The effect of common inversion polymorphisms *In(2L)t and In(3R)Mo* on patterns of transcriptional variation in *Drosophila melanogaster*

Erik Lavington[1], Andrew D. Kern[1,2]

1. Department of Genetics, Rutgers University, Piscataway, New Jersey

2. Human Genetics Institute of New Jersey, Rutgers University, Piscataway, New Jersey

Abstract

Chromosomal inversions are an ubiquitous feature of genetic variation. Theoretical models describe several mechanisms by which inversions can drive adaptation and be maintained as polymorphisms. While inversions have been shown previously to be under selection, or contain genetic variation under selection, the specific phenotypic consequences of inversions leading to their maintenance remain unclear. Here we use genomic sequence and expression data from the Drosophila Genetic Reference Panel to explore the effects of two cosmopolitan inversions, *In(2L)t* and *In(3R)Mo*, on patterns of transcriptional variation. We demonstrate that each inversion has a significant effect on transcript abundance for hundreds of genes across the genome. Inversion affected loci (IAL) appear both within inversions as well as on unlinked chromosomes. Importantly, IAL do not appear to be influenced by the previously reported genome-wide expression correlation structure. We found that five genes involved with sterol uptake, four of which are Niemann-Pick Type 2 orthologs, are upregulated in flies with *In(3R)Mo* but do not have SNPs in LD with the inversion. We speculate that this upregulation is driven by genetic variation in *mod(mdg4)* that is in LD with *In(3R)Mo*. We find that there is little evidence for regional or position effect of inversions on gene expression at the chromosomal level but do find evidence for the distal breakpoint of *In(3R)Mo* interrupting one gene and possibly disassociating the two flanking genes from regulatory elements.

Introduction

Chromosomal inversions, in which a portion of linear DNA sequence is flipped in its orientation, are a common member of the menagerie of DNA polymorphisms, and have been found in diverse organismal populations such as humans, plants, and fruit flies(KRIMBAS and POWELL 1992; KIDD *et al.* 2010; LOWRY and WILLIS 2010). In many cases, large chromosomal inversions have profound impacts on phenotype and disease(FEUK 2010). For instance recurrent inversions are responsible for an estimated 43% of hemophilia A cases(LAKICH *et al.* 1993). Inversions can also have beneficial effects. A 900kb inversion on human chromosome 17 (q21.31) has been shown to be associated with higher female fecundity in the Icelandic population (STEFANSSON *et al.* 2005). In populations of the malaria vector *An. gambiae* a large chromosomal inversion on chromosome 2L (2La) is associated with desiccation resistance and thus segregates at high frequencies in arid environments (FOUET *et al.* 2012). These examples are

the very tip of the iceberg—inversion polymorphisms have been implicated in numerous phenotypic differences among a host of organisms, however little is known about the mechanisms by which inversions confer their phenotypic effects.

Perhaps the single best studied inversions are those from *Drosophila*, in part made famous by the pioneering work of Dobzhansky (DOBZHANSKY and STURTEVANT 1938). Dobzhansky focused much attention on spatial and temporal variation in frequency of large inversions of *D. pseudoobscura* and showed in broad strokes that clear fitness differences were responsible for the regular patterns of frequency change observed. These findings in turn spurred a large body of population genetics theory to explain the establishment and selective persistence of inversions in natural populations (LEVENE and DOBZHANSKY 1958; FRASER *et al.* 1966; ANDERSON *et al.* 1967; TOBARI and KOJIMA 1967). As postulated by Sturtevant (1921), crossover suppression induced in inversion heterozygotes can mean that a single adaptive allele within an inversion may suffice for the selective invasion of that rearrangement (Haldane 1937). Such lowered levels of recombination and attendant increases in linkage disequilibrium (LD) could thus present the opportunity for subsequent coadaptation of multiple genes near inversion breakpoints (STURTEVANT and MATHER 1938; DOBZHANSKY 1947). Conversely locally adapted alleles that predate the rearrangement on the same chromosome might aid the establishment of an inversion simply because of the reduction in recombination rates between such loci (KIRKPATRICK and BARTON 2006). Further, inversions might have direct fitness effects, for instance by deletion or changes in gene expression near the inversion breakpoints (KIRKPATRICK and KERN 2012). At present we have precious little information as to the variants responsible for differential fitness effects associated with inversions.

In *Drosophila melanogaster* paracentric inversions spanning several megabases are common and have been found in populations across the globe (Stalker 1976, 1980; Knibb *et al.* 1981; Sezgin *et al.* 2004; Anderson *et al.* 2005; Umina *et al.* 2005). Much of this segregating inversion polymorphism is associated with latitudinal clines in *D.melanogaster*, an historically tropical species adapting along tropical-to-temperate climatic gradients in Australia and North America (KNIBB *et al.* 1981; WEEKS *et al.* 2002; DE JONG and BOCHDANOVITS 2003; SEZGIN *et al.* 2004; REINHARDT *et al.* 2014; SCHRIDER *et al.* 2016). Clinally varying phenotypes that are associated with inversions include heat resistance, cold tolerance, and body size (Weeks *et al.* 2002; Anderson *et al.* 2003; de Jong and Bochdanovits

2003). Clinal variation of inversion frequency in *D.melanogaster* has been shown via population genetic approaches to be due to selection independent of demography (Reinhardt *et al.* 2014; Kapun *et al.* 2016), though migration has been suggested to generate these patterns along with local adaptation (Bergland *et al.* 2016). Indeed, inversions have been observed to have a major effect on several phenotypes that vary between temperate and tropical populations across several *Drosophila* species and these clines have been stable since their discovery roughly 80 years ago (Hoffmann *et al.* 2004; Cogni *et al.* 2017). Unfortunately, while the associations are known, the molecular mechanisms at work determining differential phenotypes as a result of inversion status are still unknown.

Recent population genomic projects in *D.melanogaster*, such as the Drosophila Genetic Reference Panel (DGRP) and Drosophila Population Genomics Project (DPGP), have opened the opportunity to study inversions systematically as these resources have captured segregating inversions from North America and Africa (Pool *et al.* 2012; Mackay *et al.* 2012; Langley *et al.* 2012; Houle and Márquez 2015). Corbett-Detig *et al*. (2012) bioinformatically mapped previously unknown breakpoints of several inversions, a task that was tedious for even single inversions prior to whole genome sequencing (Wesley and Eanes 1994; Andolfatto *et al.* 1999; Matzkin *et al.* 2005). For instance Corbett-Detig *et al*. (2012) discovered the breakpoints associated with numerous inversions and demonstrated the expected increase in LD near inversion breakpoints and elevated differentiation between inverted and standard arrangement chromosomes at the nucleotide level. In parallel with the exponential increase in population genomic resources, large-scale phenotypic association studies of these same genotypes have been accumulating(Mackay *et al.* 2012; Vonesch *et al.* 2016; Telonis-Scott *et al.* 2016). These include numerous phenotypes previously associated with inversion polymorphism such as body size (Weeks *et al.* 2002) and desiccation resistance (Hoffmann *et al.* 2005).

A logical place to look for inversion effects that may influence suites of phenotypes would be transcript level variation. Previous findings strongly suggest that inversions could be important drivers of adaptation with gene expression variation as a potential molecular mechanism (Chambers 1991; López-Maury *et al.* 2008; Fraser 2013). Indeed inversions could affect patterns of transcript variation in a number of ways: **1)** genes at or near inversion breakpoints may become disabled or separated from their regulatory apparatus, thus inversions may have direct

effects on transcription, **2)** increased LD in inversions due to crossover suppression may increase linkage with gene expression Quantitative Trait Loci (eQTL), and thus alternative alleles of the inversion may be associated with differential expression of genes within the inversion (i.e. indirect, cis-eQTL associated with the inversion), **3)** eQTL in LD with the inversion might themselves regulate genes outside of the inversion (i.e. indirect, trans-eQTL associated with the inversion), or **4)** the large-scale nature of *Drosophila* inversions may create global changes in the organization of chromatin or nuclear localization of the chromosomes such that genes are differentially regulated between inversion and standard karyotypes. Thus inversions may have a direct effect on global patterns of transcription, and act as trans eQTL themselves. Indeed earlier studies of transcriptional variation in *D.melanogaster* have hinted at the influence of inversions on genome wide patterns of gene expression (AYROLES *et al.* 2009; MASSOURAS *et al.* 2012; HUANG *et al.* 2015). Here we address the effect of two cosmopolitan inversions, *In(2L)t* and *In(3R)Mo*, on patterns of transcription by using whole-genome sequence, gene expression, and inversion call data from the DGRP.

Methods/Materials

Materials: Processed expression data previously reported in (Ayroles et al. 2009) was downloaded from ArrayExpress (KOLESNIKOV *et al.* 2015). We accepted inversion state calls for each of the DGRP lines where cytological and bioinformatic inversion calls for *In(2L)t* and *In(3R)Mo* agree and removed lines from analyses where there was any disagreement (CORBETT-DETIG *et al.* 2012a; HUANG *et al.* 2014; HOULE and MÁRQUEZ 2015). Using the same databases, we removed Individuals from lines likely heterozygous for *In(2L)t* or *In(3R)Mo*. Expression analyses were performed with 34 lines (136 individuals), with two lines homozygous for *In(2L)t* and seven lines homozygous for *In(3R)Mo*. There were 26 lines homozygous for the Standard arrangement at both *In(2L)t and In(3R)Mo*, as one line was homozygous for both inversions. We calculated LD between *In(2L)t* and SNPs using 181 lines including 19 inversion bearing lines. We calculated LD between *In(3R)Mo* and SNPs using 197 lines including 17 inversion bearing lines. To maintain consistency with Affymetrix library files, dm3/BDGP release 5 genomic coordinates and annotations corresponding to BDGP version 5.49 were used in conjunction with the Affymetrix Drosophila 2 Release 35 library file update.

