Evolution of Plasticity in Response to Ethanol between Sister Species with Different Ecological Histories (*Drosophila melanogaster* and *D. simulans*)

Sarah A. Signor*

Molecular and Computational Biology, University of Southern California, Los Angeles, California 90089; Biological Sciences, North Dakota State University, Fargo, North Dakota 58108

Submitted September 2, 2019; Accepted July 7, 2020; Electronically published September 15, 2020

Online enhancements: Excel files.

ABSTRACT: When populations evolve adaptive reaction norms in response to novel environments, it can occur through a process termed genetic accommodation. Under this model, the initial response to the environment is widely variable between genotypes as a result of cryptic genetic variation, which is then refined by selection to a single adaptive response. Here, I empirically test these predictions from genetic accommodation by measuring reaction norms in individual genotypes and across several time points. I compare two species of *Drosophila* that differ in their adaptation to ethanol (*D. melanogaster* and *D. simulans*). Both species are human commensals with a recent cosmopolitan expansion, but only *D. melanogaster* is adapted to ethanol exposure. Using gene expression as a phenotype and an approach that combines information about expression and alternative splicing, I find that *D. simulans* exhibits cryptic genetic variation in the response to ethanol, while *D. melanogaster* has almost no genotype-specific variation in reaction norm. This is evidence for adaptation to ethanol through genetic accommodation, suggesting that the evolution of phenotypic plasticity could be an important contributor to the ability to exploit novel resources.

Keywords: phenotypic plasticity, *Drosophila*, ethanol, genetic accommodation.

Introduction

Genetic accommodation describes the adaptive evolution of phenotype plasticity. Genetic accommodation is thought to proceed through two stages, beginning when a population encounters a novel environment and ending when the population has become adapted to that environment (fig. 1*A*). At the beginning of the process of genetic accommodation, when an environment is novel, different individuals or genotypes will respond differently to the environment (West-Eberhard 2005; Ghalambor et al. 2007; Robinson 2013; Morris et al. 2014; Schlichting and Wund 2014). Because the environment is novel, the plastic response of the individuals would not have been shaped by natural selection, and so the initial set of responses may be adaptive, deleterious, or neutral (Schlichting 2008; Gibson 2009; Hayden et al. 2011; Paaby and Rockman 2014). As the population adapts to the novel environment, selection should result in the loss of variation in the plastic response in favor of a single plastic response that maximizes fitness in that new environment (Baldwin 1896). A requirement of this process is that it is adaptive to maintain a plastic response (Via and Lande 1985; Guntrip and Sibly 1998; Lande 2009; Matzkin 2012; Huang and Agrawal 2016).

Levis and Pfennig (2016) and Jones and Robinson (2018) provide four guidelines for establishing that evolution of plasticity has occurred by genetic accommodation. First, the focal trait must be able to be environmentally induced in a derived lineage and in an ancestral-proxy lineage. Second, cryptic genetic variation is uncovered when the ancestral proxy lineage is exposed to the derived environment. Third, the focal trait must exhibit evidence of evolutionary change in regulation or form in the derived lineage. Last, the focal trait must exhibit evidence of "adaptive refinement" in the derived lineage. In this study I have used ethanol as the environmental variable, *Drosophila simulans* as an ancestor proxy, and *Drosophila melanogaster* as the derived lineage. I then determined whether the responses of these two species to ethanol meet the criteria for genetic accommodation.

The different adaptive histories of *D. simulans* and D. *melanogaster* to ethanol make them ideal for testing the predictions of genetic accommodation. *Drosophila melanogaster* is considerably more ethanol tolerant than *D.*

* Email: sarah.signor@ndsu.edu.
ORCIDs: Signor, http://orcid.org/0000-0003-2401-0644.

Am. Nat. 2020. Vol. 196, pp. 620–633.
DOI: 10.1086/710763

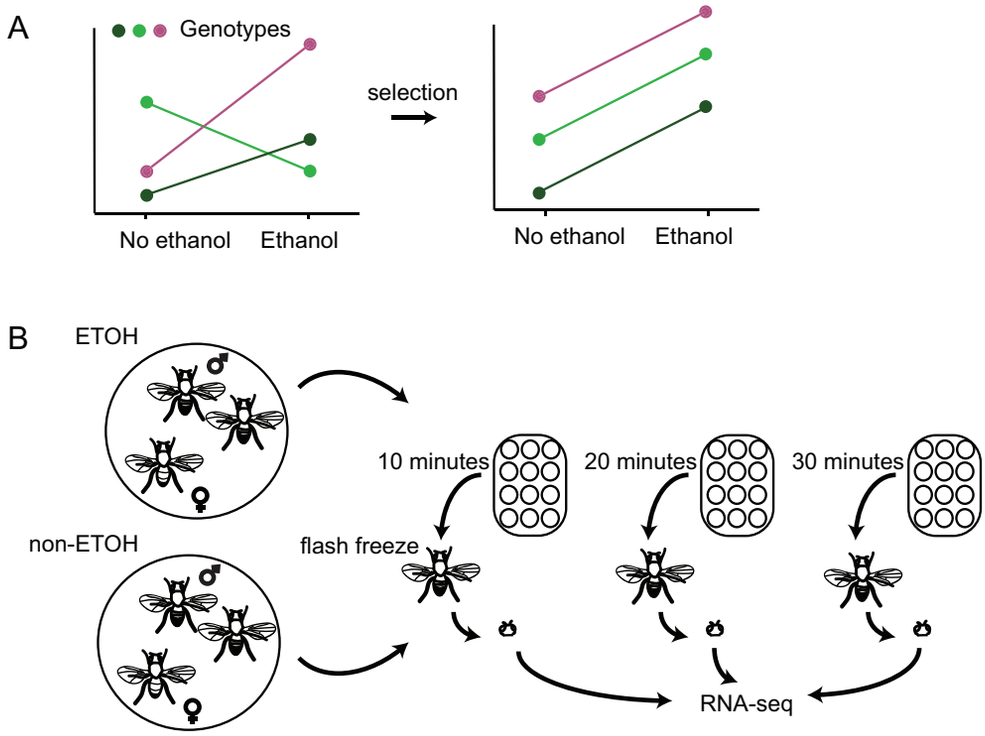

Figure 1: *A*, Illustration of the predictions for the evolution of phenotype plasticity. Shown are predictions for a single trait, such as one gene whose expression responds to ethanol. In a species that is not adapted to ethanol, the expectation is that different genotypes would respond differently, that is, exhibit genotype-by-environment interactions (*left*). In a species that is adapted to ethanol, each genotype will respond the same way (*right*). *B*, Illustration of the environment that each *Drosophila* male was exposed to during the experiment. Each chamber contained two male flies and one female fly. Within a plate there were 12 chambers, and the males from a single plate were pooled to create a sequencing library. Males from each of the 12 chambers were collected and flash frozen after 10, 20, or 30 min. After flash freezing, their heads were isolated for RNA sequencing (RNA-seq). This was done for six genotypes each of *D. melanogaster* and *D. simulans*. ETOH = ethanol.

simulans and is found regularly feeding on and ovipositing in resources with ethanol concentrations greater than 8% (fig. A1; Chodroff et al. 2010; Ulitsky et al. 2011; Quinn et al. 2016; Ulitsky 2016). In addition, lower ethanol tolerance is the "ancestral" state, as most drosophilids in the *D. melanogaster* species group have low ethanol tolerance (David and Van Herrewege 1983). Therefore, the expectation is as follows: *D. simulans* will exhibit extensive variation between genotypes in the plastic response to ethanol, evidence that cryptic genetic variation has not been removed by selection. *Drosophila melanogaster* will have a single plastic response to ethanol, evidence that selection has removed differences between genotypes.

The trait I measure to test this prediction is exon expression. Genes produce multiple messenger RNA (mRNA) transcripts through alternative splicing, which contain different exons, but short-read sequencing fundamentally cannot reconstruct entire transcripts. Exon-level analysis incorporates information about both alternative splicing and expression (see "Methods"). This is necessary because different isoforms of a gene often have different regulatory attributes and stability, as well as potentially different protein products. Indeed, different isoforms of the same gene can have opposite effects on their downstream targets, such as up- and downregulation (Hakre et al. 2006; Shen et al. 2019). Alternative splicing has been previously implicated in the response to ethanol, and in *D. melanogaster* it may be a more important component of the response than gene expression changes (Oomizu et al. 2003; Newton et al. 2004; Pietrzykowski et al. 2008; Sasabe and Ishiura 2010; Hemby 2012; Zaharieva et al. 2012; Robinson and Atkinson 2013).

