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Insertion of the retroposable element, *jockey*, near the *Adh* gene of *Drosophila melanogaster* is associated with altered gene expression

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Summary

The *alcohol dehydrogenase* (*Adh*) gene of *Drosophila melanogaster* is well suited to be a gene expression reporter system. *Adh* produces a measurable phenotype at both the enzyme and mRNA levels. We recovered a spontaneous transposable element (TE) insertion mutation near the *Adh* gene. The insertion is a truncated retroposable element, *jockey*, inserted upstream of the adult *Adh* enhancer region. Comparisons between the Adh^{jockey} allele and its direct wild-type ancestral allele were made in an isogenic background (i.e. identical *cis* and *trans* factors). Differences in Adh^{jockey} expression compared with the wild-type can be attributed solely to the presence of the *jockey* element. This *jockey* insertion results in a decrease in adult mRNA transcript levels in the Adh^{jockey} homozygous lines relative to the wild-type counterpart and accounts for a correlated decrease in alcohol dehydrogenase (ADH) enzyme activity. The larval ADH activity levels are not detectably different.

1. INTRODUCTION

Transposable elements (TEs) are DNA sequences that have the ability to replicate and move from site to site within a host genome. This inherent mobility can introduce a plethora of effects on the host genome that range from molecular structural changes (Lim, 1988; Engels, 1989; Tsubota & Dang-Vu, 1991; Lytte and Haymer, 1993) to temporal or spatial alterations of gene regulation (Levis, *et al.*, 1984; Itoh *et al.*, 1988; Stavenhagen & Robins, 1988; Strand and McDonald, 1989; Geyer & Corces, 1992; Mogila *et al.*, 1993; Dunn & Laurie, 1995); and from decreased fitness to increased genetic variation. Increases in genetic variation can lead to an increase in an organism's ability to adapt to changes in the environment (MacKay, 1985; McDonald, 1993).

Our ability to study the effects of TEs is hampered by several factors. Most studies of TE–host interactions are comparisons of a mutated allele from one stock of flies with a standard wild-type stock. The problems presented by this approach are: (a) the mutant allele and the wild-type allele in the comparison are not genetically related and (b) the genetic background of (*cis* and *trans* factors that produce the total expression phenotype) of the two strains is not identical. These problems may be circumvented by comparing the expression of a mutant allele with that of its ancestral wild-type form in flies of the same genetic background.

The *Alcohol dehydrogenase* (*Adh*) gene of *Drosophila melanogaster* is an excellent model system for studying the effects of a TE-induced mutation on differential gene expression. *Adh* is expressed in a temporal and spatial manner and has been studied extensively. The expression of *Adh* is measurable at both the mRNA and enzyme level, and is one of the few examples where a phenotype is directly correlated with an environmental factor – alcohol. Previous work has shown that *Adh* is controlled by multiple *trans*- and *cis*-acting factors, and therefore homogeneity of genetic background is essential when making comparisons between *Adh* alleles (Maroni *et al.*, 1982; Maroni & Laurie-Ahlberg, 1983). We have recovered a spontaneous insertion mutation of the *Adh* gene in an isogenic laboratory stock of *D. melanogaster* (White & Jacobson, 1996). The insertion mutation is a 296 bp truncated 3′ end of a *jockey* transposable element (Priimägi *et al.* 1988) designated Adh^{jockey} (White & Jacobson, 1996). The *jockey* insertion is located 670 bp upstream of the *Adh* distal promoter in a region previously identified as a putative larval enhancer

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region (Corbin & Maniatis, 1990). This mutation apparently did not exist when the line was established (Laurie-Ahlberg *et al.*, 1980). When discovered, the new mutant allele was in a heterozygous state with the original wild-type allele. Crosses were carried out to isolate the mutant and wild-type alleles into isogenic lines of the two types. These lines are genetically identical except for the presence or absence of the insertion mutation.

Because of the way the lines were constructed and maintained, any differences in gene expression between the two strains should be attributable solely to the presence of the insertion mutation and not to heterogeneity of genetic background. This system provides a unique way to study the effects of spontaneous insertions on gene expression.

2. MATERIALS AND METHODS

(i) *Drosophila strains*

Canton S and Oregon R are standard wild-type laboratory stocks obtained from the Mid-American Drosophila Stock Center (Bowling Green, OH). RI21,III and RI22,III are isogenic third chromosome substitution stocks constructed by C. Laurie (Laurie-Ahlberg *et al.*, 1980) and obtained from C. Laurie in 1987. CL55 is the *Ho-R* strain used by C. Laurie to construct the isogenic third chromosome substitution lines. The *CyO/PmSp* chromosomes were extracted into the CL55 isogenic background by C. Laurie and this strain is designated I255 (or isogenic second chromosome substitution into CL55). Adh^{nLA248} (obtained from C. Laurie) is a null *Adh* allele (Aaron *et al.*, 1979) in a *b*, *black*; *cn*, *cinnabar*; *bw*, *brown* background resulting in white-eyed black-bodied flies. Other mutant alleles referred to can be found in Lindsley & Zimm (1992).