Methods:

Statistical analyses were performed in R (R CORE TEAM 2013) using the functions (`lm`), (`anova`), (`quantile`), (`qvalue`), (`phyper`), and (`ggplot2`).

3' UTR Array Analysis:

Correlation structure: Pairwise gene expression correlation coefficients were calculated by linear regression on all unique pairwise combinations of probe sets, excepting self-comparisons. Correlation coefficients reported here as adjusted r^2 from the R function (`lm`). Gene expression modules used here were reported in Ayroles et al (2009). Null distributions of uniquely occupied clusters for each inversion were generated by permuting the occupied cluster for each gene 100,000 times and calculating the total unique clusters occupied by inversion affected loci (IAL) for each permutation.

Inversion effect on expression: Linear regressions of sex, inversion, and Line effects with expression as the response were performed for each probe set:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + D_{kl} + \varepsilon_{ijkl}$$

for individual expression value, Y_{ijkl}, in response to i^{th} Sex, A_i, j^{th} *In(2L)t* state, B_j, k^{th} *In(3R)Mo* state, C_k, and the l^{th} Line in the j^{th} *In(2L)t* state, D_{kl}, with ε_{ijkl} as the error term in Lines. Model testing was performed using (`add1`) and (`drop1`) in R to add interaction terms or remove main effect terms from the above model, respectively. Interaction terms were added one at a time to the main effects and tested for each probe set. An AIC is reported for each model with a lower absolute value being preferred when comparing two models. The effect of adding an interaction term between Sex and *In(2L)t* or Sex and *In(3R)Mo* varied by probe set, but the above model was the best fit for 10,082 of the probe sets. Models with and without an interaction term between inversions performed the same for all loci thus we chose the less complex model. Similarly, the above model was the best fit for 12,994 probe sets when compared to dropping any of the main effect terms. We calculated *p*-values of the observed *F* values as percentiles of the *F* distributions generated by 10,000 permutations sampling each inversion independently without replacement. Multiple testing correction was performed by calculating *q*-values using the R

package (`qvalue`) with FDR=0.05 (STOREY and TIBSHIRANI 2003) on the permutation-derived *p*-values. Proportion of variance explained by each effect was calculated as η^2. Magnitude and direction of inversion effect was calculated as Cohen's d (COHEN 1988). Cohen's d is a description of the difference between two standardized distributions and the expected proportion of overlap between the distributions can be estimated given a false positive rate. For example, for two distributions, each with a standard deviation of 1, a Cohen's d of 1 represents a difference of 1 standard deviation between the means and a ~62% expected overlap given a false positive rate of 5%.

Functional Annotation Enrichment: Functional annotation profiling was performed using the g:Profiler online portal of g:GOSt using default settings (REIMAND *et al.* 2016) (version r1622_e84_eg31). Ambiguous 3'UTR probe sets were resolved manually if possible, or ignored if they overlapped transcripts for more than one gene.

SNP-Inversion LD: Linkage disequilibrium (LD) was calculated as $r^2=D'/p_S(1-p_S)p_I(1-p_I)$ for each diallelic SNP S and inversion I, with major allele frequencies p_S and p_I, using a custom bash script. For each SNP, significance was calculated as a chi-squared (χ^2) transformation with 1 degree of freedom of r^2 as $\chi^2=Nr^2$, where N is the sample size. Significant LD was defined as a SNP with sample size of at least 60, minor allele frequency of 10% for both the SNP and inversion state, and χ^2 > critical value (p=0.05, d.f.=1) with Bonferroni correction for all SNPs on that chromosome arm (n=967774, χ^2 > 29.65329 and 947970, χ^2 > 29.61321 for chr2L and chr3R, respectively). The sample size and minor allele frequency cutoffs ensured that there were at least six representative lines bearing minor alleles. Genes were considered in significant LD with inversion state if at least one significant SNP was found within the annotated gene region (FlyBase v5.49).

IAL physical clustering: To see if inversion affected loci (IAL) were physically clustered within the genome, we examined physical clustering by measuring the coefficient of variance (CV) of distances between genes by chromosome arm. Location and length of each gene was used from FlyBase v5.49. Distance between neighboring genes was calculated as the distance between ends of gene annotated regions of neighboring genes. Distance from the most distal or proximal genes to the distal or proximal endpoint, respectively, was not included. CV was calculated as the standard deviation divided by the mean of the distribution of intergenic distances for each

chromosomal arm (SOKAL and ROHLF 1995). We defined the intergenic distance between overlapping gene regions as zero. Null distributions of intergenic distances for each chromosome and each inversion were generated by 100,000 random samples, without replacement, of the same number of genes from a chromosome arm as the number of IAL for that chromosome arm and inversion, then calculating the distances between those genes. Confidence intervals were calculated as 2.5%-97.5% quantiles from the corresponding random sample distribution.

Transcription factor-target gene interactions: Transcription factors (TFs) and target genes (TGs) were defined by Drosophila Interaction Database modMine (CONTRINO *et al.* 2012) (v2015_12). Genes in this analysis were those that are present in Affymetrix Drosophila2 genome array annotation (Release 35), DroID TF-TG database v2015_12, and FlyBase v5.49 annotation. Over/under-representation of genes with significant inversion effect on expression as targets of transcription factors with SNPs in LD with inversion state was calculated as the probability of the observation given the hypergeometric distribution.

Custom scripts, data, and analysis results can be found online at https://github.com/kern-lab/lavingtonKern , including file descriptions in the AnalysisFiles.readme document.

Results

To examine what influence, if any, common inversion polymorphisms have on patterns of transcription in the Drosophila genome we combined publically available genome sequences(MACKAY *et al.* 2012), their associated karyotype calls (CORBETT-DETIG *et al.* 2012a; HUANG *et al.* 2014; HOULE and MÁRQUEZ 2015), and previously published microarray based expression data(AYROLES *et al.* 2009). We validated the use of a model with only main effects of Sex, Line, and two cosmopolitan inversions, *In(2L)t* and *In(3R)Mo* using the R functions (add1) and (drop1). This main effects model performed better than any model having any of the main effect terms removed or with the addition of any interaction term (see Methods). After correction for multiple testing (see Methods), we found 229 and 498 total probe sets with significant inversion effects for *In(2L)t* and *In(3R)Mo*, respectively, hereafter referred to as inversion affected loci (IAL) (Figure 1). These IAL occur both within the inversions themselves (40 *In(2L)t*, 111 *In(3R)Mo*), outside the inversions but near the breakpoints (3 *In(2L)t*, 38 *In(3R)Mo* within 1Mb of the breakpoint), as well as scattered throughout the genome (134 *In(2L)t*, 181 *In(3R)Mo*; see Table 1). This is a large number of loci

with transcript abundance variation correlating with inversion state; however, we note that the inversion effect contribution to variance is relatively small for the vast majority of loci (Supplemental data).

One explanation for the large number of IAL found across the genome is that few loci are directly affected by the inversion and the remaining loci are affected indirectly by an expression variation correlation structure, previously described by Ayroles et al (2009). We addressed this correlation structure by the numbers of unique expression modules occupied by, and the distribution of correlation coefficients of, IAL as compared to all genes. If a significant portion of the IAL we observe are due to expression variation correlation, then we would expect that IAL occupy fewer expression modules than the same number of genes drawn at random. We would also expect the mean correlation of IAL between IAL to be higher than the genome wide average. We observe IAL occupy more modules than expected at random for both *In(2L)t* (71 obs; 38-56 95% c.i.) and *In(3R)Mo* (108 obs ; 65-87 95% c.i.). For both inversions, we did not observe higher mean correlation between IAL between IAL and non-IAL or the genome-wide mean (Table 2).

We then examined the inversion effect on gene expression variation for four distinct categories of effect: 1.) *cis*- or 2.) *trans*-inversion effects of SNPs in LD with the inversion, 3.) direct effects of the inversion by interrupting genes, and 4.) regional effects of chromosomal rearrangement.

Cis-Inversion effect of SNPs in LD with the inversion

Our model explicitly tests the effect of chromosomal arrangement on expression variation and here we focus on SNP variation as the main driver of the inversion effect by taking advantage of LD between the inversion state and SNP variants. LD with inversions is highest at the breakpoints and decays in both directions from each breakpoint as expected (Figure 2) (Wesley and Eanes 1994; Andolfatto *et al.* 2001; Langley *et al.* 2012; Corbett-Detig *et al.* 2012b). To determine *cis*-effects of the inversions we tested for over-representation of IAL among loci in LD with inversion state. This ignores the location of the loci and focuses on the correlation of SNP alleles with the inversion state. As expected, few loci in LD with the inversion were located on a different chromosomal arm (3 with *In(2L)t*, and 2 with *In(3R)Mo*)) and IAL in LD are located only on the same chromosomal arm (Table 3). We observed a significant overrepresentation of IAL with SNPs in LD with inversion state (Table 4).

202 ***Trans*-inversion effect of SNPs in LD with the inversion**

203 Our expectation of a *cis*-inversion effect is dependent on SNP variation in LD with the inversion. By the same
204 rationale, a *trans*-inversion effect may be detected as an IAL without SNP variation in LD with the inversion, as we
205 observe with a majority of IAL for each inversion (181 of 192 for *In(2L)t* and 323 of 425 for *In(3R)Mo* (see Table 4).
206 Assuming SNP variation is the basis of expression variation, one trivial explanation of a *trans*-inversion effect is SNP
207 variation in transcription factors in LD with the inversion acting on downstream targets. The TFs in this case need
208 not be IAL as SNPs in protein coding regions of TFs can give rise to expression variation in downstream targets.
209 However, we found no over- or underrepresentation of IAL that are targets of TFs with SNPs in LD with either
210 inversion (Table 5). A possible example of *trans*-inversion effect was found by functional analysis of IAL and
211 discussed below.