Ethanol response in *D. melanogaster* and *D. simulans* is particularly well suited for studying genetic accommodation for two reasons. First, the availability of inbred lines allows replicated measurements of genotype-specific responses to the environment, which allows a direct test of the predictions of the genetic accommodation theory. Second, the use of inbred lines allows for replicated measurements of the reaction norm at multiple time points to

understand the temporal component of the response to the environment (Saltz et al. 2018).

Methods

Fly Lines

The six genotypes used for male flies from *Drosophila simulans* were collected from the Zuma Organic Orchard in Zuma Beach, California, in the winter of 2012 and made nearly isogenic by 15 generations of full sibling inbreeding (Signor et al. 2017*a*, 2017*c*). Male flies from six genotypes of *Drosophila melanogaster* originated from an orchard in Winters, California, in 1998 and were made isogenic by 40 generations of full sibling inbreeding (Yang and Nuzhdin 2003; Campo et al. 2013; Signor et al. 2017*a*, 2017*c*). All procedures used here were the same as described previously and will be briefly summarized (Signor et al. 2017*a*, 2017*b*). *Drosophila* in natural conditions are heterozygous compared with inbred laboratory strains (Nuzhdin et al. 2012). Therefore, each genotype was crossed to a white-eyed "tester" strain to create the heterozygous F_1 flies used in the ethanol exposure assays (*D. simulans*, w^{501}, Cornell species stock 14021-0251.011; *D. melanogaster*, w^{1118}, Bloomington stock 3605). With this design I can replicate observations because the crosses are genetically identical within a genotype, but the flies more closely resemble wild flies (Wahlsten 2001; Brakefield 2003). Rearing occurred on a standard medium at 25°C with a 12L:12D photoperiod. To control for maternal effects and variation in offspring quality, female parents were collected as virgins, aged 1 day, and then density matched with male flies (10 per sex). The F_1 offspring were then collected as virgins, reared in single-sex vials with standardized density, and aged for 3–4 days prior to the assay. A portion of this experiment was intended to analyze behavioral differences in ethanol; thus, during the ethanol exposure a mated female was included as a stimulus but was not collected for RNA sequencing (y^1w^1, Cornell species stock center 14021-0251.013; Signor et al. 2017*a*, 2017*b*).

Experimental Setup

In this experiment, intoxication occurs through inhalation of ethanol vapors, and previous publications have established the efficacy of this approach (Signor et al. 2017*a*, 2017*b*). Ethanol exposure took place in a circular arena, each of which was part of a larger chamber containing 12 arenas each with a diameter of 2.54 cm (VWR 89093-496; fig. 1*B*). The bottom of each chamber contained a standard amount of either grapefruit medium or medium in which 15% of the water has been replaced with ethanol (table A1). Replicates were conducted randomly under standardized conditions (25°C, 70% humidity). To set up the assay, the flies were sedated at 4°C for 10 min, to avoid the physiological effects of CO_2 (Perron et al. 1972; Barron 2000). Then the flies were placed in the chambers, which were left upside down at room temperature for 10 min while the flies oriented themselves (Signor et al. 2017*a*, 2017*b*; Signor and Nuzhdin 2018). After the initial acclimation period, the flies were exposed to ethanol for 10, 20, or 30 min for three replicates of each of the two conditions (fig. 1*B*). Flies are most active during the hours following dawn; thus, to standardize behavior and circadian rhythms all assays were conducted within a 2-h window after dawn. At the conclusion of the assay the chambers were flash frozen in liquid nitrogen, allowed to freeze through, and transferred to dry ice, and then all of the males were collected for RNA sequencing (fig. 1*B*).

Sample Preparation and RNA Sequencing

Flash-frozen flies were freeze dried, and 10–12 heads were placed in a 96-tube plate (Axygen MTS-11-C-R). mRNA purification, complementary DNA (cDNA) synthesis, and library preparation were carried out by RAPiD Genomics (http://rapid-genomics.com) using a robot. mRNA was purified using the Dynabeads mRNA Direct Micro kit (Invitrogen 61021) with slight modifications. To fragment the RNA, mRNA beads were resuspended in 10 μL of 2× first-strand buffer (Invitrogen 18064-014), incubated at 80°C for 2 min, and placed on ice; then the supernatant was collected after 5 min on a magnetic stand. First-strand synthesis was performed using standard protocols for Superscript II (Invitrogen 18640-014) and reverse transcription (25°C for 10 min, 42°C for 50 min, 70°C for 15 min, 10°C hold). Second, strand synthesis was carried out using standard protocols with DNA Pol I and incubation at 16°C for 2.5 h. cDNA was purified with a 1.8 volume of AMPure XP following the manufacturer's instructions (Beckman Coulter A63880). Sequencing was performed using the Illumina HiSeq 2500 system as both 2 × 150-bp and 2 × 50-bp reads, resulting in two technical replicates per sample. The two run lengths (and runs) were intended to provide extra coverage, and all replicates were sequenced in both runs.

Exon Expression Analysis

It is common in organisms with alternative splicing for exons from different isoforms of a single gene to overlap with one another or be shared between all or most isoforms (fig. 2). Short-read data fundamentally cannot resolve these exons to individual isoforms; however, one approach is to quantify each exon separately and decompose exons overlapping between isoforms into those that

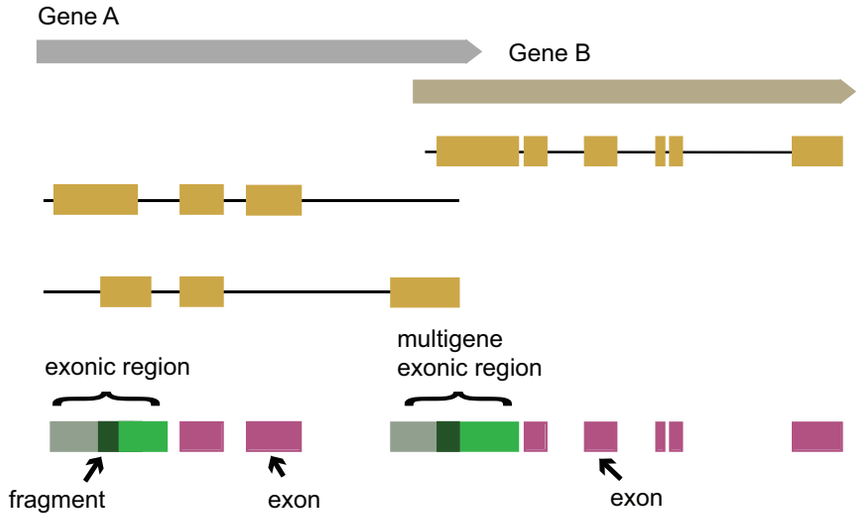

Figure 2: Illustration of the classification scheme for exons, exonic regions, and exon fragments. Exons either do not overlap other exons in different isoforms (exons, shown in purple) or are fused regions consisting of a set of overlapping exons (exonic regions, shown in green). Exonic regions can be decomposed into exon fragments, depending on their overlap between different isoforms.

are shared and unique. When the differences between overlapping exons are less than 10 bp, there is no appreciable amount of information loss in not decomposing overlapping exons, and this approach has been taken in the past (Dalton et al. 2013; Graze et al. 2014; Newell et al. 2016; Fear et al. 2016). However, in many cases the differences in exon overlap are much larger than this, so I use a classification scheme where reads may be assigned to "exons," "exonic regions," or "exon fragments" and then compare the abundance of each exon/exonic region/exon fragment in each condition (i.e., the abundance of an exon with and without ethanol; Signor and Nuzhdin 2018; fig. 2). Here, an exon does not overlap any other exons. If exons from different isoforms overlap, they are grouped into an exonic region. Exon fragments are classified by decomposing exonic regions based on the 5′ and 3′ positions of the exons within the region. Thus, all exon fragments are subregions of exonic regions. Exon boundaries for *D. simulans* were determined using the *D. simulans* 2.02 genome features file. Alignment was performed using BWA-MEM version 0.7.15 (which has been shown to perform better than split-read mappers such as STAR; Newman et al. 2017), and BED files were used to count reads in each region and obtain the length-adjusted read count (reads in region divided by the length of region) and the average per nucleotide (APN; Li 2015).

