Temporal Stability of Molecular Diversity Measures in Natural Populations

of *Drosophila pseudoobscura* and *Drosophila persimilis*

by

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Thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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ABSTRACT

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Abstract

Many molecular ecological and evolutionary studies sample wild populations at a single point in time, failing to consider that data they collect represents genetic variation from a potentially unrepresentative snapshot in time. Variation across time in genetic parameters may occur quickly in species that produce multiple generations of offspring per year. However, many studies of rapid contemporary microevolution examine phenotypic trait divergence as opposed to molecular evolutionary divergence. Here, we compare genetic diversity in wild caught populations of *Drosophila persimilis* and *D. pseudoobscura* collected 16 years apart at the same time of year and same site at four X-linked and two mitochondrial loci to assess genetic stability. We found no major changes in nucleotide diversity in either species, but we observed a drastic shift in Tajima's D between *D. pseudoobscura* timepoints at one locus associated with the increased abundance of a set of related haplotypes. Our data also suggests that *D. persimilis* may have recently accelerated its demographic expansion. While the changes we observed were modest, this study reinforces the importance of considering potential temporal variation in genetic parameters within single populations over short evolutionary timescales.

Dedication

This work is dedicated to my amazing family and support system: my fiancé John, sister Megan, and wonderful parents Tim and Patti. Their unwavering love, encouragement, and guidance is the reason I am the person I am today, and I could not have made it this far without them.

I would also like to dedicate this work to the following key individuals that have been instrumental in my career as a scientist and my decision to go to graduate school:

Mrs. Cynthia Williams- Mrs. Williams was way more than just my high school biology teacher. She was like a second mother to me after many years of playing soccer with her daughter, and is one of the most influential people in my life who fostered my passion for science. Her class was by far the most fun, engaging, exciting, and inspiring class I ever took in public school, and is one of the main reasons I chose to teach science, as opposed to math, as my future career.

Dr. Miriam Ferzli- Dr. Ferzli was not only the best undergraduate biology teacher I had during my time at NC State, she was my role model. Dr. Ferzli shared my passion for both science and teaching and gave me the opportunity to teach my first class as sole instructor as a TA for an introductory biology lab. She has been a constant source of encouragement and support, and I cannot thank her enough.

Dr. David Threadgill- Dr. Threadgill gave me the opportunity to work on my first independent research project. My time working with David and the members of the Threadgill lab opened my eyes as to just how fun and exciting it was to conduct research and helped solidify my decision to go to graduate school. It was here that I learned many of the skills needed to be a successful scientist, and I do not believe I would be here today had it not been for David.

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I would also like to thank B. Manzano-Winkler for all of her help training me in Noor lab protocol and fly handling and K. Ritz for her technical assistance with the project. Many thanks go to M. Turelli for his generous support for the 2013 field collections. I also thank C. Rushworth, J. Coughlan, T. Markow, and three anonymous reviewers for their helpful comments on the manuscript. Lastly, I thank the National Institutes of Health for funding this project through grant GM086445 awarded to Mohamed, as well as the UPGG training grant.

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AJ Hish's contribution to this work was the collection of the 2013 *D. persimilis* and *D. pseudoobscura* fly lines used in this study.

1. Introduction

1.1 Context of Study

Evolution can occur over relatively short periods of time, on the order of tens of years (Hendry and Kinnison 1999; Carroll et al. 2007), and examples of rapid adaptive phenotypic evolution have been documented in a wide variety of species, including birds (Berthold et al. 1992; Grant and Grant 2002), fish (Stearns 1983; Reznick et al. 1997; Hendry et al. 2000; Barrett 2008; Cureton and Broughton 2014), lizards (Losos et al. 1997), insects (Turelli and Hoffmann 1995; Huey et al. 2000; Carroll et al. 2001), zooplankton (Cousyn et al. 2001), and plants (Franks et al. 2007). Both drift and selection can play important roles in changing allele frequencies over short timeframes, with drift's role being particularly pronounced in species with small effective population sizes.