212 **Direct effect of inversions by disrupting genes at breakpoints**

213 Nucleotide sequence variation at each breakpoint of an inversion can truncate transcribed gene regions and
214 disassociate transcribed regions from transcription factor binding sites and other regulatory elements. Truncation
215 most likely leads to down-regulation of genes and rearrangement of regulatory elements and can lead to up- or
216 downregulation of genes at either breakpoint. The closest IAL to any of the four breakpoints are near the distal
217 breakpoint of *In(3R)Mo*. *CG1951* and *βGalNAcTB* are within 2.5kb inside and outside of the inversion region,
218 respectively, and *Ssl2* is interrupted by the breakpoint. All three are downregulated in the presence of the
219 inversion. The next closest IAL is 24 kb away. The closest IAL to the proximal breakpoint of *In(3R)Mo* are 32kb
220 away. It is also important to note that loci immediately surrounding the proximal breakpoint are not
221 transcriptionally affected by the rearrangement, so the presumed disassociated regulatory elements from the
222 distal end are not altering expression of loci at the proximal end. The closest IAL to the proximal and distal
223 breakpoints of *In(2L)t* are 34kb and 37kb away, respectively, and thus probably too far to have been affected by
224 direct effects of the breakpoint.

225 **Regional effect of chromosomal rearrangement**

We tested for regional effects of chromosomal rearrangement by looking for over- or underrepresentation of genes in LD with the inversion as IAL. We did observe more IAL than expected in LD with each inversion (Table 4) and a trend of transcriptional downregulation across the *In(2L)t* region (Figure 3 A & D), but note that the effect size is relatively small. Near the breakpoints, patterns of inversion effect direction are generally small and not likely significantly different from zero (Figure 3 B,C,E & F). The pattern of downregulation immediately surrounding the distal breakpoint of *In(3R)Mo* is moderate and appears to be driven by three loci: *CG1951, Ssl2*,and *β4GalNAcTB*. This is likely a direct effect of the inversion on *Ssl2*, as the breakpoint interrupts this gene proximal to *CG1951* (CORBETT-DETIG *et al.* 2012a), and possibly disassociation of regulatory elements from coding sequence with respect to *CG1951* and *β4GalNAcTB*.

We also examined whether IAL tend to cluster together by physical location along chromosomes, which could arise from more localized regional effects. We measured physical clustering as the coefficient of variation (CV) of distances between genes, for the global CV, and between IAL by chromosome arm. For each arm, IAL for both inversions were less clustered than the distribution of all genes, although *In(3R)Mo* IAL are more clustered on chromosome 3R than we would expect for the same number of randomly drawn genes, but are still less clustered than the genomic background generally (Table 6).

Functional analysis

Coadapted alleles segregating with an inversion should also be in LD with the inversion breakpoints. We used gProfiler g:GOSt functional profiling to detect overrepresentation of functional groups in sets of IAL. The sets of IAL that we analyzed were those IAL in LD with the inversion (Table 2) or targets of another gene with variation segregating with the inversion (Table 4). Functional analysis of IAL for each inversion yielded significant groups only when considering all *In(3R)Mo* IAL or only IAL where inversion state explains at least 15% of expression variance (Supplemental data). Sterol transport is significant in both cases (p=0.022 for all, p=0.000116 for ≥15% variance) and catalytic activity term (GO:0003824) is significant when considering all IAL(p=0.000146). We found no significant functional groups when considering any similar grouping of *In(2L)t* IAL.

The sterol transport group found to be enriched among *In(3R)Mo* IAL includes four of the eight Niemann-Pick type 2 orthologs (*Npc2b*, *Npc2c*, *Npc2f*, *Npc2g*) (HUANG *et al.* 2007), along with *Apoltp*. All five genes are upregulated in association with the inverted arrangement, suggesting an increase of sterol uptake in *In(3R)Mo* bearing flies. In the context of the expression samples, most of these genes are preferentially expressed in the adult gut (modENCODE, Contrino *et al.* 2012). While the four NPC2s are on chromosome 3R (Figure 4), *Apoltp* is on chromosome 2L, and none of these genes contain a SNP in the gene region that is in significant LD with *In(3R)Mo*, or each other, and only *Npc2f* is within the inversion region (Figure 5). It is possible that the significant upregulation of the sterol transport group is a downstream effect of *mod(mdg4)*, which is an IAL near the proximal breakpoint (Figure 4) and in LD with *In(3R)Mo* (Figure 5). *mod(mdg4)* is a chromatin protein associated with *Npc2b* as well as other chromatin proteins and TFs associated with all eight *Npc2* orthologs (Supplemental data). We address this exciting finding in further detail in the Discussion.

Discussion

Despite decades of research on polymorphic inversions in *Drosophila melanogaster,* and despite an overwhelming consensus that inversions are maintained due to selection, we have little understanding of the targets of selection in these inversions that leads to their maintenance (cf. Kirkpatrick and Kern 2012). Here we examine the role of transcriptional variation induced by cosmopolitan inversions to explore what effect, if any, inversions might have on gene expression that itself might be selectively favored. Besides gross rearrangement effects, we examined gene disruption at inversion breakpoints, IAL in LD with the inversion, and IAL not in LD with the inversion. We assumed that multiple functionally linked IAL to be potentially coadapted so long as they contained SNPs in LD with the inverted arrangement. We also assumed that *trans*-inversion effects, IAL not in LD with the inversion, could be the result of these loci interacting with TFs in LD with the inversion or epistatic interactions with loci in LD. We note that while sample size of *In(2L)t* bearing individuals in the expression analysis is small (8 of 136), we were still able to detect a relatively large number of significant loci across the genome. We believe that our methods were conservative and limit the number of false positives at the expense of a likely high number of false negatives. We argue that we are accounting for the small sample sizes in our analyses and interpretations. We also

note that we could not address over- or under-dominance in this study as we examined only lines known to be homozygous for either arrangement of *In(2L)t* or *In(3R)Mo*.

Of particular concern was what role, if any, expression correlation had on our findings. To explore this we considered pairwise correlation coefficients and previously described expression modules (AYROLES *et al.* 2009). One could imagine that the true false discovery rate would be much higher than anticipated due to expression correlation of multiple loci from the same expression module. We found that IAL for both inversions occupied more expression modules than we would expect when drawing genes at random. Even when considering just the pairwise expression correlation, we found that IAL for both inversions were slightly less correlated on average than the genome wide mean. These observations suggest that the inversion effects arose independent of the underlying correlation structure.

We found many loci of significant effect, yet most have a modest contribution to expression variation. Importantly, the only evidence of direct structural influence on patterns of expression was at a single breakpoint that appears to be due to the inversion mutation interrupting gene regions but not moving genes to a different region of the chromosome. That is, there was no appreciable pattern of up- or down-regulation of genes along the chromosome with respect to the location of inversion breakpoints. Perhaps unsurprisingly, we did find an overabundance of expression perturbation within the inversion regions themselves. This would suggest the effect is possibly due to genetic, rather than the structural, variation in LD with the inversion. That is, transcription variation associated with the inversion is due to genetic variation at the gene level and not the rearrangement of loci.

Inversion polymorphism can be maintained by reduced recombination between locally coadapted alleles within inversions (DOBZHANSKY and OTHERS 1970), both with (NEI *et al.* 1967; PEPPER 2003) or without epistasis (KIRKPATRICK and BARTON 2006). Clinal inversion variation in *D.melanogaster* within *In(3R)Payne* in Australian populations and *In(3R)Payne, In(2R)NS, and In(2L)t* in American populations has been shown to be due to selection rather than demographic history and indicative of coadaptation (KENNINGTON *et al.* 2006; KOLACZKOWSKI *et al.* 2011; KAPUN *et al.* 2016). We reasoned that one might be able to detect epistatic coadaptation through functional analysis of IAL. Coadaptation of non-interacting loci would likely result in either no significant functional groups or multiple,

unrelated significant groups. We found functional annotation enrichment only when we considered all IALs with respect to *In(3R)Mo*. This would suggest that either one locus or a few coadapted, but non-interacting, loci segregate with these inversions to maintain polymorphic inversions. However, it is still possible that coadapted loci could be overlooked in our analysis; certainly the existing annotation is incomplete. Moreover, our statistical power to detect IAL suffers due to the constraints of the number of inversions captured in the DGRP dataset. Nevertheless our observation that there are multiple IAL within the inversion with argues strongly for the role that inversions play as modifiers of recombination which may hold adaptive haplotypes together.

Our strongest functional finding, that sterol uptake associated with *In(3R)Mo*, appears to be driven by genetic variation in a single locus as a *trans*-inversion effect. Four of the five genes in this cluster are located on chromosome 3R, however none of these genes have a SNP in significant LD with *In(3R)Mo*, or with each other (Supplemental data), and only one is found within the inversion region (*Npc2f)*. This would rule out effective coadaptation of these genes and suggest that the location of these genes on the same chromosome as the inversion is coincidence. Assuming the upstream effector of the sterol transport is a transcription factor, it could contain a SNP that alters either its protein-coding sequence or expression. Either scenario would require that the genetic variant responsible for the upregulation of the four Npc2s in question would have to be in LD with *In(3R)Mo.* Only one TF, *mod(mdg4),* annotated in DroIDb as interacting with any (*Npc2b*) of the five sterol transport IAL contains a SNP in LD with *In(3R)Mo*. Furthermore, *mod(mdg4)* is itself an IAL and also located near the proximal breakpoint of *In(3R)Mo*. We speculate that inversion associated expression variation detected in this functional group is under control of *mod(mdg4)*.