The APN was calculated separately for each read length and then combined between read lengths to handle the mixture of read lengths for each sample (2 × 150 bp and 2 × 50 bp). If the APN was greater than zero in at least half of all samples per condition, the exon or exonic region was considered detected. Several approaches to normalize coverage counts were considered. Upper quartile normalization with log transformation and median centering within genotype by time by treatment were selected because of better performance of the residuals (Bullard et al. 2010; Dillies et al. 2013).

To test the significance of components of expression variation, the log APN for each exon, exonic region, or exon fragment was modeled as

$$\Upsilon_{ijkl} = \mu + g_i + t_j + (gt)_{ik} + m_k + (gm)_{ik} + (tm)_{ijk} + \epsilon_{ijkl}$$

for the ith genotype (g_i), the jth treatment (t_j; j = ethanol or no ethanol), the kth time point (m_k; k = 10, 20, 30 min), μ mean expression across samples, and the lth replicate (supplemental files 1, 2; supplemental files 1–3 are available online). For the interaction between treatment and time point, the log APN for each exon, exonic region, or exon fragment was modeled as

$$\Upsilon_{ij} = \mu + t_j + \epsilon_{ij}$$

for the ith condition (time by treatment), μ mean expression across samples, and the jth replicate. Contrasts to compare treatments within time point (ethanol vs. no ethanol for 10, 20, and 30 min) were conducted (supplemental files 1, 2). Residuals were evaluated for conformation

with normality assumptions, and assumptions were met in excess of 95% of the models.

In contrast to exon abundance, I evaluated alternative splicing specifically by comparing the abundance of all exons within a gene to each other, as this is more direct evidence of a change in isoform abundance than a change in exon/exonic region/exon fragment abundance (fig. 2). For example, a change in relative abundance might be detected if the last exon of a gene was the most abundant compared with all other exons/exonic regions/exon fragments without ethanol and the least abundant with ethanol. Exons and exonic regions for each gene and for each sample were ranked, and the most expressed region was ranked as 1, the least expressed region was ranked as 3, and all others were ranked as 2 (fig. 2). Exon ranks for each gene were modeled as

$$\Upsilon_{ijk} = \mu + r_i + t_j + (rt)_{ij} + \epsilon_{ijkl},$$

where Υ_{ijk} is the exon rank (1, 2, or 3) of the ith exonic region of the gene, the jth condition (time by treatment), and the kth replicate; μ is mean expression across samples; r_i is the exonic region of the gene; t_j is the condition; and $(rt)_{ij}$ is the interaction between exonic region and condition (supplemental file 3). More traditional generalized linear model approaches can be taken only if their assumptions are met, and in this case they are not because of a lack of normality in the distribution of model residuals. Accordingly, a nonparametric test must be relied on to look for changes in exon or exonic region representation between exons of a gene, and I used a rank test to summarize changes in exon representation. *F*-tests for the significance of the mean square attributed to the effect tested versus the mean square attributed to error (or the appropriate interaction term) were used. The false discovery rate was controlled using the Benjamini-Hochberg procedure, with a significance cutoff of $\alpha = .05$ (Benjamini and Hochberg 1985).

Functional Class Enrichment

The response to ethanol could be enriched for genes with different functional classes, such as pseudogenes or non-protein-coding genes. Genes were assigned to functional classes on the basis of the *D. melanogaster* 6.17 and *D. simulans* 2.02 assemblies. I tested the significant sets of exons and exonic regions for enrichment with non-protein-coding genes. Multigene exonic regions were not included, meaning exons that belong to more than one gene, as they often do not correspond to the same functional class of gene. Every test of functional class enrichment compared the frequency of a given subcategory among all exons and exonic regions detected in the data set compared with the frequency within a significant list of exons and exonic regions. A χ^2 test was performed in R to test the significance of the enrichment of each of these categories.

Results

To assess the predictions from genetic accommodation, I have first compared the response to ethanol for exons, exonic regions, and exon fragments between *Drosophila simulans* and *Drosophila melanogaster*. Note that because isoforms evolve quite rapidly, the comparisons cannot be entirely direct—for example, figure 3*A* illustrates the gene *cabut*, where in *D. melanogaster* there are three annotated transcripts and four exon fragments (one exonic region). In *D. simulans cabut* there are two transcripts, one exon, and two exon fragments (one exonic region). I then looked for shared genes involved in the response to ethanol as well as differences in reaction norms over time. I estimated the contribution of alternative splicing to each species reaction to ethanol by looking at changes in the abundance of exons within a gene. Last, I examined the genes involved in the response to ethanol to better understand what types of genes are involved in cryptic variation in *D. simulans* and what types of genes are involved in adaptation in *D. melanogaster*.

Changes in Exon Expression

Table 1 summarizes the number of exons, exonic regions, and exon fragments that altered their expression in response to ethanol (table A1; supplemental file 1). The most striking difference in the *D. simulans* data set compared with the *D. melanogaster* data set is the number of exons and exonic regions that were significant for components of variance that interact with genotype: 1,457 for the interaction among genotype, treatment, and time and 486 for the interaction between genotype and treatment, compared with two and three exons and exonic regions, respectively, in *D. melanogaster* (table 1; fig. 3*B*). One explanation for the large effect of genotype in *D. simulans* compared with that in *D. melanogaster* would be more variation attributable to genotype overall in *D. simulans*. However, this does not appear to be the case, as in *D. melanogaster* 2,076 exons and exonic regions are expressed differently between genotypes, and in *D. simulans* this number is 2,400 (table 1). There is abundant genotype-specific variation in *D. melanogaster*, but it does not alter how *D. melanogaster* responds to ethanol. The disparity between *D. melanogaster* and *D. simulans* in the number of exons and exonic regions that respond to ethanol is also unique to categories that include an interaction with genotype—for example, the interaction between ethanol and