Many of the studies of rapid evolution have focused on phenotypic change, rather than molecular change, and examine populations with small effective population sizes and/ or the adaptive history of species after introduction to a new habitat (Berry 1964; Johnston and Selander 1964; Williams and Moore 1989). Since these phenotypic traits have a clear underlying genetic basis, molecular evolutionary changes in the genomes of these species must necessarily have resulted as well. However, rapid molecular evolution can also

occur quickly in populations with large effective population sizes via selection. For instance, the spread of cytoplasmically transmitted Wolbachia microbes in *Drosophila simulans* caused a mitochondrial haplotype to spread quickly to high frequencies in natural populations in California in less than ten years (Turelli and Hoffmann 1995).

Thus, commonly used diversity measurements employed in many molecular population genetic studies may change over short timeframes. Such changes could potentially confound studies that sample from natural populations, which tend to focus on spatial diversity measures (e.g. F_{ST}) at specific points in time as opposed to temporal diversity in single locales. This spatial-focused approach assumes that allelic variation within populations is stable over time, which may not be true given the idiosyncratic nature of natural selection and presence of genetic drift. Temporal allele frequency data can also help researchers monitor population demographic dynamics, such as growth and migration. If molecular evolutionary parameters change over short timeframes, conclusions gathered from studies of a single time point may not reflect historic evolutionary processes (Grant and Grant 2002). Further, some studies detect the effects of natural selection across much of the genomes of species with large effective population sizes (Andolfatto 2005; Sella et al. 2009), which could suggest

that genetic parameters derived from particular timepoints are unrepresentative at many loci across the genome. While some studies assess temporal shifts in allele frequencies and consider the roles of selection and drift in causing these changes (Coletta-Filho et al. 2014; Holmes 2015; Lewontin et al. 1981), comparatively few examine temporal changes using DNA sequence data and site frequency spectra (e.g., Bergland et al. 2014).

1.2 Goal of Study

To better understand the temporal variation seen in species with large effective population sizes, we evaluate the stability of the frequently used evolutionary genetic parameters pi (nucleotide diversity) and Tajima's D in populations of *Drosophila persimilis* and *Drosophila pseudoobscura* collected sixteen years apart from the exact same site. Given that approximately one hundred generations separate our sample collections, these populations may differ in the genetic parameters assayed. We selected four random loci spanning the X chromosome and two loci within the mitochondrial genome as test cases.

2. Materials and Methods

2.1 Fly Stocks

This study examines the genetic diversity seen in wild cohorts of *Drosophila pseudoobscura* and *Drosophila persimilis* obtained in both 1997 and 2013 from the Robert Louis Stevenson State Park in Mount St. Helena, California during the month of July. DNA preparations of the individuals collected in 1997 were stored in the -80°C freezer until needed for further analysis. A total of 13 *D. pseudoobscura* and *7 D. persimilis* isofemale lines collected in 1997 and 25 *D. pseudoobscura* and 15 *D. persimilis* isofemale lines collected in 2013 were analyzed, though not all samples amplified at every locus examined.

2.2 DNA Prep, Amplification, and Sequencing

Genomic DNA from adult males of the *D. pseudoobscura* and *D. persimilis* cohorts was extracted using the single fly squish protocol (Gloor and Engels 1992). Primers were designed to amplify approximately 800bp to 1kb regions of four X-linked loci (DPSX008, DPSX009, XLgrp1e, XRgrp8) and two mitochondrial loci (cytochrome oxidase subunit I and NADH dehydrogenase subunits 4 and 5) by PCR in 20µl reactions. See Supplementary Table 1 for primers. Sizes of the PCR products were confirmed on a 1% agarose gel, and samples were purified for sequencing using ExoSAP-IT (Affymetrix). Purified PCR products were

sequenced by Eton Bioscience. Sequences from this study can be found in the GenBank database under the accession numbers KM887512-KM887838.

