## GEPHE SUMMARY

**Gephebase Gene**
alcohol dehydrogenase (Adh)

GephelD
GP00001962

Entry Status
Published

Main curator
Courtier

## PHENOTYPIC CHANGE

**Trait Category**
Physiology

**Trait**
Xenobiotic resistance (alcohol)

**Trait State in Taxon A**
**Drosophila melanogaster - slow allele - lower enzyme activity**

**Trait State in Taxon B**
**Drosophila melanogaster - fast allele - higher enzyme activity**

**Ancestral State**
Taxon A

**Taxonomic Status**
Intraspecific

### Taxon A

*Latin Name*
*Drosophila melanogaster*

Common Name
fruit fly

Synonyms
Sophophora melanogaster; fruit fly; Drosophila melanogaster Meigen, 1830; Sophophora melanogaster (Meigen, 1830); Drosophila melangaster

Rank
species

Lineage
cellular organisms; Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Holometabola; Diptera; Brachycera; Muscomorpha; Eremoneura; Cyclorrhapha; Schizophora; Acalyptratae; Ephydroidea; Drosophilidae; Drosophilinae; Drosophilini; Drosophila; Sophophora; melanogaster group; melanogaster subgroup

Parent
melanogaster subgroup () - (Rank: species subgroup)

**NCBI Taxonomy ID**
7227

**is Taxon A an Infraspecies?**
No

### Taxon B

*Latin Name*
*Drosophila melanogaster*

Common Name
fruit fly

Synonyms
Sophophora melanogaster; fruit fly; Drosophila melanogaster Meigen, 1830; Sophophora melanogaster (Meigen, 1830); Drosophila melangaster

Rank
species

Lineage
cellular organisms; Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Holometabola; Diptera; Brachycera; Muscomorpha; Eremoneura; Cyclorrhapha; Schizophora; Acalyptratae; Ephydroidea; Drosophilidae; Drosophilinae; Drosophilini; Drosophila; Sophophora; melanogaster group; melanogaster subgroup

Parent
melanogaster subgroup () - (Rank: species subgroup)

**NCBI Taxonomy ID**
7227

**is Taxon B an Infraspecies?**
No

## GENOTYPIC CHANGE

**Generic Gene Name**
Adh

Synonyms
adh; ADH; Adh3; BG:DS01486.8; CG32954; CG3481; dADH; DM-ADH; DmADH; Dmel\CG3481; Dreg-1; Reg-1; T16

String
7227.FBpp0100048

Sequence Similarities
Belongs to the short-chain dehydrogenases/reductases (SDR) family.

GO - Molecular Function
GO:0042803 : protein homodimerization activity
GO:0008774 : acetaldehyde dehydrogenase (acetylating) activity
GO:0004022 : alcohol dehydrogenase (NAD) activity
GO:0016491 : oxidoreductase activity

GO - Biological Process
GO:0006117 : acetaldehyde metabolic process

**UniProtKB** Drosophila melanogaster
P00334

**GenebankID or UniProtKB**
M22210

GO:0046164 : alcohol catabolic process
GO:0006066 : alcohol metabolic process
GO:0048149 : behavioral response to ethanol
GO:0006067 : ethanol metabolic process
GO:0006069 : ethanol oxidation
GO:0055114 : oxidation-reduction process

GO - Cellular Component
GO:0005829 : cytosol
GO:0032991 : protein-containing complex

**Mutation #1**
**Presumptive Null**
No

**Molecular Type**
Coding

**Aberration Type**
SNP

**SNP Coding Change**
Nonsynonymous

**Molecular Details of the Mutation**
**Thr192Lys. Historical note : this discovery is actually based on progressive mapping started before 1980; and on early population genetics work by Kreitman 1983 (PMID: 6410283). There are three other (noncoding) substitutions in Adh coding regions. In vitro tests showed that none of the other three noncoding substitutions contributed a detectable effect. The K192T substitution is thus sufficient to explain the entirety of the activity difference from the coding region.**

**Experimental Evidence**
Linkage Mapping

| | Taxon A | Taxon B | Position |
|---|---|---|---|
| Codon | - | - | - |
| Amino-acid | - | - | - |