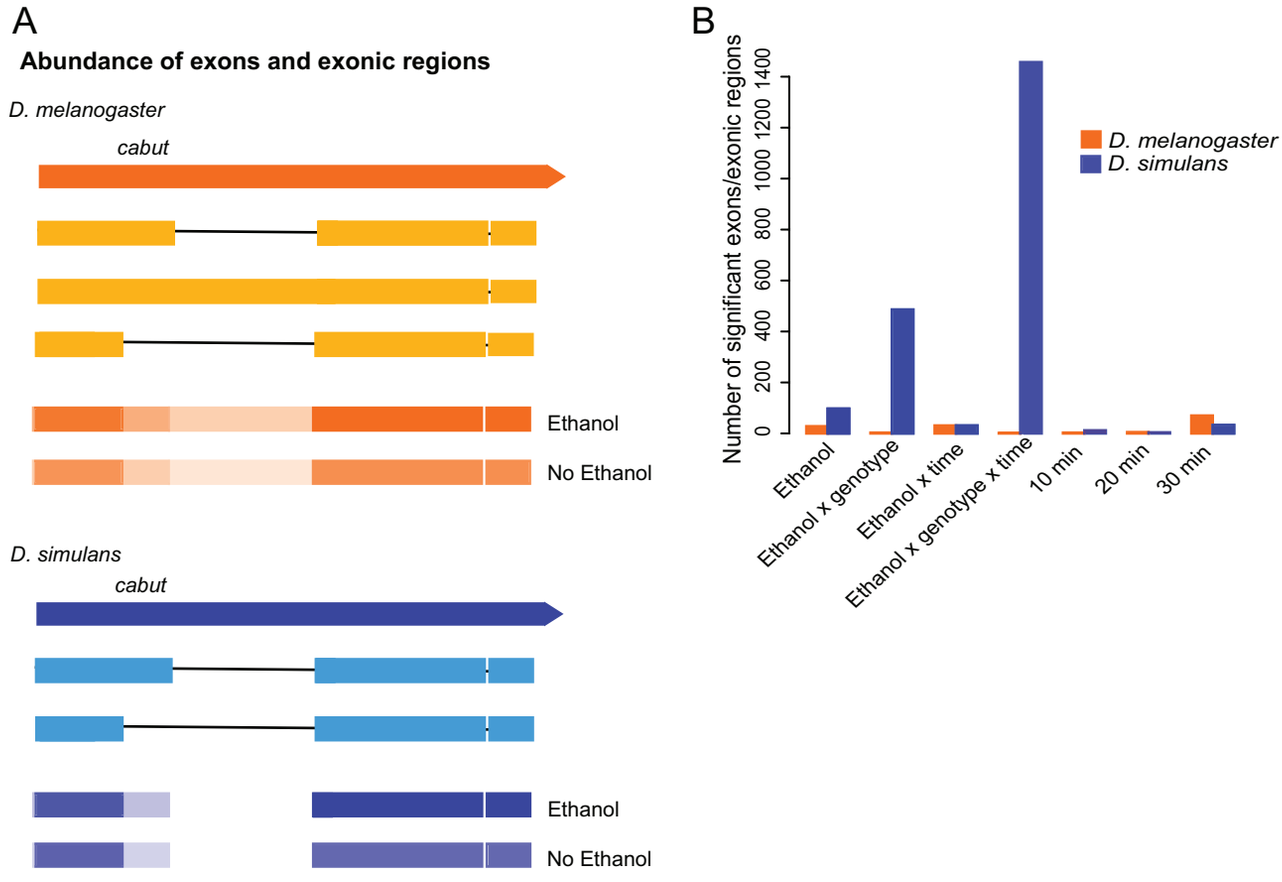

Figure 3: *A*, Example of the information about gene expression and isoform abundance that can be inferred from measures of differential exon abundance. The *cabut* gene is a relatively simple example, having few isoforms and exons, yet it is still a complicated interspecies comparison given that *Drosophila melanogaster* has more annotated isoforms than *Drosophila simulans*. Below each gene model the frequency of different exons and exon fragments in each environment is shown, with frequency indicated by the darkness of the color. In *D. simulans* the first of two isoforms shown has an exon fragment that is more abundant in the presence of ethanol, suggesting that that isoform is more abundant. In *D. melanogaster* only the second of the three isoforms has a unique fragment, which is also more abundant in ethanol and suggests differential isoform usage. *B*, Number of exons or exonic regions that are significant for each component of variance in *D. melanogaster* and *D. simulans*. Many more exons and exonic regions are significant for *D. simulans* than for *D. melanogaster*, but only for components of variance that contain an interaction with genotype.

Table 1: Summary of the exons, exonic regions, and exon fragments that were significantly differently expressed for each component of variance

	G	ETOH	G × ETOH	ETOH × T	G × ETOH × T	10 min	20 min	30 min
Drosophila simulans:								
Exons	1,994	76	387	24	1,158	9	3	29
Exonic regions	406	23	98	7	299	3	1	4
Exon fragments	608	18	81	6	225	4	2	6
Drosophila melanogaster:[a]								
Exons	1,445	15	3	22	0	2	1	46
Exonic regions	631	13	0	8	2	1	4	24
Exon fragments	1,135	21	0	12	4	3	5	20

Note: Ethanol in this case is the environment. ETOH = ethanol; G = genotype; T = time.

[a] Reproduced from Signor and Nuzhdin 2018.

time has 31 significant exons and exonic regions in *D. simulans* and 30 in *D. melanogaster* (table 1).

Core Components of the Response to Ethanol

Exons and exonic regions that respond to ethanol by changing their expression in *D. simulans* and *D. melanogaster* are more likely to represent core components of the ethanol response pathway than those that are not shared (table 2). For example, in response to ethanol seven exons and exonic regions belonged to genes that were shared between species: *cabut*, *CG11741*, *CG32512*, *CG4607*, *Drat*, *Pinocchio*, and *sugarbabe* (Signor and Nuzhdin 2018). *Drosophila simulans* and *D. melanogaster* shared *cabut*, *CG32512*, *CG407*, *Drat*, *Pinocchio*, and *sugarbabe* for multiple components of variance, which suggests that they are important for the response to ethanol. *Drat*, *cabut*, *sugarbabe*, *Pinocchio*, *CG32512*, and *CG407* have also been implicated in the response to ethanol previously (Morozova et al. 2006; Kong et al. 2010; Awofala et al. 2012).

Reaction Norms over Time

When reaction norms are measured, it is most typically at a single time point; however, many responses are dose dependent and will change over time. Therefore, I examined the number of exons and exonic regions with differences in expression at 10, 20, and 30 min. The number of exons and exonic regions with differences in expression increases at 30 min for both *D. melanogaster* and *D. simulans*, although the increase is more marked for *D. melanogaster* (table 1). For example, in *D. simulans* 29 exons and four exonic regions changed their expression at 30 min, compared with nine and three at 10 min. In *D. melanogaster*, 46 exons and 24 exonic regions changed their expression at 30 min, compared with two and one at 10 min. Furthermore, in *D. simulans* the largest component of variation in terms of the number of exons and exonic regions that change their expression is the interaction among genotype, environment, and time (table 1; fig. 3). The differences observed between time points in *D. simulans* and *D. melanogaster* underscores the importance of time for studying reaction norms.

Table 2: Genes that have exons and exonic regions that change expression in both *Drosophila melanogaster* and *Drosophila simulans* for each component of variance

	Shared gene(s)
ETOH	*cabut*, *CG11741*, *CG32512*, *CG4607*, *Drat*, *Pinocchio*, *sugarbabe*
G × ETOH	...
ETOH × T	*cabut*, *CG43366*, *Drat*
G × ETOH × T	...
10 min	*cabut*
20 min	*cabut*
30 min	*cabut*, *CG32103*, *CG32512*, *CG4607*, *Drat*, *Pinocchio*, *sugarbabe*

Note: ETOH = ethanol; G = genotype; T = time.

Changes in the Relative Abundance of Exons within a Gene

There is evidence for changes in splicing in response to ethanol in *D. simulans* for 54 genes and in response to the interaction between ethanol and time for 94 genes (fig. 4; supplemental file 3). In *D. melanogaster* there is evidence for changes in splicing in response to ethanol for 71 genes and in response to the interaction between ethanol and time for 145 genes (Signor and Nuzhdin 2018). For the interaction between treatment and time, *lola*, *Mhc*, and *Prm* were shared between species; *lola* has been implicated in several genomic screens for genes involved in complex behaviors, including ethanol sensitivity (Morozova et al. 2007; Gates et al. 2011; Fukui et al. 2012).

Interactions with Genotype Are Enriched for Nested Non-Protein-Coding Genes in D. simulans

The type of gene that is involved in the response to ethanol in *D. simulans* may also reflect the types of genes where cryptic variation is more likely to accumulate. I observed that a large proportion of exons and exonic regions that changed their expression in response to ethanol in *D. simulans* were non-protein-coding. Protein-coding genes still represent the majority of genes whose exons and exonic regions change their expression in response to ethanol; however, there appeared to be more non-protein-coding genes than would be expected by chance. In *D. simulans* the number of non-protein-coding genes whose exons or exonic regions changed their expression in response to ethanol was enriched for ethanol, ethanol by genotype, and ethanol by genotype by time ($\chi^2 = 49.598$, $P < .0005$; $\chi^2 = 235.21$, $P < 2.2 \times 10^{-16}$; $\chi^2 = 727.17$, $P < 2.2 \times 10^{-16}$). *Drosophila melanogaster* was not enriched for non-protein-coding genes in response to ethanol, ethanol by time, ethanol by genotype, ethanol by genotype by time; 10 or 20 min; exons or exonic regions expressed only in one environment; or genes implicated in changes in relative abundance (Signor and Nuzhdin 2018). In contrast, for *D. melanogaster*, non-protein-coding exons and exonic regions were enriched in expression only at 30 min after exposure to ethanol ($\chi^2 = 12.831$, $P = .005$).