We focused on X-linked and mitochondrial loci because amplifying them in male samples prevented the need for subcloning to distinguish heterozygotes. For reference, the X-chromosome in these species comprises 40% of the overall genome and extends for over 200cM (Anderson 1993). DPSX008 and DPSX009 are noncoding regions containing microsatellites that have been previously examined in Machado *et al.* (2002). XLgrp1e and XRgrp8 are intergenic regions that we picked arbitrarily. COI and ND4/5 are protein-coding regions involved in oxidative phosphorylation that have been previously examined in Machado and Hey (2003). The DPSX008 and XLgroup1e loci are approximately 3.71 Mb apart, the XLgrp1e and DPSX009 loci are approximately 23.62 Mb apart, and the DPSX009 and XRgroup8 loci are approximately 15.62 Mb apart. Assuming a recombination rate of 4 cM/Mb along the X chromosome (McGaugh et al. 2012), only the DPSX008 and XLgroup1e loci would be linked at all (<50cM), and this pair of loci would be separated by roughly 15cM of recombination. See Supplementary Figure 1 for the physical map of the X chromosome.

2.3 Data Analyses

DNA sequences were aligned using ClustalW in BioEdit 7.0.9 (Hall 1994), followed by manually shifting bases further to the left or right (usually around gaps) to further improve the alignment. A 9-bp micro-inversion found in the DPSX009 locus in *D. persimilis* from alignment bases 687-695 was removed from all sequences in the dataset to prevent biases in the calculation of genetic diversity measures. DnaSP 5.10.01 (Rozas et al. 2003) was then used to calculate estimates of nucleotide diversity (pi) and Tajima's D. We also used this to calculate D_{XY} as a measure of molecular differentiation between the timepoints within each species, as well as the number of segregating sites within each cohort and shared by both cohorts of each species. These estimates excluded sites where gaps in the sequence sets were found.

To better visualize the nature of the change in Tajima's D at the DPSX009 locus in *D. pseudoobscura*, we created a minimum spanning network (Fig 2) using PopART version 1.7 (http://popart.otago.ac.nz). We also performed a Fisher's Exact Test to assess the statistical significance of difference in the relative abundance of a haplotype group at *DPSX009* in *D. pseudoobscura* (see Results section).

To account for differences in sample size between the 1997 and 2013 cohorts and test statistical significance of the differences in pi between the timepoints or having a larger D_{xy} than expected by chance, we created a custom Perl script. This script uses the output file from the BioEdit Sequence difference count Matrix application to calculate pi and D_{xy} for both the 1997 and 2013 groups of sequences, followed by random sampling without replacement. It then randomly assigns sequences in the dataset to one of two groups, each the same size as the original 1997 and 2013 cohorts, calculates a new pi and D_{xy}, and assesses whether these measures are greater than the original pi and D_{xy}. We set the code to sample without replacement 10,000 times. The number of times the simulated D_{xy} and difference in pi between the 2013 and 1997 cohorts were greater than the original difference in pi and D_{xy} for each locus was recorded as our p value. This script was made flexible so that anyone can run it with their own custom datasets without having to modify the code. The script is available upon request.

3. Results

We measured the genetic diversity across four X-linked (DPSX008, DPSX009, XLgrp1e, XRgrp8) and two mitochondrial loci (COI and ND4/ND5) in populations of *D. pseudoobscura* and *D. persimilis* collected in 1997 and 2013 to assess the temporal stability of commonly used molecular evolutionary parameters over short periods of evolutionary time. All measures of pi, D_{XY}, and Tajima's D are presented in Table 1. We observed no drastic changes in values of nucleotide diversity (pi) in either *D. persimilis* or *D. pseudoobscura* between the two timepoints. Likewise, there are no overall trends of increased or decreased pi values in the 2013 cohorts in comparison to the 1997 cohorts across all six loci.