(ii) *Strain construction and background of lines*

The spontaneous insertion occurred in a third chromosome substitution line, RI22,III. The third chromosome substitution lines were constructed so that third chromosomes from wild populations were substituted into an isogenic background, using a laboratory strain CL55 (*Ho-R*) as the isogenic background stock and a balancer stock, *TM6*, *Ubx/Sb* (Laurie-Ahlberg *et al.*, 1980). Construction of strains that are homozygous for either the mutant allele or the wild-type allele was necessary for the precise determination of any differences caused by the presence of the mutation (Fig. 1). *Adh* is located on the second chromosome of *D. melanogaster* and an isogenic second chromosome balancer stock, *CyO/PmSp* (I255), was used to extract the second chromosome from the heterozygous stock into a controlled genetic background. Individual female RI22,III flies were mated with individual

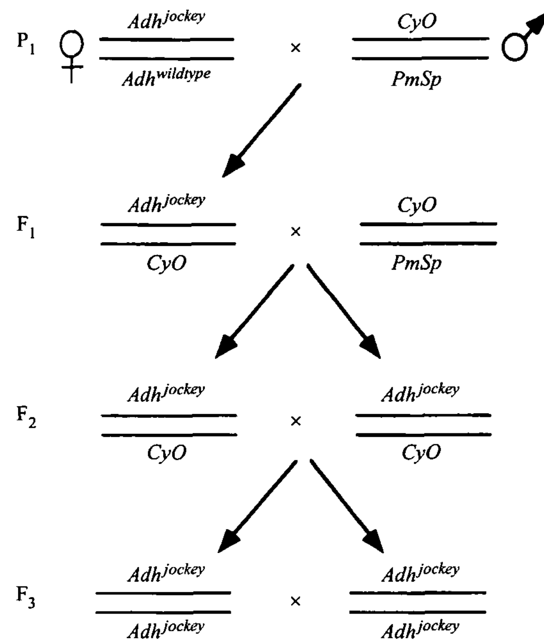

Fig. 1. Crossing scheme to isolate the Adh^{jockey} and $Adh^{wild\text{-}type}$ alleles into isogenic lines. Female RI22,III were crossed to an isogenic second chromosome *CyO/PmSp* balancer strain (I255). F_1 progeny were backcrossed to I255 and F_2 Adh^{jockey}/CyO females were crossed to their Adh^{jockey}/CyO brothers. Homozygous Adh^{jockey} females were crossed to their homozygous brothers. The resultant strains Adh^{jockey} and $Adh^{wild\text{-}type}$ are identical except for the presence or absence of the *jockey* insertion mutation.

CyO/PmSp males. *CyO*/+ F_1 females were collected and individually backcrossed to single *CyO/PmSp* males. *CyO*/+ F_2 females were crossed with their *CyO*/+ F_2 male siblings. The F_3 +/+ flies from each line were then crossed together and maintained as separate stocks to test for Adh^{jockey} or $Adh^{wild\text{-}type}$ homozygosity. Each stock was tested by Southern blot analysis for the presence or absence of the mutant allele. Several lines of each strain (Adh^{jockey} or $Adh^{wild\text{-}type}$) were retained.

(iii) *Genomic DNA isolation and analysis*

Genomic DNA was isolated and analysed as described previously (White & Jacobson, 1996).

(iv) *Alcohol dehydrogenase activity*

The level of alcohol dehydrogenase (ADH) enzyme activity was quantified using a spectrophotometric measure of the reduction of NAD+ to NADH over time (Stam & Laurie-Ahlberg, 1982) with isopropanol as a substrate (David *et al.*, 1981). Animals from two different developmental stages were assayed. Adult male flies were aged to 7–9 days post-eclosion, separated into groups of three, weighed for live weight values on a Cahn microbalance, homogenized in