As I investigated the genes that responded to ethanol, I noticed what seemed to be an unusual number that

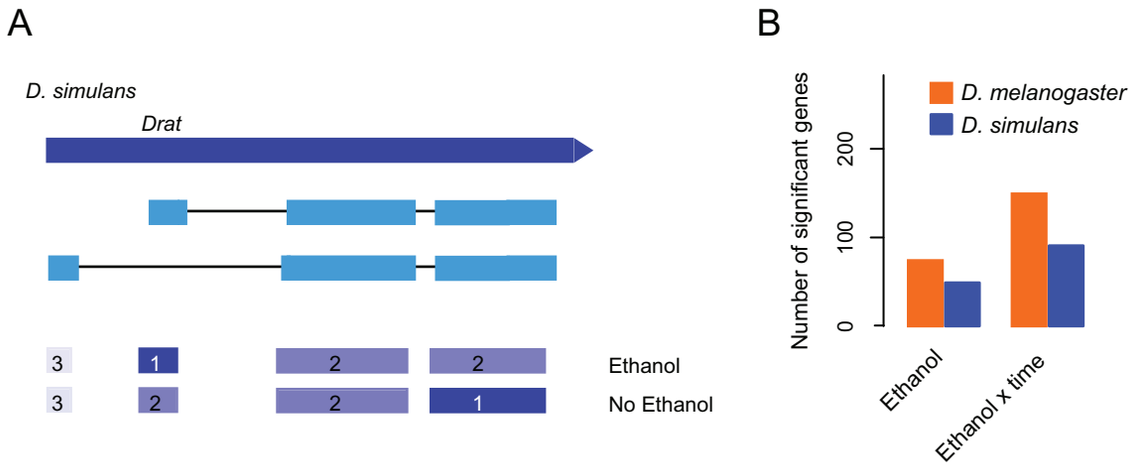

Figure 4: *A*, Illustration of a change in the relative abundance of the gene *Drat*. With and without ethanol, different exons are the most abundant compared with the other exons within the gene (abundance is indicated by the darkness of the color). That one of these exons is not shared among all isoforms suggests a change in isoform usage between environments. *B*, Number of genes that have a significant change in relative abundance for each component of variance in *Drosophila melanogaster* and *Drosophila simulans*.

were nested in the introns of other genes. To determine whether more genes were nested in the introns of other genes than expected by chance, I first determined the overall frequency of gene nesting. I used the criterion that an exon nested in an intron must overlap the intron by at least 80 bp or 10%. I found that in *D. melanogaster* 9.2% of exons were nested within introns, while in *D. simulans* 9.7% were nested, similar to what has been reported previously (Lee and Chang 2013). However, among exons or exonic regions that changed their expression in response to ethanol, in *D. melanogaster* 18.6% were nested, and in *D. simulans* 33.5% were nested (Signor and Nuzhdin 2018). This is a significant enrichment of exons that are nested within other introns, for both *D. melanogaster* ($\chi^2 = 12.344$, $P < .0004$) and *D. simulans* ($\chi^2 = 1{,}721.1$, $P < 2.2 \times 10^{-16}$). Not all of the exons or exonic regions that are nested and changed their expression in response to ethanol were non-protein-coding; therefore, I also tested whether there are more nested non-protein-coding genes that change their expression in response to ethanol. The number of exons or exonic regions that changed expression in response ethanol and were nested and non-protein-coding was enriched in *D. simulans* ($\chi^2 = 237.49$, $P < 2.2 \times 10^{-16}$) but not in *D. melanogaster*.

Discussion

The prediction from theory on genetic accommodation was that in *Drosophila simulans* there would be abundant variation in how each genotype responds to ethanol and in *Drosophila melanogaster* there would be very little variation (fig. 1*A*). As such, my results strongly support genetic accommodation as a mechanism explaining the adaptive plastic response of *D. melanogaster* to ethanol. Without exposure to ethanol-rich resources, in *D. simulans* environmentally induced variants are not exposed to selection and accumulate as cryptic genetic variation. The result of this lack of selection in *D. simulans* manifests as greater variation between genotypes in the novel environment (Rutherford 2000; Gibson and Dworkin 2004; Hermisson and Wagner 2004; Schlichting 2008). In *D. melanogaster* ethanol is used as a resource; however, the environment is patchy, and therefore response to ethanol is expected to be selected on to have an optimal plastic response (Via and Lande 1985; Guntrip and Sibly 1998; Lande 2009; Matzkin 2012; Huang and Agrawal 2016). The use of inbred lines is a unique opportunity to assess these predictions, as the contribution of interactions with genotype can be directly assessed.

There is a caveat to this work in that it is possible that there is simply more genetic variation between genotypes in *D. simulans* than in *D. melanogaster*. This could potentially make the differences seen between the two species the result of background differences in genetic variation. Approximately the same number of genes have a significant effect of genotype in each species, but this cannot be entirely ruled out. In addition, there is potentially an alternative explanation for the observation that *D. simulans* has many more exons and exonic regions that respond in a genotype-specific manner. If the effect size of ethanol in *D. simulans* is larger (but not large enough to effect all categories of variance) and the effect size of genotype is also much larger, this could cause more genes to be significant for interaction effects independent of any genetic

accommodation. However, there is no evidence that the effect size of genotype is much larger in *D. simulans*.

This system fits the criteria described in Levis and Pfennig (2016) and Jones and Robinson (2018) for establishing evolution by genetic accommodation. The focal trait, response to ethanol, can be environmentally induced in an ancestor proxy lineage (*D. simulans*). Cryptic genetic variation is also uncovered when the ancestor proxy lineage experiences this environment—this is the massive increase in genotype-specific responses to ethanol observed in *D. simulans*. Furthermore, the focal trait exhibits evidence of evolutionary change in the derived lineage—the loss of genotype-specific responses in *D. melanogaster*. The enrichment of alternative splicing in response to ethanol in *D. melanogaster* is also evidence of evolutionary change in the regulation or form in derived lineages. The focal trait, response to ethanol, also exhibits evidence of adaptive refinement in the derived lineage—*D. melanogaster* is adapted to ethanol, it preferentially oviposits on ethanol-rich substrate, and it is thought to be both a caloric advantage and a response to wasp parasitism (Milan et al. 2012; Pohl et al. 2012; Zhu and Fry 2015; Gao et al. 2018).

The response to ethanol in *D. simulans* is enriched for non-protein-coding genes and genes that are nested in the introns of other genes. Long noncoding RNAs are commonly associated with introns and are complex and important mediators of gene expression (Louro et al. 2009; Rearick et al. 2010; Chen et al. 2016). It could be that cryptic genetic variation preferentially accumulates for the expression of intronic non-protein-coding genes as a result of lower selective constraint on expression. If variation in the expression of intronic non-protein-coding genes is more likely to be neutral, preferential accumulation within less constrained sequences would be expected. Alternatively, intronic long noncoding RNAs could simply be disproportionately important for the response to ethanol in *D. simulans* for unknown functional reasons. Intronic long noncoding RNAs have been shown to be responsive to environmental stimuli in terms of expression level, and they can have complex interactions with the promoter elements, transcription factors, and alternative splicing of their host genes (Louro et al. 2009).

Alternative splicing is a more important component of the response to ethanol in *D. melanogaster* than in *D. simulans*, as evidenced by differences in the frequency of significant changes in relative abundance of exons. Alternative splicing does not require de novo transcription of genes (which requires time for transcriptional activation and accumulation of precursor mRNA [pre-mRNA]) or changes in overall expression levels. The ability to change rapidly makes alternative splicing likely to contribute substantially to plastic phenotypes that change in response to the environment. In plants, for example, some stress-response genes produce nonfunctional transcripts in the absence of stress. During stress response they are rapidly converted to functional transcripts, avoiding the negative effect on growth when they are not needed but also avoiding the time necessary for pre-mRNA accumulation and transcription (Mastrangelo et al. 2012). Alternatively, the fact that alternative splicing is less important in *D. simulans* could suggest that there is selection against the accumulation of cryptic genetic variation for splicing patterns.

In *D. simulans*, the largest number of exons and exonic regions changed their expression in response to genotype by ethanol by time. There is often no empirical reason for measuring reaction norms at a particular time point; however, there is considerable evidence that the time points measured will change the conclusions of the study. These observations about the importance of time are supported in other studies; for example, in a study of heat-stressed *Drosophila* different isoforms of a single gene were found to be expressed at seven time points over 48 h (Telonis-Scott et al. 2014). In several other studies initial transcriptome changes are large in response to an environmental change but return to baseline levels after prolonged exposure (termed transcriptional resilience; Brennan et al. 2015; Franssen et al. 2015; Moya et al. 2015; Seneca and Palumbi 2015). If these studies and the one conducted here had terminated after a single time point, the conclusions may have been very different. For example, in *D. simulans* the inclusion of time allowed me to see the pattern of genotype-specific enrichment evident in the genotype-by-ethanol and genotype-by-time-by-ethanol effects that I would not have seen if I had included only genotype by ethanol.