Increased sterol uptake fits nicely with the positive correlation of *In(3R)Mo* frequency with latitude (KAPUN *et al.* 2014). *Npc2* genes control sterol homeostasis via uptake of dietary sterols in *D.melanogaster*(HUANG *et al.* 2007; CARVALHO *et al.* 2010; NIWA *et al.* 2011). Two species of *Drosophila* differentially express *Npc2's* in response to cold acclimation, though the patterns differ between the two (PARKER *et al.* 2015). Increased dietary cholesterol increases cold tolerance in *Drosophila melanogaster* (SHREVE *et al.* 2007), however, *D.melanogaster* takes up phytosterols more efficiently than cholesterol (COOKE and SANG 1970). Furthermore, *D.melanogaster* is a sterol auxotroph (CARVALHO *et al.* 2010; NIWA *et al.* 2011) that utilizes dietary sterols preferentially to biosynthesizing

different sterols from dietary sterols (CARVALHO *et al.* 2012). This would suggest that *In(3R)Mo* carrying *D.melanogaster* could be cold-acclimated due to increased uptake of dietary sterols, rather than the upregulation of cholesterol production.

It is difficult to interpret results for *In(2L)t* without a clear functional annotation group associated with the inverted state. Assuming *In(2L)t* is under selection (KAPUN *et al.* 2016), the simplest interpretation is that *In(2L)t* polymorphism is maintained by only a small number of loci in LD with the inversion. It is possible that one or more loci in LD with *In(2L)t* contain protein coding variation under selection and no appreciable transcript abundance variation with respect to chromosomal arrangement. We note that there are only 14 IAL in LD with *In(2L)t* (Supplemental data). While a lack of a significant functional group may be dissatisfying, this does provide a manageable candidate list for validation of single targets.

Conclusion

We found that two different cosmopolitan inversions in *D.melanogaster* have some effect on the expression of hundreds of genes across the genome. While we caution that our sample sizes, particularly for *In(2L)t*, are very small, the permutation approach that we have taken is conservative. The genetic variation responsible for the observed transcriptional variation is only in small part due to the inversion event itself, with the majority of the variation being the result of either allelic variation in LD with the inversions, trans-effects of regulators that also are in LD with the inversions, or as of yet uncharacterized, indirect effects of the inversions. Our results mirror those of a recent report on transcriptional variation caused by inversions in *Drosophila pseudoobscura* (FULLER *et al.* 2016). Fuller et al. demonstrated quite convincingly that the well-studied polymorphic inversions of *D. pseudoobscura* are modulating levels of transcription at multiple life history stages and even found hints of trans-effects. However, due to experimental design constraints they could not examine inversion effects on unlinked chromosomes. Our findings extend this pattern to *Drosophila melanogaster* and show that inversions are affecting loci genome-wide. While we have begun to parse the possible causes of IAL, our focus on available data limits the statistical power of our study. Thus it will be important to conduct carefully designed experiments on inversion

350 polymorphism in *D.melanogaster* to elucidate the true extent of influence of inversions on genome-wide patterns

351 of transcription.

352 **Acknowledgements**

353 We thank Dan Schrider and Steve Schaeffer and two anonymous reviewers for feedback on the manuscript. A.D.K.

354 and E.L. were supported in part by NIH award no. R01GM078204.

355

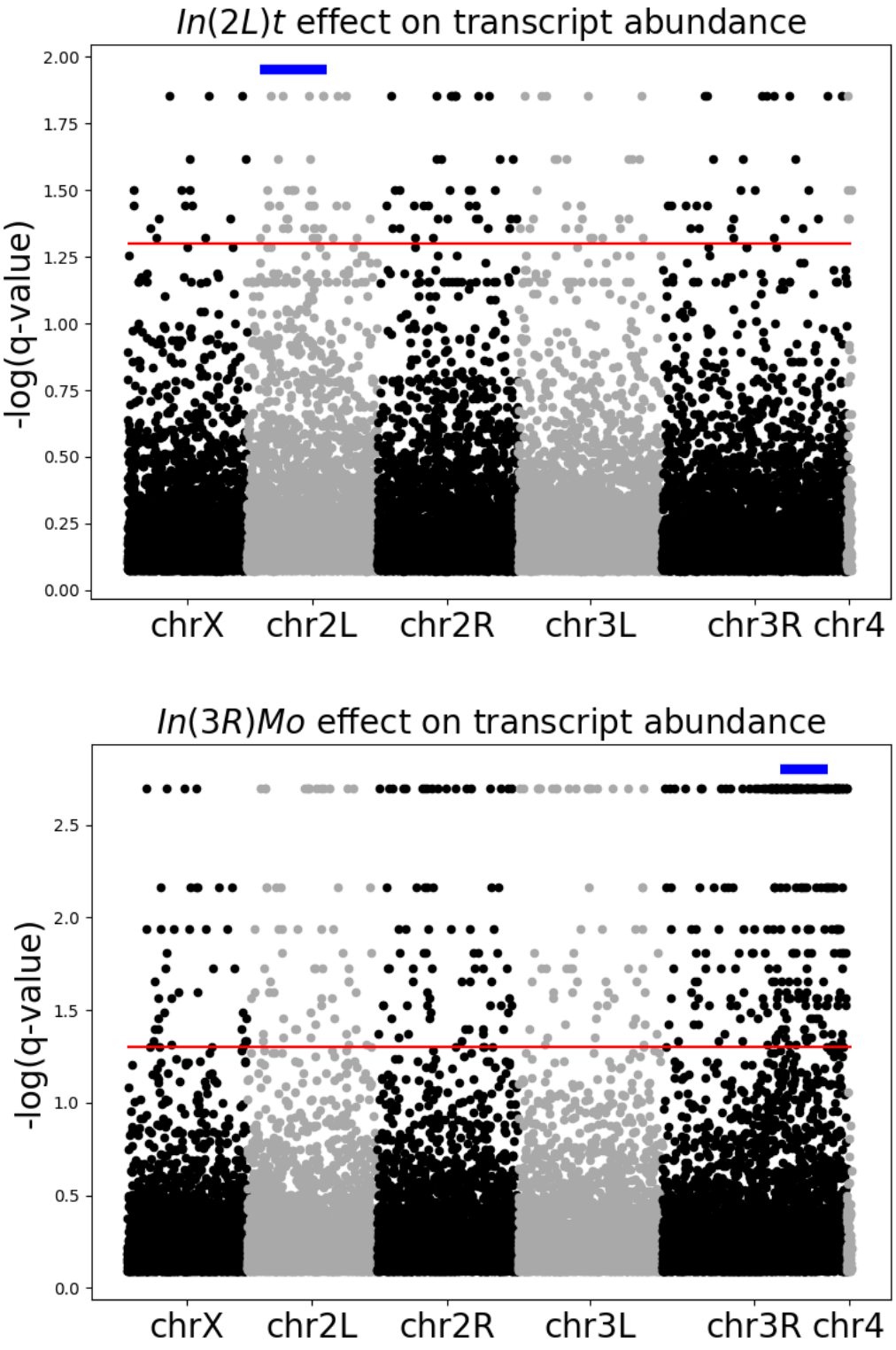


Figure 1. Manhattan plot of log transformed, Bonferroni corrected p-values for an *In(2L)t* (top) and *In(3R)Mo* (bottom) inversion effect on transcription for each probe set across the genome. The blue bar above the points indicates the genomic location of the inversion. The red horizontal line represents the genome-wide significance threshold of p=5x10-8