Table 1: Molecular diversity measures from samples of *D. persimilis* and *D. pseudoobscura* collected in 1997 and 2013. N represents the number of individuals sequenced. S represents the number of segregating sites present in each cohort, S_{total} represents the total number of segregating sites that were found in the 1997 and 2013 cohorts combined, and S_{shared} represents the number of segregating sites that were common between both of the cohorts. The asterisk (*) indicates values that were found to be statistically significant ($p < 0.05$).

Locus	Species	N	pi	Tajima's D	S	S_{total}	S_{shared}	D_{XY}
DPSX008	1997-pseudo	11	0.01669	-1.22385	55	107	41	0.01750
	2013-pseudo	22	0.01895	-1.47765	93			
	1997-persimilis	6	0.00803	-0.48641	18	34	11	0.00826*
	2013-persimilis	12	0.00673	-1.25940	27			
DPSX009	1997-pseudo	12	0.01116	-1.46051	40	47	28	0.01272
	2013-pseudo	25	0.01378	0.73806	35			
	1997-persimilis	5	0.01413	-0.28656	25	41	15	0.01244
	2013-persimilis	15	0.01103	-0.55262	31			
XLgroup1e	1997-pseudo	10	0.02096	-0.91774	57	134	39	0.02359
	2013-pseudo	22	0.02575	-1.55367	116			
	1997-persimilis	6	0.00738	-0.77134	16	43	10	0.00754
	2013-persimilis	14	0.00803	-1.79642	37			
XRgroup8	1997-pseudo	13	0.00811	-1.30480	35	45	25	0.00744
	2013-pseudo	21	0.00729	-1.24694	35			
	1997-persimilis	5	0.00396	-0.07339	8	29	4	0.00580
	2013-persimilis	15	0.00610	-0.99667	25			
COI	1997-pseudo	13	0.00640	-0.71686	18	26	13	0.00639
	2013-pseudo	23	0.00615	-0.68706	21			
	1997-persimilis	7	0.00658	-1.34103	16	24	8	0.00543
	2013-persimilis	15	0.00460	-1.18713	16			
ND4/ND5	1997-pseudo	12	0.00177	-0.90540	6	12	5	0.00185
	2013-pseudo	23	0.00203	-1.65217	11			
	1997-persimilis	7	0.00075	-1.23716	2	7	2	0.00102
	2013-persimilis	13	0.00141	-1.98196	7			

Given that our 1997 cohorts of *D. persimilis* and *D. pseudoobscura* had fewer samples than our 2013 cohorts, we needed to ensure that our genetic diversity measures were not skewed as a result of sampling error. Using our custom Perl script, we used random sampling without replacement to assess the statistical significance of the difference in pi and D_{XY} amongst the 1997 and 2013 sequence sets. The number of times the simulated difference in pi or value of D_{XY} was greater than the actual difference in pi or value of D_{XY} in all six loci after sampling without replacement 10,000 times was documented as our p value. No significant differences in pi were found at any of the six loci (p value > 0.05), and only the D_{XY} value at the DPSX008 locus in *D. persimilis* was found to be significantly greater than our randomized samples ($p = 0.0094$).

There are also no consistent trends in Tajima's D in *D. pseudoobscura*, but locus DPSX009 appears to have changed, as evidenced by its drastic switch from a negative to a positive Tajima's D value (Fig 1). This finding can be explained by the increase of a set of related haplotypes spreading sometime between 1997 and 2013 in *D. pseudoobscura* (Fig 2). These haplotypes have detectable linkage disequilibrium across their length, with more than fifteen SNPs near the middle of the sequenced area (spanning approximately 500 bases) in almost complete LD. About 25% of sequences in the 1997 cohort have the haplotype group found

in 40% of the sequences of the 2013 cohort (NS, Fisher's exact test p=0.47). The high LD could suggest the spread may have been recent evolutionarily. However, we do see a consistent decrease in Tajima's D between the 1997 and 2013 cohorts of *D. persimilis* across all loci (mitochondrial loci analyzed together).