The genes containing exons or exonic regions that changed their expression in response to ethanol in both species (*Drat*, *cabut*, *sugarbabe*, *Pinocchio*, *CG32512*, and *CG407*) have also been implicated in the response to ethanol in other studies. The gene *cabut* has been previously observed as being upregulated in response to ethanol, and *cabut* is in general responsive to changes in metabolic conditions (Awofala et al. 2012; Havula and Hietakangas 2012; Bartok et al. 2015). The genes *sugarbabe* and *cabut* are both downstream of the *Mondo-Mlx* sugar-sensing pathway (Mattila et al. 2015), which has been linked to severe obesity, high circulating triglycerides, and tumorigenesis (Kathiresan et al. 2008; Kooner et al. 2008; Herman et al. 2012; Carroll et al. 2015). *Drat* has been implicated in ethanol-related cell death (Chen et al. 2012). Their specific roles in the response to ethanol are not known.

Inferring that gene expression differences are adaptive or nonadaptive remains a major challenge in the study of gene expression reaction norms. The patterns observed in *D. simulans* suggest that abundant genotype-by-environment interactions have accumulated neutrally

and become uncovered in response to a novel environment. In contrast, in *D. melanogaster* this ethanol environment is not novel, and variation in plasticity has been selected out in favor of an adaptive phenotypic response. Non-protein-coding genes are overrepresented in *D. simulans* in response to ethanol potentially because their expression is less constrained and can accumulate more neutral variation. This is an excellent illustration of genetic accommodation, where phenotypic plasticity has facilitated adaptation to a novel resource within a patchy environment.

Acknowledgments

I thank Sergey Nuzhdin, Jeremy Newman, and Lauren McIntyre for contributions to the manuscript. I thank my undergraduate researchers for assistance in producing these data: N. Shadman, V. Paterson, Z. Polonus, K. Cortez, L. Hassanzadeh, L. Cline, A. Khokhar, E. Lee, K. L. Yee, M. Ling, S. Sarva, O. Akintonwa, A. Gupta, R. Manson, P. Hassanzadeh, and K. Kavoussi. This work was supported by the National Institutes of Health (grants GM102227 and MH09156) and by the National Science Foundation Established Program to Stimulate Competitive Research (grant NSF-EPSCoR-1826834).

Data and Code Availability

Drosophila melanogaster and *D. simulans* sequence data have been submitted to Bioproject (accession nos. PRJNA482662 and PRJNA646232).

APPENDIX

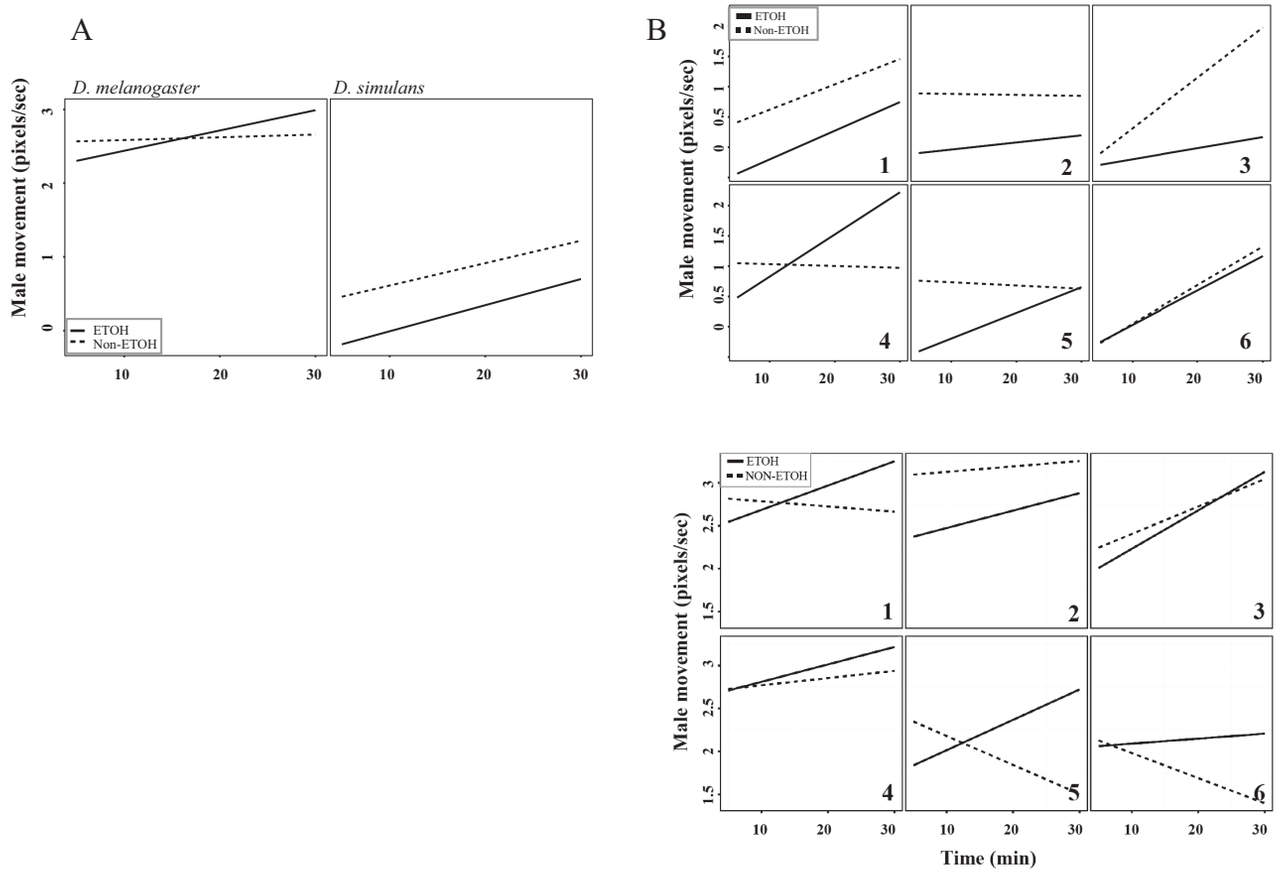

Figure A1: The effect of ethanol on behavior and gene expression in the brain is not directly translatable; however, there are genotype-specific differences in the effect of ethanol on behavior in both *Drosophila melanogaster* and *Drosophila simulans* (reproduced from Signor et al. 2017*a*, 2017*c*). *A*, Average movement for all genotypes with and without ethanol for both *D. simulans* and *D. melanogaster*. *Drosophila melanogaster* is on average more active than *D. simulans*. Data are for males only. *B*, Average movement for each of six genotypes with and without ethanol. The pattern of movement variation in response to ethanol is genotype specific, with some genotypes responding very little in their activity level (*D. simulans* genotype 6) and others having widely different movement trajectories (*D. melanogaster* genotype 5). ETOH = ethanol.

Table A1: Fly food recipe

	Value
Nonethanol:	
Agar (g)	5.7
Malt sugar (g)	8.1
Yeast (g)	10.8
Water (mL)	150
Grapefruit juice (mL)	150
Ethanol:	
Agar (g)	5.7
Malt sugar (g)	8.1
Yeast (g)	10.8
Water (mL)	105
Grapefruit juice (mL)	150
Ethanol (mL)	45

Literature Cited

Awofala, A. A., J. A. Davies, and S. Jones. 2012. Functional roles for redox genes in ethanol sensitivity in *Drosophila*. Functional and Integrative Genomics 12:305–315.

Baldwin, J. M. 1896. A new factor in evolution. American Naturalist 30:441–451.

Barron, A. B. 2000. Anaesthetising *Drosophila* for behavioural studies. Journal of Insect Physiology 46:439–442.

Bartok, O., M. Teesalu, R. Ashwall-Fluss, V. Pandey, M. Hanan, B. M. Rovenko, M. Poukkula, et al. 2015. The transcription factor *Cabut* coordinates energy metabolism and the circadian clock in response to sugar sensing. EMBO Journal 34:1538–1553.

Benjamini, Y., and Y. Hochberg. 1985. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B 57:289–300.

Brakefield, P. M. 2003. Artificial selection and the development of ecologically relevant phenotypes. Ecology 84:1661–1671.

Brennan, R. S., F. Galvez, and A. Whitehead. 2015. Reciprocal osmotic challenges reveal mechanisms of divergence in phenotypic plasticity in the killifish *Fundulus heteroclitus*. Journal of Experimental Biology 218:1212–1222.

