate standing genetic variation are capable of diverging from their ancestral state and converging with populations undergoing similar selection, all within as little as 45 generations. An unexpected result was that there was a clear stage of non-aging in our postponed reproduction populations. Prior studies did not reveal a similar pattern (Rose et al. 2002), but I believe this was likely due to how the experiment was conducted. Our previous mortality assays were conducted in vials, whereas in my work it was conducted in cages.

Chapter 2 of my thesis is similar to Chapter 1, in that I measured different phenotypes from the same 30 experimentally evolved populations to examine the rapidity and repeatability of selection in sexual populations. I measured egg hatching time, larval development, and pupal development. Larval and pupal development data were similar to the data of Chapter 1, in that I saw evidence of convergence within selection regime and evidence of divergence between selection regimes. I did not see the same pattern for egg hatching. Instead, what I found was that only the accelerated development populations showed evidence of convergence within selection regime, and divergence from the other selection regimes. For the remaining populations, it appeared as if recent evolutionary history played a larger role in determining egg hatching time.

This led me to the conclusion that the nature of the selection imposed on the populations has a clear implication for how phenotypes will evolve. If a phenotype is strongly affected by selection, then evolutionary history has a diminished effect on the phenotype. The converse is also true, in that if selection does not impact the phenotype, then evolutionary history takes precedent.

Chapter 3 of my thesis analyzed transcriptomic data from 20 of the previously described populations at two different time points. Specifically, I took female whole-body samples from populations selected for the accelerated development and postponed reproduction. To determine differentiation between the two selection regimes, I compared the RNA abundance at the gene level for all 20 populations. I found that within-selection regime there is very little differentiation, while between selection regimes there were hundreds of genes that were differentially expressed. These results are very similar to those seen in Graves et al. (2017) for genomic differentiation. Unexpectedly, I found that a high proportion of non-coding RNA genes were differentially expressed. Although, I currently do not have much insight into why this is the case, it is likely that non-coding RNA genes play some role in phenotypic differentiation.

Chapter 4 of my thesis sought to examine the interactions between the genome, transcriptome, and organismal phenotype using statistical learning tools (Mueller et al. 2018). Genomic data were taken from Graves et al. (2017), transcriptomic data were taken from Chapter 3 (Barter et al. 2019), and phenotypic data were taken from Chapter 1 (Burke et al 2016). All measures are from the populations selected for accelerated development and postponed reproduction. First, I compared how well transcriptomic measures predict phenotypes at many different ages, using the transcriptomic measures from just one age. I found that once the population has exited the developmental stage, the transcriptome can reliably predict mortality at

seven of the eight ages tested. Second, I compared whether the genome or the transcriptome can better predict phenotypic measures. I found that neither the genome or the transcriptome had a clear advantage in predicting age-specific mortality and fecundity. Their specific value varied depending on which phenotypic measure I used as an input. Lastly, I sought to find genomic regions that were good predictors for the expression of each transcript that was differentiated between the two selection regimes. I found that causal genomic regions for the expression of a differentiated gene were found across the genome, rather than being restricted to the locale of the differentiated gene. In addition, I found that most candidate genomic regions were predictive for the expression of multiple transcripts, suggesting these regions have pleiotropic effects.

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