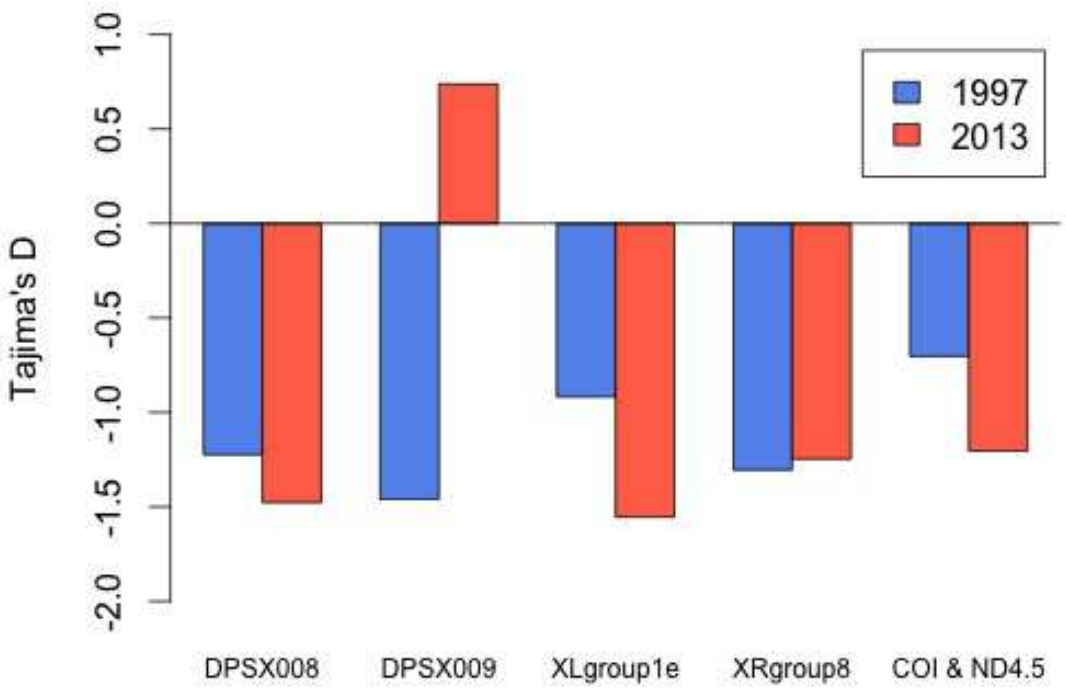