Bullard, J. H., E. Purdom, K. D. Hansen, and S. Dudoit. 2010. Evaluation of statistical methods for normalization and differential expression in mRNA-Seq experiments. BMC Bioinformatics 11:94.

Campo, D., K. Lehmann, C. Fjeldsted, T. Souaiaia, J. Kao, and S. V. Nuzhdin. 2013. Whole-genome sequencing of two North American *Drosophila melanogaster* populations reveals genetic differentiation and positive selection. Molecular Ecology 22:5084–5097.

Carroll, P. A., D. Diolaiti, L. McFerrin, H. Gu, D. Djukovic, J. Du, P. F. Cheng, et al. 2015. Deregulated Myc requires MondoA/Mlx for metabolic reprogramming and tumorigenesis. Cancer Cell 27:271–285.

Chen, P., X. Tu, F. Akdemir, S. K. Chew, A. Rothenfluh, and J. M. Abrams. 2012. Effectors of alcohol-induced cell killing in *Drosophila*. Cell Death and Differentiation 19:1655–1663.

Chen, X., C. C. Yan, X. Zhang, and Z.-H. You. 2016. Long noncoding RNAs and complex diseases: from experimental results to computational models. Briefings in Bioinformatics 59:bbw060-19.

Chodroff, R. A., L. Goodstadt, T. M. Sirey, P. L. Oliver, K. E. Davies, E. D. Green, Z. Molnár, et al. 2010. Long noncoding RNA genes: conservation of sequence and brain expression among diverse amniotes. Genome Biology 11:R72.

Dalton, J. E., J. M. Fear, S. Knott, B. S. Baker, L. M. McIntyre, and M. N. Arbeitman. 2013. Male-specific Fruitless isoforms have different regulatory roles conferred by distinct zinc finger DNA binding domains. BMC Genomics 14:659.

David, J. R., and J. Van Herrewege. 1983. Adaptation to alcoholic fermentation in *Drosophila* species: relationship between alcohol tolerance and larval habitat. Comparative Biochemistry and Physiology 74:283–288.

Dillies, M. A., A. Rau, J. Aubert, C. Hennequet-Antier, M. Jeanmougin, N. Servant, C. Keime, et al. 2013. A comprehensive evaluation of normalization methods for Illumina high-throughput RNA sequencing data analysis. Briefings in Bioinformatics 14:671–683.

Fear, J. M., L. G. León-Novelo, A. M. Morse, A. R. Gerken, K. Van Lehmann, J. Tower, S. V. Nuzhdin, and L. M. McIntyre. 2016. Buffering of genetic regulatory networks in *Drosophila melanogaster*. Genetics 203:1177–1190.

Franssen, S. U., V. Nolte, R. Tobler, and C. Schlötterer. 2015. Patterns of linkage disequilibrium and long range hitchhiking in evolving experimental *Drosophila melanogaster* populations. Molecular Biology and Evolution 32:495–509.

Fukui, A., M. Inaki, G. Tonoe, H. Hamatani, M. Homma, T. Morimoto, H. Aburatani, et al. 2012. *Lola* regulates glutamate receptor expression at the *Drosophila* neuromuscular junction. Biology Open 1:362–375.

Gao, H.-H., Y.-F. Zhai, H. Chen, Y.-M. Wang, Q. Liu, Q.-L. Hu, F.-S. Ren, et al. 2018. Ecological niche difference associated with varied ethanol tolerance between *Drosophila suzukii* and *Drosophila melanogaster* (Diptera: Drosophilidae). Florida Entomologist 101:498–504.

Gates, M. A., R. Kannan, and E. Giniger. 2011. A genome-wide analysis reveals that the *Drosophila* transcription factor, *Lola*, promotes axon growth in part by suppressing expression of the actin nucleation factor, *Spire*. Neural Development 6:37.

Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology 21:394–407.

Gibson, G. 2009. Decanalization and the origin of complex disease. Nature Reviews Genetics 10:134–140.

Gibson, G., and I. Dworkin. 2004. Uncovering cryptic genetic variation. Nature Reviews Genetics 5:681–690.

Graze, R. M., L. M. McIntyre, A. M. Morse, B. M. Boyd, S. V. Nuzhdin, and M. L. Wayne. 2014. What the X has to do with it: differences in regulatory variability between the sexes in *Drosophila simulans*. Genome Biology and Evolution 6:818–829.

Guntrip, J., and R. M. Sibly. 1998. Phenotypic plasticity, genotype-by-environment interaction and the analysis of generalism and specialization in *Callosobruchus maculatus*. Heredity 81:198–204.

Hakre, S., M. I. Tussie-Luna, T. Ashworth, C. D. Novina, J. Settleman, P. A. Sharp, and A. L. Roy. 2006. Opposing functions of TFII-I spliced isoforms in growth factor-induced gene expression. Molecular Cell 24:301–308.

Havula, E., and V. Hietakangas. 2012. Glucose sensing by ChREBP/MondoA–Mlx transcription factors. Seminars in Cell and Developmental Biology 23:640–647.

Hayden, E. J., E. Ferrada, and A. Wagner. 2011. Cryptic genetic variation promotes rapid evolutionary adaptation in an RNA enzyme. Nature 474:92–95.

Hemby, S. E. 2012. Alternative splicing of AMPA subunits in prefrontal cortical fields of cynomolgus monkeys following chronic ethanol self-administration. Frontiers in Psychiatry 2:72.

Herman, M. A., O. D. Peroni, J. Villoria, M. R. Schön, N. A. Abumrad, M. Blüher, S. Klein, et al. 2012. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature 484:333–338.

Hermisson, J., and G. P. Wagner. 2004. The population genetic theory of hidden variation and genetic robustness. Genetics 168:2271–2284.

Huang, Y., and A. F. Agrawal. 2016. Experimental evolution of gene expression and plasticity in alternative selective regimes. PLoS Genetics 12:e1006336.

Jones, B. M., and G. E. Robinson. 2018. Genetic accommodation and the role of ancestral plasticity in the evolution of insect eusociality. Journal of Experimental Biology 221:jeb153163-11.

Kathiresan, S., O. Melander, C. Guiducci, A. Surti, N. P. Burtt, M. J. Rieder, G. M. Cooper, et al. 2008. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nature Genetics 40:189–197.

Kong, E. C., L. Allouche, P. A. Chapot, K. Vranizan, M. S. Moore, U. Heberlein, and F. W. Wolf. 2010. Ethanol-regulated genes that contribute to ethanol sensitivity and rapid tolerance in *Drosophila*. Alcoholism: Clinical and Experimental Research 34:302–316.

Kooner, J. S., J. C. Chambers, C. A. Aguilar-Salinas, D. A. Hinds, C. L. Hyde, G. R. Warnes, F. J. Gómez Pérez, et al. 2008. Genome-wide scan identifies variation in MLXIPL associated with plasma triglycerides. Nature Genetics 40:149–151.

Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. Journal of Evolutionary Biology 22:1435–1446.

Lee, Y. C. G., and H. H. Chang. 2013. The evolution and functional significance of nested gene structures in *Drosophila melanogaster*. Genome Biology and Evolution 5:1978–1985.

Levis, N. A., and D. W. Pfennig. 2016. Evaluating "plasticity-first" evolution in nature: key criteria and empirical approaches. Trends in Ecology and Evolution 31:563–574.

Li, H. 2015. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv, 1303.3997.

Louro, R., A. S. Smirnova, and S. Verjovski-Almeida. 2009. Long intronic noncoding RNA transcription: expression noise or expression choice? Genomics 93:291–298.

Mastrangelo, A. M., D. Marone, G. Laidò, A. M. De Leonardis, and P. De Vita. 2012. Alternative splicing: enhancing ability to cope with stress via transcriptome plasticity. Plant Science 185/186:40–49.

Mattila, J., E. Havula, E. Suominen, M. Teesalu, I. Surakka, R. Hynynen, H. Kilpinen, et al. 2015. *Mondo-Mlx* mediates organismal sugar sensing through the *Gli*-similar transcription factor *sugarbabe*. Cell Reports 13:350–364.

Matzkin, L. M. 2012. Population transcriptomics of cactus host shifts in *Drosophila mojavensis*. Molecular Ecology 21:2428–2439.