359

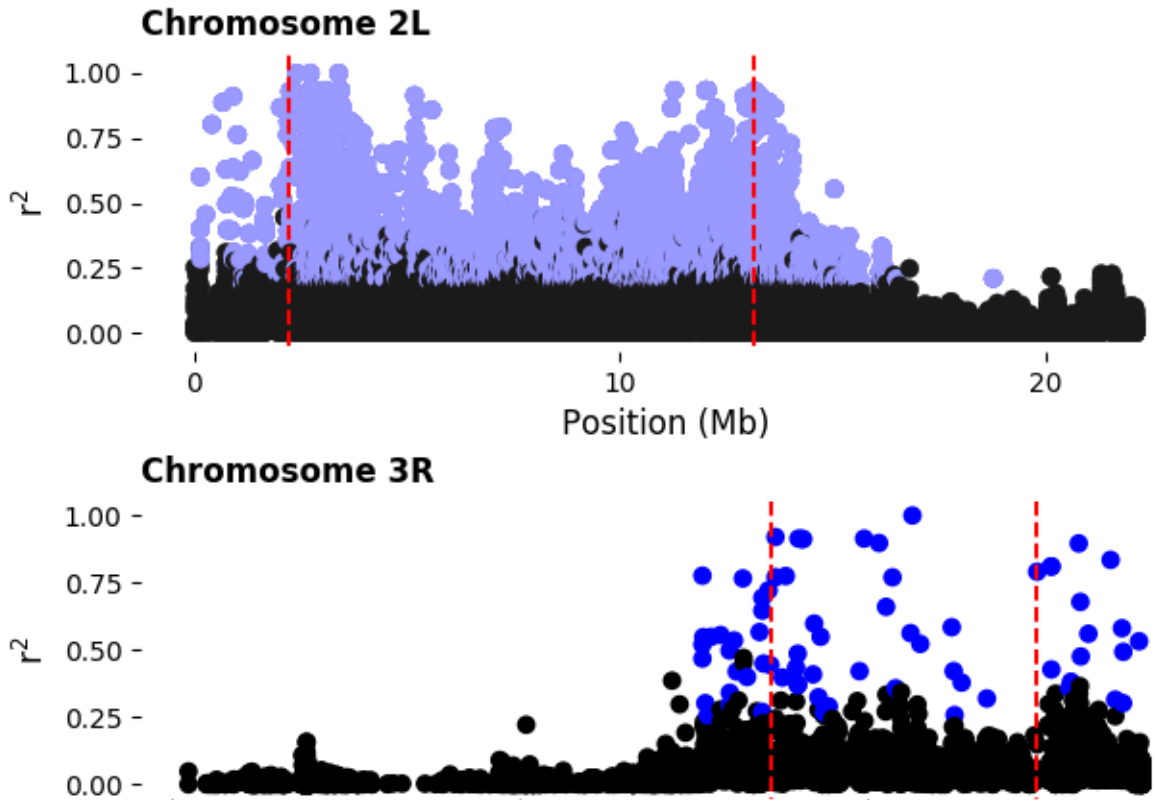


Figure 2. Linkage disequilibrium between SNPs and inversion state. Disequilibrium is measured as r^2.
360 Inversion breakpoints are depicted as red vertical lines. SNPs with at least 10% minor allele frequency, for
361 Inversion and SNP, and are in significant LD with the inversion are shown in light blue ($p<0.05$, Bonferroni correction for all SNPs detected by chromosome arm).

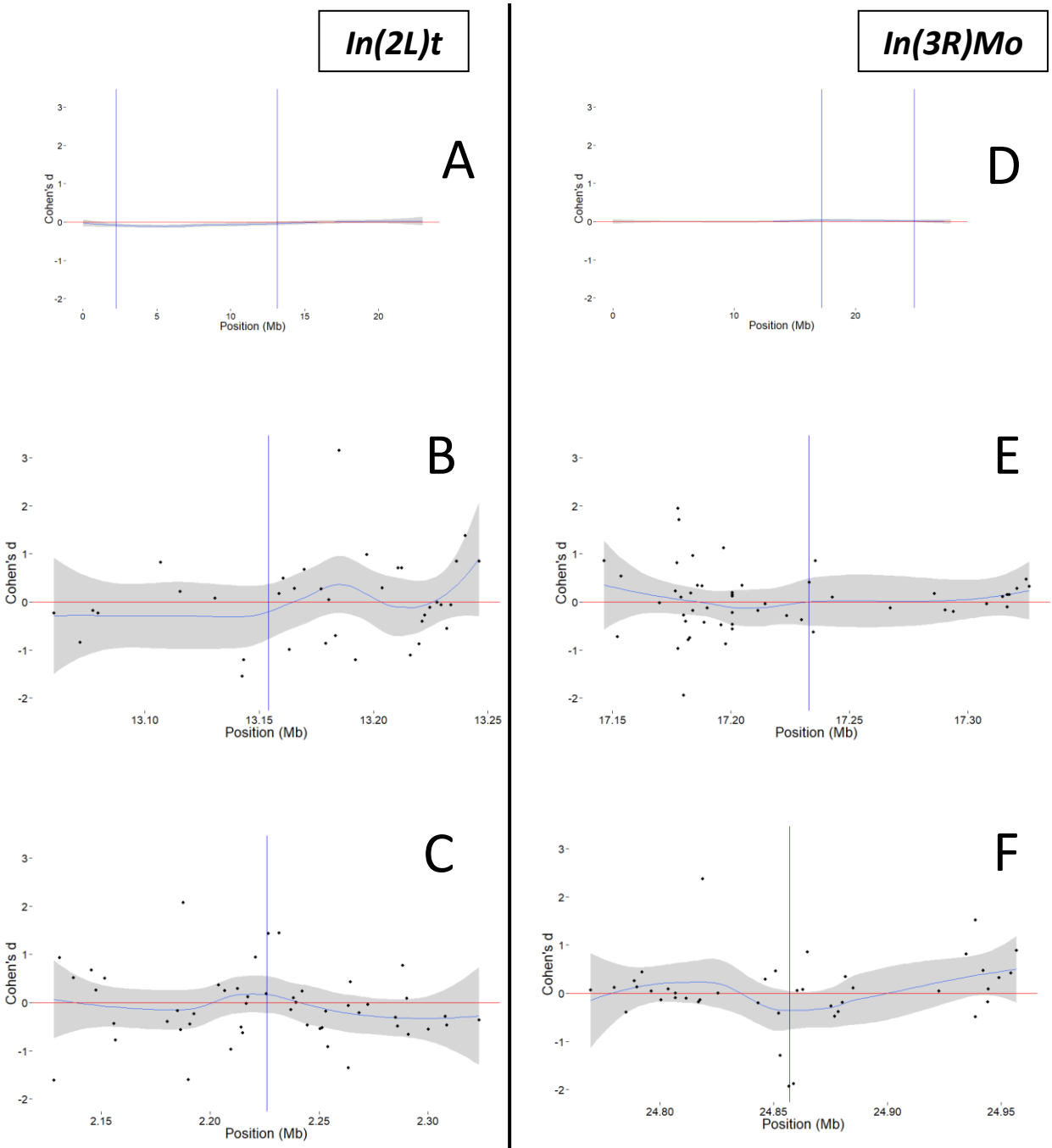
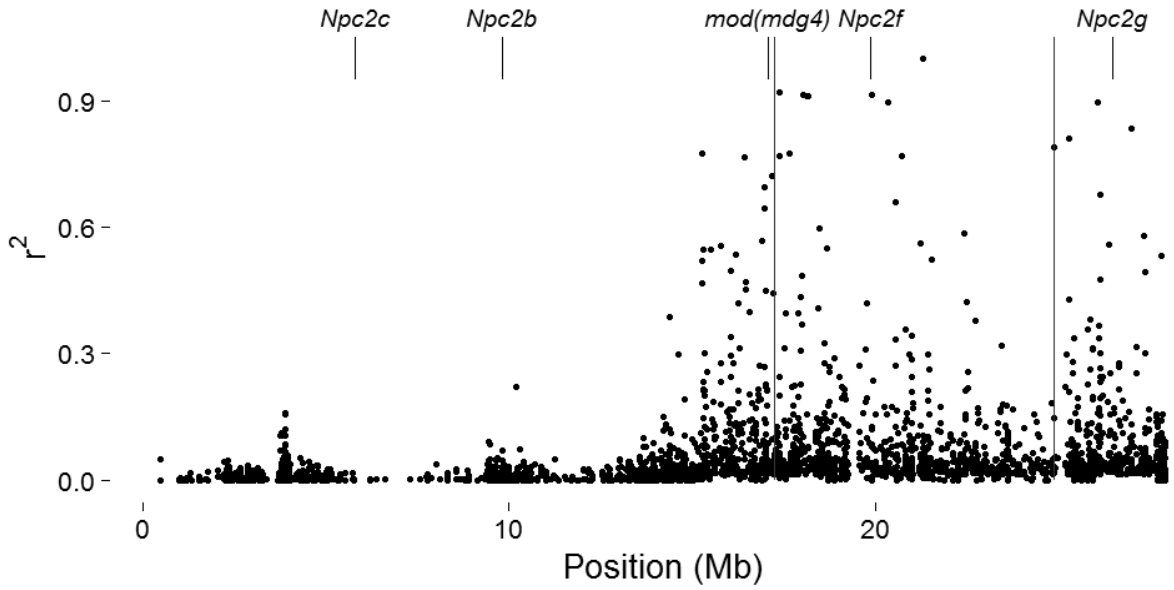


Figure 3. Cohen's d of inversion effect for probe sets by location. Cohen's d for *In(2L)t* effect across chromosome 2L (A) and for *In(3R)Mo* effect across chromosome 3R (D). Loess curves (polynomial line) and 95% confidence intervals (shaded area) for 200kb surrounding each breakpoint: *In(2L)t* (B) proximal and (C) distal, *In(3R)Mo* proximal (E) and distal (F). Inversion breakpoints are depicted as vertical lines. Loess curves and confidence intervals generated by `(geom_smooth)` function in R package (`ggplot2`). Positive values of Cohen's d represent increased transcript levels associated with the inverted chromosome state and negative values represent decreased transcript levels.



389

390 Figure 4. Location of *Npc2s* and *mod(mdg4)* on chromosome 3R. Long vertical lines represent breakpoints of
391 *In(3R)Mo*. Only *mod(mdg4)* contains a SNP in LD with *In(3R)Mo*.