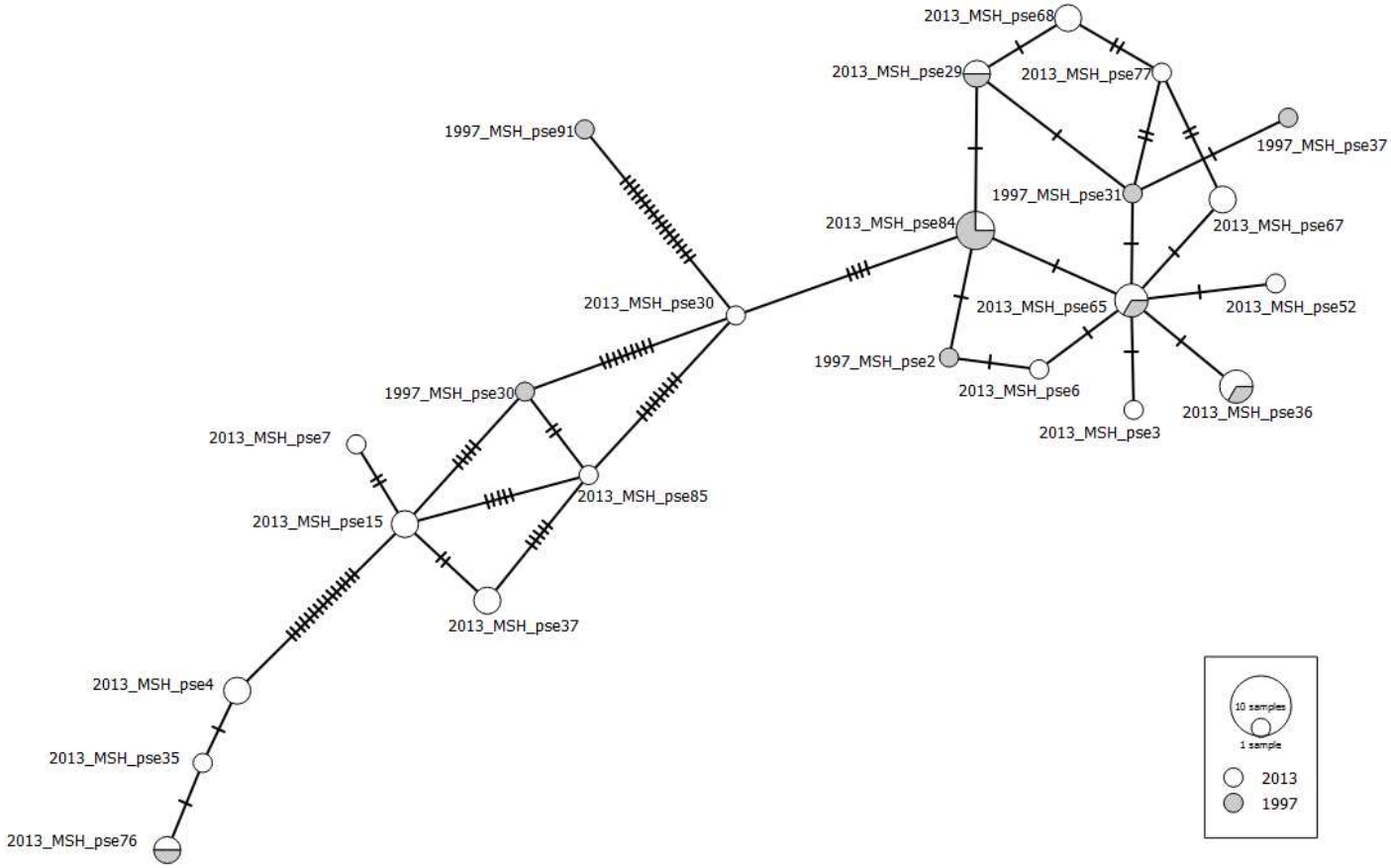