Milan, N. F., B. Z. Kacsoh, and T. A. Schlenke. 2012. Alcohol consumption as self-medication against blood-borne parasites in the fruit fly. Current Biology 22:488–493.

Morozova, T. V., R. R. H. Anholt, and T. F. C. Mackay. 2006. Transcriptional response to alcohol exposure in *Drosophila melanogaster*. Genome Biology 7:R95.

———. 2007. Phenotypic and transcriptional response to selection for alcohol sensitivity in *Drosophila melanogaster*. Genome Biology 8:R231.

Morris, M. R. J., R. Richard, E. H. Leder, R. D. H. Barrett, N. Aubin-Horth, and S. M. Rogers. 2014. Gene expression plasticity evolves in response to colonization of freshwater lakes in threespine stickleback. Molecular Ecology 23:3226–3240.

Moya, A., L. Huisman, S. Forêt, J. P. Gattuso, D. C. Hayward, E. E. Ball, and D. J. Miller. 2015. Rapid acclimation of juvenile corals to CO_2-mediated acidification by upregulation of heat shock protein and Bcl-2 genes. Molecular Ecology 24:438–452.

Newell, N. R., F. N. New, J. E. Dalton, L. M. McIntyre, and M. N. Arbeitman. 2016. Neurons that underlie *Drosophila melanogaster* reproductive behaviors: detection of a large male-bias in gene expression in *fruitless*-expressing neurons. Genes, Genomes, Genetics 6:2455–2465.

Newman, J. R. B., A. Conesa, M. Mika, F. N. New, S. Onengut-Gumuscu, M. A. Atkinson, S. S. Rich, et al. 2017. Disease-specific biases in alternative splicing and tissue-specific dysregulation revealed by multitissue profiling of lymphocyte gene expression in type 1 diabetes. Genome Research 27:1807–1815.

Newton, P. M., K. Tully, T. McMahon, J. Connolly, J. Dadgar, S. N. Treistman, and R. O. Messing. 2004. Chronic ethanol exposure induces an N-type calcium channel splice variant with altered channel kinetics. FEBS Letters 579:671–676.

Nuzhdin, S. V., M. L. Friesen, and L. M. McIntyre. 2012. Genotype-phenotype mapping in a post-GWAS world. Trends in Genetics 28:421–426.

Oomizu, S., N. Boyadjieva, and D. K. Sarkar. 2003. Ethanol and estradiol modulate alternative splicing of dopamine D2 receptor messenger RNA and abolish the inhibitory action of bromocriptine on prolactin release from the pituitary gland. Alcoholism: Clinical and Experimental Research 27:975–980.

Paaby, A. B., and M. V. Rockman. 2014. Cryptic genetic variation: evolution's hidden substrate. Nature Reviews Genetics 15:247–258.

Pietrzykowski, A. Z., R. M. Friesen, G. E. Martin, S. I. Puig, C. L. Nowak, P. M. Wynne, H. T. Siegelmann, et al. 2008. Posttranscriptional regulation of BK channel splice variant stability by miR-9 underlies neuroadaptation to alcohol. Neuron 59:274–287.

Perron, J. M., L. Huot, G. W. Corrivault, and S. S. Chawla. 1972. Effects of carbon dioxide anaesthesia on *Drosophila melanogaster*. Journal of Insect Physiology 18:1869–1874.

Pohl, J. B., B. A. Baldwin, B. L. Dinh, P. Rahman, D. Smerek, F. J. Prado, N. Sherazee, et al. 2012. Ethanol preference in *Drosophila melanogaster* is driven by its caloric value. Alcoholism: Clinical and Experimental Research 36:1903–1912.

Quinn, J. J., Q. C. Zhang, P. Georgiev, I. A. Ilik, A. Akhtar, and H. Y. Chang. 2016. Rapid evolutionary turnover underlies conserved lncRNA-genome interactions. Genes and Development 30:191–207.

Rearick, D., A. Prakash, A. McSweeny, S. S. Shepard, L. Fedorova, and A. Fedorov. 2010. Critical association of ncRNA with introns. Nucleic Acids Research 39:2357–2366.

Robinson, B. G., and N. S. Atkinson. 2013. Is alcoholism learned? insights from the fruit fly. Current Opinion in Neurobiology 23:529–534.

Robinson, B. W. 2013. Evolution of growth by genetic accommodation in Icelandic freshwater stickleback. Proceedings of the Royal Society B 280:20132197.

Rutherford, S. L. 2000. From genotype to phenotype: buffering mechanisms and the storage of genetic information. BioEssays 22:1095–1105.

Saltz, J. B., A. M. Bell, J. Flint, R. Gomulkiewicz, K. A. Hughes, and J. Keagy. 2018. Why does the magnitude of genotype-by-environment interaction vary? Ecology and Evolution 8:6342–6353.

Sasabe, T., and S. Ishiura. 2010. Alcoholism and alternative splicing of candidate genes. International Journal of Environmental Research and Public Health 7:1448–1466.

Schlichting, C. D. 2008. Hidden reaction norms, cryptic genetic variation, and evolvability. Annals of the New York Academy of Sciences 1133:187–203.

Schlichting, C. D., and M. A. Wund. 2014. Phenotypic plasticity and epigenetic marking: an assessment of evidence for genetic accommodation. Evolution 68:656–672.

Seneca, F. O., and S. R. Palumbi. 2015. The role of transcriptome resilience in resistance of corals to bleaching. Molecular Ecology 24:1467–1484.

Shen, S.-M., C. Zhang, M.-K. Ge, S.-S. Dong, L. Xia, P. He, N. Zhang, et al. 2019. PTENα and PTENβ promote carcinogenesis through WDR5 and H3K4 trimethylation. Nature Cell Biology 21:1436–1448.

Signor, S. A., M. Abbasi, P. Marjoram, and S. V. Nuzhdin. 2017*a*. Conservation of social effects (Ψ) between two species of *Drosophila* despite reversal of sexual dimorphism. Ecology and Evolution 7:10031–10041.

———. 2017*b*. Social effects for locomotion vary between environments in *Drosophila melanogaster* females. Evolution 71:1765–1775.

Signor, S. A., F. N. New, and S. Nuzhdin. 2017*c*. A large panel of *Drosophila simulans* reveals an abundance of common variants. Genome Biology and Evolution 10:189–206.

Signor, S., and S. Nuzhdin. 2018. Dynamic changes in gene expression and alternative splicing mediate the response to acute alcohol exposure in *Drosophila melanogaster*. Heredity 121:342–360.

Telonis-Scott, M., A. S. Clemson, T. K. Johnson, and C. M. Sgrò. 2014. Spatial analysis of gene regulation reveals new insights into the molecular basis of upper thermal limits. Molecular Ecology 23:6135–6151.

Ulitsky, I. 2016. Evolution to the rescue: using comparative genomics to understand long non-coding RNAs. Nature Reviews Genetics 17:601–614.

Ulitsky, I., A. Shkumatava, C. H. Jan, H. Sive, and D. P. Bartel. 2011. Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. Cell 147:1537–1550.

Via, S., and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505–522.

Wahlsten, D. 2001. Standardizing tests of mouse behavior: reasons, recommendations, and reality. Physiology and Behavior 73:695–704.

West-Eberhard, M. J. 2005. Developmental plasticity and the origin of species differences. Proceedings of the National Academy of Sciences of the USA 1:6543–6549.

Yang, H.-P., and S. V. Nuzhdin. 2003. Fitness costs of *Doc* expression are insufficient to stabilize its copy number in *Drosophila melanogaster*. Molecular Biology and Evolution 20:800–804.

Zaharieva, E., J. K. Chipman, and M. Soller. 2012. Alternative splicing interference by xenobiotics. Toxicology 296:1–12.

Zhu, J., and J. D. Fry. 2015. Preference for ethanol in feeding and oviposition in temperate and tropical populations of *Drosophila melanogaster*. Entomologia Experimentalis et Applicata 155:64–70.

Associate Editor: Jeff Leips
Editor: Russell Bonduriansky

"The authoress has been fortunate in her sources of information and in her artists. She wields an easy and graceful pen, has the art of vivid description and good generalizing power. . . . It is just the book to use in schools as a reader, or for collateral reading by classes in zoölogy." From the review of Buckley's *Winners in Life's Race* (*The American Naturalist*, 1883, 17:47–50).