392

393

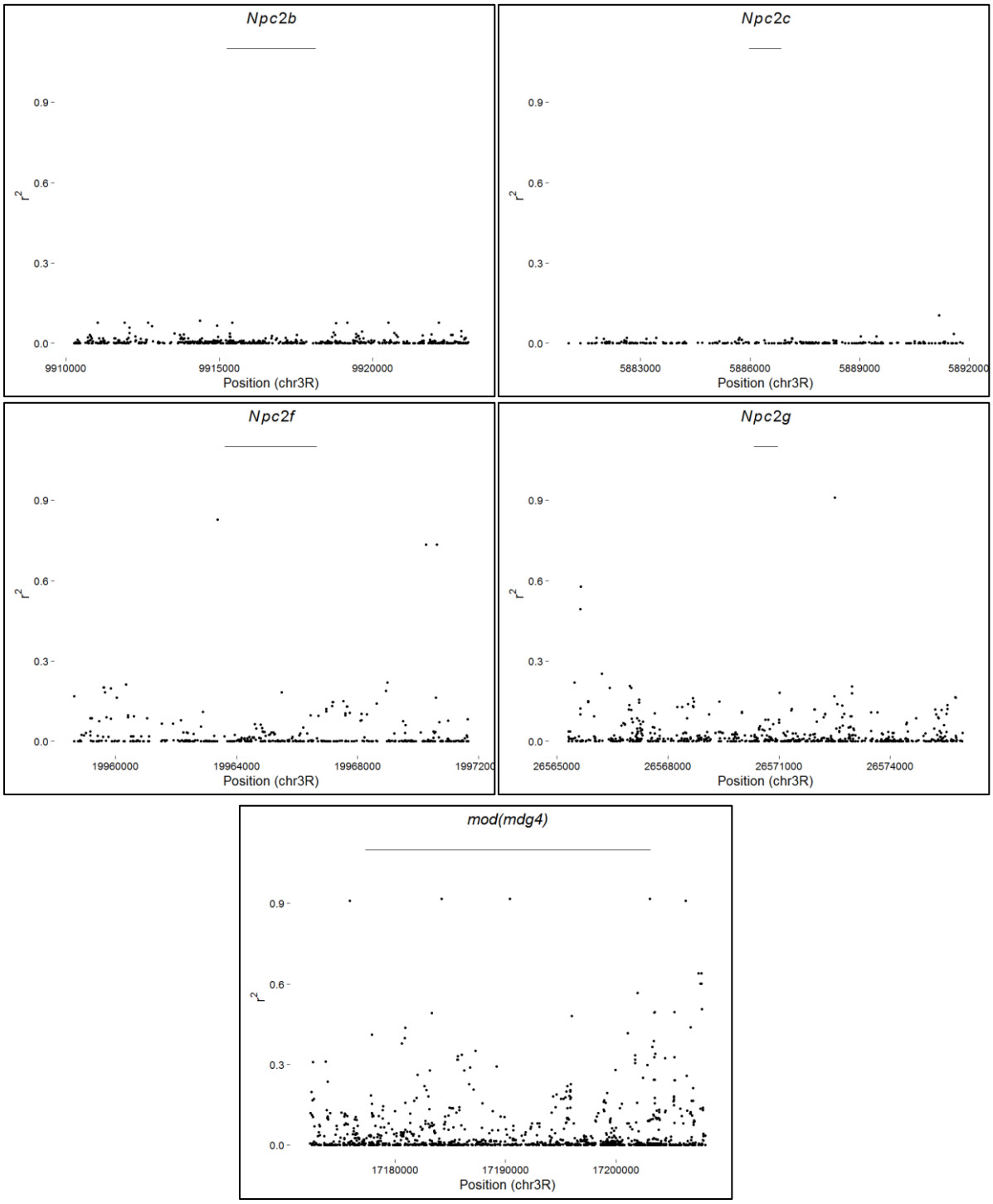


Figure 5. Correlation of SNPs with *In(3R)Mo* by gene. All SNPs within gene regions and within 5kb up- and down-stream of each gene: *Npc2b, Npc2c, Npc2f, Npc2g,* and *mod(mdg4)*. Thin horizontal bars above each plot represent the gene region for that gene.

	Within Inversion	Outside Inversion, on same Chr	< 1Mb outside Inversion	Chr 2L	Chr 2R	Chr 3L	Chr 3R	Chr X	Chr 4
In(2L)t	40	18	3	61	42	36	35	17	4
In(3R)Mo	111	133	38	44	58	49	244	30	0

Table 1. Location of IAL. Counts of IAL between breakpoints, on the same chromosome as the inversion but outside of the breakpoints, and those on the same chromosome as the inversion but outside, and within 1Mb, of the breakpoints, as well as total IAL on each chromosome arm for each inversion.

		Mean r^2
In(2L)t	**IAL x IAL**	0.0965
	IAL x other	0.0962
In(3R)Mo	**IAL x IAL**	0.1025
	IAL x other	0.1042
	Genome-wide	0.1181

405 Table 2.Pairwise correlation of gene expression. Mean r^2 was calculated from all pairwise correlation for the set
406 description. IAL x IAL is the set of all pairwise correlation coefficients of expression between IAL for that inversion.
407 IAL x other is the set of correlation coefficients of expression between IAL and all other (non-IAL).
408

		chr2L	chr2R	chr3L	chr3R	chrX
In(2L)t	**All loci**	370	1	0	1	1
	IAL	11	0	0	0	0
In(3R)Mo	**All loci**	0	2	0	663	0
	IAL	0	0	0	110	0

409

410 Table 3. Location of loci with SNPs in LD with inversions. Loci are identified as BDGP v5.49 and Affymetrix library
411 v.35 annotated gene regions containing SNPs in significant LD with the inversion (see Methods).

412

Inversion	Total Genes	Total IAL	IAL in LD with Inversion	Genes in LD with Inversion	E[X]	p(E[X]>x)
In(2L)t	11969	192	11	372	6	*0.01683*
In(3R)Mo		425	102	744	26	6.08×10^{-35}

Table 4. IAL in LD with inversion. Expected counts (E[X]) and p-values are calculated by hypergeometric distribution with uncorrected p-value cutoff of p=0.025 for the two-tailed test. Genes included in analyses are annotated in both BDGP v5.49 and Affymetrix library version 35.

Inversion	Total Genes	DroID TFs with Sig SNPs	Total DroID TF targets	Total DroID TF targets also IAL	Targets of Sig SNP DroID TFs also IAL	E[X]	p(E[X]>x)
In(2L)t	10623	3	3802	181	53	65	0.96260
In(3R)Mo		8	2373	385	99	86	0.04801

417 Table 5. IAL as targets of Transcription Factors with SNPs in LD with inversion. Expected counts E[X] and p-values
418 calculated by hypergeometric distribution with uncorrected p-value cutoff of p=0.025 for the two-tailed test.
419 Genes included in these analyses are annotated in BDGP v5.49, Affymetrix library v.35, and DroIDb v2015_12.
420

	In(2L)t		Genome	*In(3R)Mo*	
	95% conf. int.	**Obs**	**Obs**	**Obs**	**95% conf. int.**
chr2L	0.882-1.366	1.338	2.612	*1.417*	0.834-1.305
chr2R	0.834-1.341	1.066	3.11	1.176	0.853-1.287
chr3L	0.806-1.352	0.979	2.812	1.129	0.829-1.278
chr3R	0.786-1.341	1.015	2.681	*1.676*	1.07-1.312
chrX	0.724-1.399	1.152	2.814	0.944	0.734-1.295

421 Table 6. Gene location dispersion as described by the Coefficient of Variation (CV). Observed genome CV's
422 calculated from loci in both Flybase ver.5.49 and Affymetrix Drosophila2 r35 annotation databases by chromosome
423 arm. See Methods for details.

Supplemental Tables

Inversion	Total Genes	Total IAL	IAL Inside Inversion	Genes Inside Inversion	E[X]	p(E[X]>x)
In(2L)t	11969	192	41	1279	20	4.3045×10^{-6}
In(3R)Mo		425	112	871	31	7.6×10^{-36}

Supplemental Table 1. IAL inside inversion region. Expected counts E[X] and p-values are calculated by hypergeometric distribution with uncorrected p-value cutoff of p=0.025 for the two-tailed test. Genes included in analyses are annotated in both BDGP v5.49 and Affymetrix library version 35.

Inversion	Total Genes	Total IAL	IAL as F_{st} outliers	Total F_{st} outliers	E[X]	p(E[X]>x)
In(2L)t	11969	192	35	1992	32	0.24089
In(3R)Mo		425	79		71	0.12336

Supplemental Table 2. IAL as F_{st} outliers in clinal populations. Populations from Reinhardt et al (2014). Number of genes reflect the overlap between the unique Affymetrix Drosophila 2 annotated genes and FlyBase r5.49 annotations. Outliers are from the p=0.05 tail of the empirical distribution of 1kb windows (see Reinhardt et al (2014)).

Supplemental Methods

Pairwise LD between SNPs: was calculated with a custom Linux script as r^2 and χ^2 calculated as $\chi^2 = Nr^2$, as described in Methods. We used the DGRP and excluded lines where either *In(2L)t* or *In(3R)Mo* was suspected to be segregating, We included SNPs that fell within the specified gene regions as annotated in FlyBase v5.49. SNPs with no base calls across all tested lines were excluded. Significance level was corrected for 843,051 tests (all unique, LD calculations for 1299 SNPs).

442 **Literature Cited**

443 ANDERSON W. W., DOBZHANSKY T., KASTRITSIS C. D., 1967 Selection and inversion polymorphism in
444 experimental populations of Drosophila pseudoobscura initiated with the chromosomal
445 consstitutions of natural populations. Evolution (N. Y.). **21**: 664–671.

446 ANDERSON A. R., HOFFMANN A. A., MCKECHNIE S. W., UMINA P. A., WEEKS A. R., 2005 The latitudinal cline in
447 the In(3R)Payne inversion polymorphism has shifted in the last 20 years in Australian Drosophila
448 melanogaster populations. Mol. Ecol. **14**: 851–8.

449 ANDOLFATTO P., DEPAULIS F., NAVARRO A., 2001 Inversion polymorphisms and nucleotide variability in
450 Drosophila. Genet. Res. (Camb). **77**: 1–8.

451 ANDOLFATTO P., WALL J. D., KREITMAN M., 1999 Unusual Haplotype Structure at the Proximal Breakpoint
452 of In(2L)t in a Natural Population of Drosophila melanogaster. Genetics **153**: 1297–1311.