Main Reference
Use of in vitro mutagenesis to analyze the molecular basis of the difference in Adh expression associated with the allozyme polymorphism in Drosophila melanogaster. (1991)

Authors
Choudhary M; Laurie CC

Abstract
In natural populations of Drosophila melanogaster, the alcohol dehydrogenase (Adh) locus is polymorphic for two allozymes, designated Slow and Fast. Fast homozygotes generally have a two- to threefold higher ADH activity level than Slow homozygotes for two reasons: they have a higher concentration of ADH protein and the Fast protein has a higher catalytic efficiency. DNA sequencing studies have shown that the two allozymes generally differ by only a single amino acid at residue 192, which must therefore be the cause of the catalytic efficiency difference. A previous P element-transformation experiment mapped the difference in ADH protein level to a 2.3-kb HpaI/ClaI restriction fragment; which contains all of the Adh coding sequences but excludes all of the 5′ flanking region of the distal transcriptional unit. Here we report the results of a site-directed in vitro mutagenesis experiment designed to investigate the effects of the amino acid replacement. This replacement has the expected effect on catalytic efficiency, but there is no detectable effect on the concentration of ADH protein estimated immunologically. This result shows that the average difference in ADH protein level between the allozymic classes is due to linkage disequilibrium between the amino acid replacement and one or more other polymorphisms within the HpaI/ClaI fragment. Sequence analysis of several Fast and Slow alleles suggested that the other polymorphism might be a silent substitution at nucleotide 1443, but another in vitro mutagenesis experiment reported here shows that this is not the case. Therefore, the molecular basis of the difference in ADH protein concentration between the allozymic classes remains an open question.

Additional References
Associations between DNA sequence variation and variation in expression of the Adh gene in natural populations of Drosophila melanogaster. (1991)

Genetic dissection of a model complex trait using the Drosophila Synthetic Population Resource. (2012)

Experimental test and refutation of a classic case of molecular adaptation in Drosophila melanogaster. (2017)

A major role for noncoding regulatory mutations in the evolution of enzyme activity. (2019)

**Mutation #2**
**Presumptive Null**
No

**Molecular Type**
Cis-regulatory

**Aberration Type**
Indel

**Indel Size**
10-99 bp

**Molecular Details of the Mutation**
**Indel in 5'UTR intron. Slow alleles nearly always have the 29 bp version of delta1 and Fast alleles predominantly have the 34bp version.**

**Experimental Evidence**
Linkage Mapping

Main Reference
The effect of an intronic polymorphism on alcohol dehydrogenase expression in Drosophila melanogaster. (1994)

Authors

Laurie CC; Stam LF

Abstract

Several lines of evidence indicate that natural selection controls the frequencies of an allozyme polymorphism at the alcohol dehydrogenase (Adh) locus in Drosophila melanogaster. However, because of associations among sequence polymorphisms in the Adh region, it is not clear whether selection acts directly (or solely) on the allozymic site. This problem has been approached by using in vitro mutagenesis to distinguish among the effects on Adh expression of individual polymorphisms. This study shows that a polymorphism within the first Adh intron (delta 1) has a significant effect on the level of ADH protein. Like the allozyme, delta 1 shows a geographic cline in frequency, indicating that it may also be a target of natural selection. These results suggest that multisite selection models may be required to understand the evolutionary dynamics of individual loci.

Additional References

Genetic dissection of a model complex trait using the Drosophila Synthetic Population Resource. (2012)

Experimental test and refutation of a classic case of molecular adaptation in Drosophila melanogaster. (2017)

A major role for noncoding regulatory mutations in the evolution of enzyme activity. (2019)

**Mutation #3**

**Presumptive Null**

**No**

**Molecular Type**

**Cis-regulatory**

**Aberration Type**

**Unknown**

**Molecular Details of the Mutation**

**mutation in 3′UTR region. Two substitutions occur in the pair of fast and slow alleles from Loehlin et al 2019: a poly-A tract of length 14 in slow and length 15 in fast, and a complex substitution of sequence CA in slow and G- in fast. Authors attempted to separate the effects of the A(14)/A(15) from the CA/G- with a construct that was recombined between the two sites. Activity of this construct was not significantly different from either neighboring construct; indicating that they did not have sufficient power to determine the causative site(s). In spite of this; Stam and Laurie also mapped an activity difference with a similar effect size of 1.1-fold to a region containing the 3â€™-UTR. Their fast and slow haplotypes carry a different suite of substitutions in this region; but the C/G nucleotide substitution is shared in both studies. Additional mapping work would be required to determine if this one substitution or if multiple substitutions are responsible for the activity difference from the 3′-UTR.**