Figure 1: Change in Tajima's D in *D. pseudoobscura* over the course of sixteen years. The DPSX009 locus has undergone a noticeable change from a negative to positive Tajima's D value.

Figure 2: Minimum spanning network of the DPSX009 locus in *D. pseudoobscura*. The left side of the diagram is comprised mostly of sequences from the 2013 cohort, whereas the right side of the diagram is composed of more of a mixture of sequences from both the 1997 and 2013 cohorts.



4. Discussion

Several studies have documented large phenotypic adaptive changes occurring in under 100 generations. For instance, Reznick et al. (1997) documented significant adaptive changes in various guppy life history traits in 4-11 years (less than 20 generations) when moved from a high- to low-predation environment. Similarly, adaptive but unpredictable morphological changes were described in two populations of Darwin's finches across 30 years of study (Grant and Grant 2002). These examples highlight evolutionary changes to phenotypes with an underlying genetic basis, so presumably there were also molecular evolutionary changes associated with these phenotypes. However, we know of no tests for molecular evolutionary changes in species not exhibiting major morphological adaptations—how stable are molecular evolutionary parameters in species not known to be experiencing strong selection on particular phenotypes, and ones with large effective populations?

Here, we sample wild populations of *Drosophila pseudoobscura* and *D. persimilis* separated by approximately one hundred generations and assess how much estimates of genetic diversity changed during this timeframe. These species presumably have large effective population sizes, and allele frequency changes would presumably be more likely to result from selection than drift

(Schaeffer 1995). For all six loci examined, we observe very slight differences in measures of pi, allele frequency (as measured via D_{XY}), and Tajima's D, with only one D_{XY} value being significantly greater than under a model of no change. We initially sequenced X-chromosome and mitochondrial loci to distinguish between potential demographic and selective effects. However, we did not observe consistent changes among loci except with respect to the decrease in Tajima's D in the 2013 *D. persimilis* samples. This finding could suggest that the population has recently accelerated its growth, as has been noted in previous collection studies (Moore 1978). Additionally, we observed a set of related haplotypes in DPSX009 in 2013 *D. pseudoobscura*, potentially indicating the recent invasion and spread of a variant, possibly driven by selection.

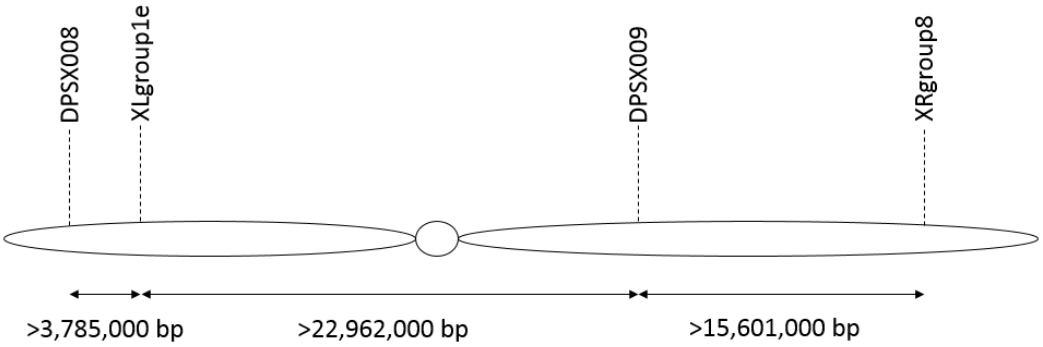
Though we find little change in most of the molecular genetic evolutionary measurements assayed, it remains critical to consider how genetic changes across time shape measures of genetic variation in extant populations. In estimating levels of gene flow or testing for selection, many studies focus only on the genetic differentiation between populations at a single time point. However, when one only considers spatial diversity measures in ecology and evolutionary studies, the crucial role of time in shaping the diversity sampled in the different locales in lost. Long-term studies in which data are collected over multiple time

points are needed to more accurately interpret the process of evolutionary change, and this small-scale study is an attempt to fill this void.

Supplementary Information

Supplementary Table 1: Primers used to amplify all six loci, including chromosomal positions of the X-linked loci. Base positions are based on the November 2004 (FlyBase 1.03/dp3) *D. pseudoobscura* alignment in the UCSC genome browser.

Locus	Scaffold	Start Position	Primer Pairs
DPSX008	XL_group1a	7,482,700 (-)	5'-CAACAAAAGGAATTGCCAAC-3' 5'-TAACTTCGCTTTCAAGTGGC-3'
DPSX009	XR_group6	4,924,218 (+)	5'-AGTGAATTATGATCGCCACAGCAAATCA-3' 5'-CATTTCGCGCTCGATAAATCTCCGC-3'
XLgroup1e	XL_group1e	10,928,711 (+)	5'-AGCGGCAGAACCGTAAAAGTG-3' 5'-CCTACATCGGCCACAGGGTAC-3'
XRgroup8	XR_group8	5,571,531 (+)	5'-GCGCTATGCGTTCAAAATGTCATCA-3' 5'-AGCCTGTCAATGCCAATGTCCT-3'
COI	mitochondrial		5'-CAACATTTATTTTGATTTTTTGG-3' 5'-TCCAATGCACTAATCTGCCATATTA-3'
ND4/ND5	mitochondrial		5'-AGCATGGTAAATTATTTTCTGG-3' 5'-TGTCTAAGAGTTGACAAAGCAA-3'



Supplementary Figure 1: Approximate distances between the four X-linked loci in *Drosophila pseudoobscura* based on the assembled scaffolds of the genome.

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