453 AULARD S., DAVID J. R., LEMEUNIER F., 2002 Chromosomal inversion polymorphism in Afrotropical
454 populations of Drosophila melanogaster. Genet. Res. (Camb). **79**: 49–63.

455 AYROLES J. F., CARBONE M. A., STONE E. A., JORDAN K. W., LYMAN R. F., MAGWIRE M. M., ROLLMANN S. M.,
456 DUNCAN L. H., LAWRENCE F., ANHOLT R. R. H., MACKAY T. F. C., 2009 Systems genetics of complex
457 traits in Drosophila melanogaster. Nat. Genet. **41**: 299–307.

458 BERGLAND A. O., TOBLER R., GONZÁLEZ J., SCHMIDT P., PETROV D., 2016 Secondary contact and local
459 adaptation contribute to genome-wide patterns of clinal variation in Drosophila melanogaster.
460 Mol. Ecol. **25**: 1157–74.

461 CARVALHO M., SAMPAIO J. L., PALM W., BRANKATSCHK M., EATON S., SHEVCHENKO A., , 2012 Effects of diet and
462 development on the Drosophila lipidome. Mol. Syst. Biol. **8**: 600.

463 CARVALHO M., SCHWUDKE D., SAMPAIO J. L., PALM W., RIEZMAN I., DEY G., GUPTA G. D., MAYOR S., RIEZMAN H.,
464 SHEVCHENKO A., KURZCHALIA T. V., EATON S., 2010 Survival strategies of a sterol auxotroph.
465 Development **137**.

466 CHAMBERS G. K., 1991 Gene expression, adaptation and evolution in higher organisms. Evidence from
467 studies of Drosophila alcohol dehydrogenases. Comp. Biochem. Physiol. Part B Comp. Biochem. **99**:
468 723–730.

469 COGNI R., KUCZYNSKI K., KOURY S., LAVINGTON E., BEHRMAN E. L., O'BRIEN K. R., SCHMIDT P. S., EANES W. F.,
470 2017 On the Long-term Stability of Clines in Some Metabolic Genes in Drosophila melanogaster.
471 Sci. Rep. **7**: 42766.

472 COHEN J., 1988 *Statistical Power Analysis for the Behavioral Sciences*.

473 CONTRINO S., SMITH R. N., BUTANO D., CARR A., HU F., LYNE R., RUTHERFORD K., KALDERIMIS A., SULLIVAN J.,
474 CARBON S., KEPHART E. T., LLOYD P., STINSON E. O., WASHINGTON N. L., PERRY M. D., RUZANOV P., ZHA Z.,
475 LEWIS S. E., STEIN L. D., MICKLEM G., 2012 modMine: flexible access to modENCODE data. Nucleic
476 Acids Res. **40**: D1082-8.

477 COOKE J., SANG J. H., 1970 Utilization of sterols by larvae of Drosophila melanogaster. J. Insect Physiol.
478 **16**: 801–812.

479 CORBETT-DETIG R. B., CARDENO C., LANGLEY C. H., 2012a Sequence-based detection and breakpoint
480 assembly of polymorphic inversions. Genetics **192**: 131–7.

481 CORBETT-DETIG R. B., HARTL D. L., , 2012b Population Genomics of Inversion Polymorphisms in Drosophila
482 melanogaster (HS Malik, Ed.). PLoS Genet. **8**: e1003056.

483 DOBZHANSKY T., 1947 Genetics of Natural Populations. Xiv. a Response of Certain Gene Arrangements in
484 the Third Chromosome of DROSOPHILA PSEUDOOBSCURA to Natural Selection. Genetics **32**: 142–
485 60.

486 DOBZHANSKY T., OTHERS, 1970 *Genetics of the evolutionary process*. Columbia University Press New York.

487 DOBZHANSKY T., STURTEVANT a. H., 1938 Inversions in the chromosomes of Drosophila pseudoobscura.
488 Genetics **23**: 28–64.

489 FEUK L., 2010 Inversion variants in the human genome: role in disease and genome architecture.
490 Genome Med. **2**: 11.

491 FOUET C., GRAY E., BESANSKY N. J., COSTANTINI C., REIG O., 2012 Adaptation to Aridity in the Malaria
492 Mosquito Anopheles gambiae: Chromosomal Inversion Polymorphism and Body Size Influence
493 Resistance to Desiccation (J Pinto, Ed.). PLoS One **7**: e34841.

494 FRASER H. B., 2013 Gene expression drives local adaptation in humans. Genome Res. **23**: 1089–96.

495 FRASER A., BURNELL D., MILLER D., 1966 Simulation of genetic systems X. Inversion polymorphism. J.
496 Theor. Biol. **13**: 1–14.

497 FULLER Z. L., HAYNES G. D., RICHARDS S., SCHAEFFER S. W., 2016 Genomics of Natural Populations: How
498 Differentially Expressed Genes Shape the Evolution of Chromosomal Inversions in Drosophila
499 pseudoobscura. Genetics **204**.

500 HALDANE J. B. S., 1957 The conditions for coadaptation in polymorphism for inversions. J. Genet. **55**:
501 218–225.

502 HOFFMANN A. A., SGRÒ C. M., WEEKS A. R., 2004 Chromosomal inversion polymorphisms and adaptation.
503 Trends Ecol. Evol. **19**: 482–8.

504 HOFFMANN A. A., SHIRRIFFS J., SCOTT M., 2005 Relative importance of plastic vs genetic factors in adaptive
505 differentiation: Geographical variation for stress resistance in Drosophila melanogaster from
506 eastern Australia. Funct. Ecol. **19**: 222–227.

507 HOULE D., MÁRQUEZ E. J., 2015 Linkage Disequilibrium and Inversion-Typing of the Drosophila
508 melanogaster Genome Reference Panel. G3 (Bethesda). **5**: 1695–701.

509 HUANG W., CARBONE M. A., MAGWIRE M. M., PEIFFER J. A., LYMAN R. F., STONE E. A., ANHOLT R. R. H., MACKAY
510 T. F. C., 2015 Genetic basis of transcriptome diversity in Drosophila melanogaster. Proc. Natl.

Acad. Sci. U. S. A. **112**: E6010-6019.

HUANG W., MASSOURAS A., INOUE Y., PEIFFER J., RÀMIA M., TARONE A. M., TURLAPATI L., ZICHNER T., ZHU D., LYMAN R. F., MAGWIRE M. M., BLANKENBURG K., CARBONE M. A., CHANG K., ELLIS L. L., FERNANDEZ S., HAN Y., HIGHNAM G., HJELMEN C. E., JACK J. R., JAVAID M., JAYASEELAN J., KALRA D., LEE S., LEWIS L., MUNIDASA M., ONGERI F., PATEL S., PERALES L., PEREZ A., PU L., ROLLMANN S. M., RUTH R., SAADA N., WARNER C., WILLIAMS A., WU Y.-Q., YAMAMOTO A., ZHANG Y., ZHU Y., ANHOLT R. R. H., KORBEL J. O., MITTELMAN D., MUZNY D. M., GIBBS R. A., BARBADILLA A., JOHNSTON J. S., STONE E. A., RICHARDS S., DEPLANCKE B., MACKAY T. F. C., 2014 Natural variation in genome architecture among 205 Drosophila melanogaster Genetic Reference Panel lines. Genome Res. **24**: 1193–208.

HUANG X., WARREN J. T., BUCHANAN J., GILBERT L. I., SCOTT M. P., 2007 Drosophila Niemann-Pick type C-2 genes control sterol homeostasis and steroid biosynthesis: a model of human neurodegenerative disease. Development **134**: 3733–42.

JONG G. DE, BOCHDANOVITS Z., 2003 Latitudinal clines inDrosophila melanogaster: Body size, allozyme frequencies, inversion frequencies, and the insulin-signalling pathway. J. Genet. **82**: 207–223.

KAPUN M., FABIAN D. K., GOUDET J., FLATT T., 2016 Genomic Evidence for Adaptive Inversion Clines in Drosophila melanogaster. Mol. Biol. Evol.: msw016-.

KAPUN M., SCHALKWYK H. VAN, MCALLISTER B., FLATT T., SCHLÖTTERER C., 2014 Inference of chromosomal inversion dynamics from Pool-Seq data in natural and laboratory populations of Drosophila melanogaster. Mol. Ecol. **23**: 1813–1827.

KENNINGTON W. J., PARTRIDGE L., HOFFMANN A. A., 2006 Patterns of diversity and linkage disequilibrium within the cosmopolitan inversion In(3R)Payne in Drosophila melanogaster are indicative of coadaptation. Genetics **172**: 1655–63.

KIDD J. M., GRAVES T., NEWMAN T. L., FULTON R., HAYDEN H. S., MALIG M., KALLICKI J., KAUL R., WILSON R. K., EICHLER E. E., 2010 A Human Genome Structural Variation Sequencing Resource Reveals Insights into Mutational Mechanisms. Cell **143**: 837–847.

KIRKPATRICK M., BARTON N., 2006 Chromosome inversions, local adaptation and speciation. Genetics **173**: 419–34.

KIRKPATRICK M., KERN A., 2012 Where's the money? Inversions, genes, and the hunt for genomic targets of selection. Genetics **190**: 1153–5.

KNIBB W. R., OAKESHOTT J. G., GIBSON J. B., 1981 Chromosome Inversion Polymorphism in Drosophila melanogaster. I. Latitudinal Clines and Associations between Inversions in Australian Populations. Genetics **98**: 833–847.

KOLACZKOWSKI B., KERN A. D., HOLLOWAY A. K., BEGUN D. J., 2011 Genomic differentiation between temperate and tropical Australian populations of Drosophila melanogaster. Genetics **187**: 245–60.