**Experimental Evidence**

**Candidate Gene**

Main Reference

Molecular dissection of a major gene effect on a quantitative trait: the level of alcohol dehydrogenase expression in Drosophila melanogaster. (1996)

Authors

Stam LF; Laurie CC

Abstract

A molecular mapping experiment shows that a major gene effect on a quantitative trait, the level of alcohol dehydrogenase expression in Drosophila melanogaster, is due to multiple polymorphisms within the Adh gene. These polymorphisms are located in an intron, the coding sequence, and the 3′ untranslated region. Because of nonrandom associations among polymorphisms at different sites, the individual effects combine (in some cases epistatically) to produce "superalleles" with large effect. These results have implications for the interpretation of major gene effects detected by quantitative trait locus mapping methods. They show that large effects due to a single locus may be due to multiple associated polymorphisms (or sequential fixations in isolated populations) rather than individual mutations of large effect.

Additional References

A major role for noncoding regulatory mutations in the evolution of enzyme activity. (2019)

**Mutation #4**

**Presumptive Null**

**No**

**Molecular Type**

**Cis-regulatory**

**Aberration Type**

**SNP**

**Molecular Details of the Mutation**

**C/T SNP in 5′ flanking region upstream of the 5′UTR is responsible for the change in Adh activity. Adh-slow: TCACCGATT; Adh-fast: TCATGCATT (where TCAC is the end of the 5′ flanking region and CGATT the beginning of the 5′UTR). The causing mutation seems to be a 3-bp multi-nucleotide mutation.**

**Experimental Evidence**

**Candidate Gene**

Main Reference

A major role for noncoding regulatory mutations in the evolution of enzyme activity. (2019)

Authors

Loehlin DW; Ames JR; Vaccaro K; Carroll SB

Abstract

The quantitative evolution of protein activity is a common phenomenon, yet we know little about any general mechanistic tendencies that underlie it. For example, an increase (or decrease) in enzyme activity may evolve from changes in protein sequence that alter specific activity, or from changes in gene expression that alter the amount of protein produced. The latter in turn could arise via mutations that affect gene transcription, posttranscriptional processes, or copy number. Here, to determine the types of genetic changes underlying the quantitative evolution of protein activity, we dissected the basis of ecologically relevant differences in Alcohol dehydrogenase (Adh) enzyme activity between and within several Drosophila species. By using recombinant Adh transgenes to map the functional divergence of ADH enzyme activity in vivo, we find that amino acid substitutions explain only a minority (0 to 25%) of between- and within-species differences in enzyme activity. Instead, noncoding substitutions that occur across many parts of the gene (enhancer, promoter, and 5′ and 3′ untranslated regions) account for the majority of activity differences. Surprisingly, one substitution in a transcriptional Initiator element has occurred in parallel in two species, indicating that core promoters can be an important natural source of the tuning of gene activity. Furthermore, we show that both regulatory and coding substitutions contribute to fitness (resistance to ethanol toxicity). Although qualitative changes in protein specificity necessarily derive from coding mutations, these results suggest that regulatory mutations may be the primary source of quantitative changes in protein activity, a possibility overlooked in most analyses of protein evolution.