KOLESNIKOV N., HASTINGS E., KEAYS M., MELNICHUK O., TANG Y. A., WILLIAMS E., DYLAG M., KURBATOVA N., BRANDIZI M., BURDETT T., MEGY K., PILICHEVA E., RUSTICI G., TIKHONOV A., PARKINSON H., PETRYSZAK R., SARKANS U., BRAZMA A., 2015 ArrayExpress update--simplifying data submissions. Nucleic Acids Res.

43: D1113-6.

KRIMBAS C. B., POWELL J. R., 1992 *Drosophila Inversion Polymorphism*. CRC Press.

LAKICH D., KAZAZIAN H. H., ANTONARAKIS S. E., GITSCHIER J., 1993 Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. Nat. Genet. **5**: 236–241.

LANGLEY C. H., STEVENS K., CARDENO C., LEE Y. C. G., SCHRIDER D. R., POOL J. E., LANGLEY S. A., SUAREZ C., CORBETT-DETIG R. B., KOLACZKOWSKI B., FANG S., NISTA P. M., HOLLOWAY A. K., KERN A. D., DEWEY C. N., SONG Y. S., HAHN M. W., BEGUN D. J., 2012 Genomic variation in natural populations of Drosophila melanogaster. Genetics **192**: 533–98.

LEVENE H., DOBZHANSKY T., 1958 New evidence of heterosis in naturally occurring inversion heterozygotes in Drosophila pseudoobscura. Heredity (Edinb). **12**: 37–49.

LÓPEZ-MAURY L., MARGUERAT S., BÄHLER J., 2008 Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. Nat. Rev. Genet. **9**: 583–93.

LOWRY D. B., WILLIS J. H., 2010 A widespread chromosomal inversion polymorphism contributes to a major life-history transition, local adaptation, and reproductive isolation. PLoS Biol. **8**: e1000500.

MACKAY T. F. C., RICHARDS S., STONE E. A., BARBADILLA A., AYROLES J. F., ZHU D., CASILLAS S., HAN Y., MAGWIRE M. M., CRIDLAND J. M., RICHARDSON M. F., ANHOLT R. R. H., BARRÓN M., BESS C., BLANKENBURG K. P., CARBONE M. A., CASTELLANO D., CHABOUB L., DUNCAN L., HARRIS Z., JAVAID M., JAYASEELAN J. C., JHANGIANI S. N., JORDAN K. W., LARA F., LAWRENCE F., LEE S. L., LIBRADO P., LINHEIRO R. S., LYMAN R. F., MACKEY A. J., MUNIDASA M., MUZNY D. M., NAZARETH L., NEWSHAM I., PERALES L., PU L.-L., QU C., RÀMIA M., REID J. G., ROLLMANN S. M., ROZAS J., SAADA N., TURLAPATI L., WORLEY K. C., WU Y.-Q., YAMAMOTO A., ZHU Y., BERGMAN C. M., THORNTON K. R., MITTELMAN D., GIBBS R. A., 2012 The Drosophila melanogaster Genetic Reference Panel. Nature **482**: 173–8.

MASSOURAS A., WASZAK S. M., ALBARCA-AGUILERA M., HENS K., HOLCOMBE W., AYROLES J. F., DERMITZAKIS E. T., STONE E. A., JENSEN J. D., MACKAY T. F. C., DEPLANCKE B., 2012 Genomic variation and its impact on gene expression in Drosophila melanogaster. PLoS Genet. **8**: e1003055.

MATZKIN L. M., MERRITT T. J. S., ZHU C.-T., EANES W. F., 2005 The structure and population genetics of the breakpoints associated with the cosmopolitan chromosomal inversion In(3R)Payne in Drosophila melanogaster. Genetics **170**: 1143–52.

NEI M., KOJIMA K. I., SCHAFFER H. E., 1967 Frequency changes of new inversions in populations under mutation-selection equilibria. Genetics **57**: 741–50.

NIWA R., NIWA Y. S., NIWA R., NIWA Y. S., 2011 The Fruit Fly Drosophila melanogaster as a Model System to Study Cholesterol Metabolism and Homeostasis. Cholesterol **2011**: 1–6.

PARKER D. J., VESALA L., RITCHIE M. G., LAIHO A., HOIKKALA A., KANKARE M., 2015 How consistent are the transcriptome changes associated with cold acclimation in two species of the Drosophila virilis group? Heredity (Edinb). **115**: 13–21.

PEPPER J. W., 2003 The evolution of evolvability in genetic linkage patterns. Biosystems **69**: 115–126.

584 POOL J. E., CORBETT-DETIG R. B., SUGINO R. P., STEVENS K. A., CARDENO C. M., CREPEAU M. W., DUCHEN P.,
585 EMERSON J. J., SAELAO P., BEGUN D. J., LANGLEY C. H., 2012 Population Genomics of sub-saharan
586 Drosophila melanogaster: African diversity and non-African admixture. PLoS Genet. **8**: e1003080.

587 R CORE TEAM, 2013 R: A Language and Environment for Statistical Computing.

588 REIMAND J., ARAK T., ADLER P., KOLBERG L., REISBERG S., PETERSON H., VILO J., 2016 g:Profiler-a web server for
589 functional interpretation of gene lists (2016 update). Nucleic Acids Res.: gkw199-.

590 REINHARDT J. A., KOLACZKOWSKI B., JONES C. D., BEGUN D. J., KERN A. D., , 2014 Parallel geographic variation
591 in Drosophila melanogaster. Genetics **197**: 361–73.

592 SCHRIDER D. R., HAHN M. W., BEGUN D. J., 2016 Parallel Evolution of Copy-Number Variation across
593 Continents in Drosophila melanogaster. Mol. Biol. Evol. **33**: 1308–1316.

594 SEZGIN E., DUVERNELL D. D., MATZKIN L. M., DUAN Y., ZHU C.-T., VERRELLI B. C., EANES W. F., 2004 Single-locus
595 latitudinal clines and their relationship to temperate adaptation in metabolic genes and derived
596 alleles in Drosophila melanogaster. Genetics **168**: 923–31.

597 SHREVE S. M., YI S. X., LEE R. E., 2007 Increased dietary cholesterol enhances cold tolerance in Drosophila
598 melanogaster. **28**: 33–37.

599 SOKAL R. R., ROHLF F. J., 1995 *Biometry*.

600 STEFANSSON H., HELGASON A., THORLEIFSSON G., STEINTHORSDOTTIR V., MASSON G., BARNARD J., BAKER A.,
601 JONASDOTTIR A., INGASON A., GUDNADOTTIR V. G., DESNICA N., HICKS A., GYLFASON A., GUDBJARTSSON D. F.,
602 JONSDOTTIR G. M., SAINZ J., AGNARSSON K., BIRGISDOTTIR B., GHOSH S., OLAFSDOTTIR A., CAZIER J.-B.,
603 KRISTJANSSON K., FRIGGE M. L., THORGEIRSSON T. E., GULCHER J. R., KONG A., STEFANSSON K., 2005 A
604 common inversion under selection in Europeans. Nat. Genet. **37**: 129–37.

605 STOREY J. D., TIBSHIRANI R., 2003 Statistical significance for genomewide studies. Proc. Natl. Acad. Sci. U.
606 S. A. **100**: 9440–5.

607 STURTEVANT A. H., MATHER K., 1938 The Interrelations of Inversions, Heterosis and Recombination. Am.
608 Nat. **72**: 447–452.

609 STURTEVART A. H., 1921 A CASE OF REARRANGEMENT OF GENES IN DROSOPHILA. Proc. Natl. Acad. Sci. **7**:
610 235–237.

611 TELONIS-SCOTT M., SGRÒ C. M., HOFFMANN A. A., GRIFFIN P. C., 2016 Cross-Study Comparison Reveals
612 Common Genomic, Network, and Functional Signatures of Desiccation Resistance in *Drosophila*
613 *melanogaster*. Mol. Biol. Evol. **33**: 1053–1067.

614 TLAND J. VAN, PUTTEN W. F. VAN, VILLARROEL H., KAMPING A., DELDEN W. Van, 2000 Latitudinal Variation for
615 Two Enzyme Loci and an Inversion Polymorphism in Drosophila melanogaster from Central and
616 South America. Evolution (N. Y). **54**: 201–209.

617 TOBARI Y. N., KOJIMA K. I., 1967 Selective modes associated with inversion karyotypes in Drosophila
618 ananassae. I. Frequency-dependant selection. Genetics **57**: 179–88.

619 UMINA P. A., WEEKS A. R., KEARNEY M. R., MCKECHNIE S. W., HOFFMANN A. A., 2005 A rapid shift in a classic
620 clinal pattern in Drosophila reflecting climate change. Science **308**: 691–3.

621 VONESCH S. C., LAMPARTER D., MACKAY T. F. C., BERGMANN S., HAFEN E., MARKOW T., JENSEN L., LEE S., WEE C.,
622 HOFFMANN A., 2016 Genome-Wide Analysis Reveals Novel Regulators of Growth in Drosophila
623 melanogaster (GS Barsh, Ed.). PLOS Genet. **12**: e1005616.

624 WEEKS A. R., MCKECHNIE S. W., HOFFMANN A. A., 2002 Dissecting adaptive clinal variation: markers,
625 inversions and size/stress associations in Drosophila melanogaster from a central field population.
626 Ecol. Lett. **5**: 756–763.

627 WESLEY C. S., EANES W. F., 1994 Isolation and analysis of the breakpoint sequences of chromosome
628 inversion In(3L)Payne in Drosophila melanogaster. Proc. Natl. Acad. Sci. **91**: 3132–3136.

629