Additional References

**Mutation #5**
**Presumptive Null**
No

**Molecular Type**
Cis-regulatory

**Aberration Type**
SNP

**Molecular Details of the Mutation**
C/G SNP in 5′UTR region

**Experimental Evidence**
Candidate Gene

Main Reference
A major role for noncoding regulatory mutations in the evolution of enzyme activity. (2019)

Authors
Loehlin DW; Ames JR; Vaccaro K; Carroll SB

Abstract
The quantitative evolution of protein activity is a common phenomenon, yet we know little about any general mechanistic tendencies that underlie it. For example, an increase (or decrease) in enzyme activity may evolve from changes in protein sequence that alter specific activity, or from changes in gene expression that alter the amount of protein produced. The latter in turn could arise via mutations that affect gene transcription, posttranscriptional processes, or copy number. Here, to determine the types of genetic changes underlying the quantitative evolution of protein activity, we dissected the basis of ecologically relevant differences in Alcohol dehydrogenase (Adh) enzyme activity between and within several Drosophila species. By using recombinant Adh transgenes to map the functional divergence of ADH enzyme activity in vivo, we find that amino acid substitutions explain only a minority (0 to 25%) of between- and within-species differences in enzyme activity. Instead, noncoding substitutions that occur across many parts of the gene (enhancer, promoter, and 5′ and 3′ untranslated regions) account for the majority of activity differences. Surprisingly, one substitution in a transcriptional Initiator element has occurred in parallel in two species, indicating that core promoters can be an important natural source of the tuning of gene activity. Furthermore, we show that both regulatory and coding substitutions contribute to fitness (resistance to ethanol toxicity). Although qualitative changes in protein specificity necessarily derive from coding mutations, these results suggest that regulatory mutations may be the primary source of quantitative changes in protein activity, a possibility overlooked in most analyses of protein evolution.



Additional References

**Mutation #6**
**Presumptive Null**
No

**Molecular Type**
Cis-regulatory

**Aberration Type**
Insertion

**Insertion Size**
10-99 bp

**Molecular Details of the Mutation**
delta-2 indel in 5′UTR region. 37-bp insertion in the fast allele.

**Experimental Evidence**
Candidate Gene

Main Reference
A major role for noncoding regulatory mutations in the evolution of enzyme activity. (2019)

Authors
Loehlin DW; Ames JR; Vaccaro K; Carroll SB

Abstract
The quantitative evolution of protein activity is a common phenomenon, yet we know little about any general mechanistic tendencies that underlie it. For example, an increase (or decrease) in enzyme activity may evolve from changes in protein sequence that alter specific activity, or from changes in gene expression that alter the amount of protein produced. The latter in turn could arise via mutations that affect gene transcription, posttranscriptional processes, or copy number. Here, to determine the types of genetic changes underlying the quantitative evolution of protein activity, we dissected the basis of ecologically relevant differences in Alcohol dehydrogenase (Adh) enzyme activity between and within several Drosophila species. By using recombinant Adh transgenes to map the functional divergence of ADH enzyme activity in vivo, we find that amino acid substitutions explain only a minority (0 to 25%) of between- and within-species differences in enzyme activity. Instead, noncoding substitutions that occur across many parts of the gene (enhancer, promoter, and 5′ and 3′ untranslated regions) account for the majority of activity differences. Surprisingly, one substitution in a transcriptional Initiator element has occurred in parallel in two species, indicating that core promoters can be an important natural source of the tuning of gene activity. Furthermore, we show that both regulatory and coding substitutions contribute to fitness (resistance to ethanol toxicity). Although qualitative changes in protein specificity necessarily derive from coding mutations, these results suggest that regulatory mutations may be the primary source of quantitative changes in protein activity, a possibility overlooked in most analyses of protein evolution.



Additional References

## RELATED GEPHE

Related Genes

17 (Acetylcholinesterase (Ace-2), Aldehyde dehydrogenase (Aldh), CG11699, Cyp12d1, Cyp28d1, Cyp28d1-Cyp28d2, cyp6d2, cyp6g1, GSS (glutathione synthetase), GSTE1-E10 cluster, kin of irre (kire), PHGPx, resistance to dieldrin, RnrS, SOD1, Ugt86Dd, CHKov1)

Related Haplotypes

4

## EXTERNAL LINKS

## COMMENTS

@SeveralMutationsWithEffect @Fitness - All six higher-activity variants appear to be derived; suggesting a history of directional selection. The three causative substitutions in the 5â€² UTR occur within 100 bp. - Entry validated by David Loehlin - http://flybase.org/reports/FBal0000310 - http://flybase.org/reports/FBal0000314