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0·01 M KPO_4, pH 7·4; 1·0 mM EDTA, and then assayed with a Hewlett-Packard spectrophotometer at 340 nm using a solution of 40·0 mM glycine, pH 9·5; 1·4 mM NAD; 0·8 M isopropanol as a substrate. Activities were obtained by taking the raw activity value and dividing by live weight to eliminate differences due to the amount of tissue per group. Activity units are in mOD/min per mg fly. Two blocks of experiments were done in different weeks. Larval ADH assays were performed on staged early third instar larvae. Larvae were collected in bulk from each isogenic line, washed free of media, separated into groups of five, homogenized in 0·01 M KPO_4, pH 7·4; 1·0 mM EDTA, and frozen at −80 °C. Assays were done in one day (to eliminate day-to-day differences) on a Thermomax Micro Plate Reader (Molecular Devices, Menlo Park, CA) at 340 nm using a solution of 40·0 mM glycine, pH 9·5; 1·4 mM NAD; 0·8 M isopropanol as a substrate. The total protein content of each group of five larvae was determined (Lowry *et al.*, 1951) and raw activity values were corrected by total protein to eliminate differences due to the amount of tissue per group of larvae. Activity units are mOD/min per mg total protein. F_1 progeny from reciprocal crosses (Adh^{jockey} females × $Adh^{wild\text{-}type}$ males and $Adh^{wild\text{-}type}$ females × Adh^{jockey} males) along with the representative parentals were aged to 7–9 days post-eclosion and assayed as described above for adults on a Thermomax Micro Plate Reader (Molecular Devices, Menlo Park, CA) at 340 nm.

(v) *RNA experiment stocks*

Transcript levels of the two strains (Adh^{jockey} and $Adh^{wild\text{-}type}$) were measured using the null allele Adh^{nLA248} as an internal control. The allele is an X-ray induced (Aaron *et al.*, 1979) duplication that makes an *Adh* transcript 200 bp longer than the wild-type allele (Chia *et al.*, 1985). Heterozygotes for Adh^{nLA248} and wild-type produce two discernible mRNA bands on an RNA gel blot (Savakis *et al.*, 1986). It has been shown that the use of 'mock' heterozygotes (equal numbers of Adh^{nLA248} and wild-type flies) give results with no significant differences compared with the use of true heterozygotes (F_1 progeny from a cross of Adh^{nLA248} and the experimental lines (Laurie & Stam, 1988). In this way, the total mRNA level from each line is compared relative to the Adh^{nLA248} transcript in the same lane. Comparisons within-lane between the Adh^{nLA248} transcript and the Adh^{jockey} or $Adh^{wild\text{-}type}$ transcripts were done.

(vi) *RNA preparation*

Adult male flies from strains Adh^{jockey}, $Adh^{wild\text{-}type}$ and Adh^{nLA248} were aged to 7–9 days post-eclosion and then separated into groups of 50 and frozen at −80 °C. Mock heterozygotes were generated by adding groups of 50 flies together. These 100 flies were homogenized in a motor-driven Dounce glass homogenizer using the RNAgents Total RNA Isolation System (Promega, Madison, WI). Tissue was disrupted in a homogenization buffer consisting of guanidine thiocyanate; 42 mM sodium citrate, pH 4·0; 0·83% *N*-lauroyl sarcosine; 0·2 mM ß-mercaptoethanol. Sodium acetate, pH 4·0, was added to a final concentration of 0·182 M. Phenol:chloroform:isoamyl alcohol was added and after 10 s of vigorous shaking, the mixture was chilled on ice for 15 min. The phases were separated by centrifugation at 10000 *g* for 20 min at 4 °C. The aqueous phase was removed to a new tube and the RNA was precipitated with an equal volume of isopropanol for 30 min at −20 °C. The precipitated RNA was pelleted by centrifugation at 10000 *g* for 15 min at 4 °C. The RNA pellet was resuspended in homogenization buffer and reprecipitated with an equal volume of isopropanol as described above. The RNA pellet was washed with 75% ice-cold ethanol and recentrifuged as described above. The pellet was dried under a vacuum and resuspended in RNAse-free water.

(vii) *Northern blot assays*

Four aliquots of each mock heterozygote RNA preparation were run on denaturing gels. Mock heterozygotes were $Adh^{jockey}/Adh^{nLA248}$ and $Adh^{wild\text{-}type}/Adh^{nLA248}$. Two replicates of each mock heterozygote sample were assayed in two blocks on different days. Formaldehyde/agarose gel electrophoresis and transfer of RNA to nylon membranes were carried out by standard procedures (Sambrook *et al.*, 1989) and according to the membrane manufacturer's specifications (Amersham, Arlington Heights, IL). Nucleic acid was fixed to the filter by baking for 2 h at 80 °C. The RNA was quantified by relative hybridization (Jacobson, 1991). Plasmid p13E3 DNA was radiolabelled by the random labelling method (Feinberg & Vogelstein, 1983). Following hybridization in 5× SSPE, 5× Denhardt's reagent and 0·5% SDS at 65 °C, the filters were washed once with 2× SSPE; 0·1% SDS at room temperature, and once at 65 °C, for 15 min before wrapping with plastic wrap and exposure to Kodak X-ray film. Exposed film was aligned with radioactive ink and pieces of membrane corresponding to the autoradiographic signals were punched out and placed in mini-scintillation vials. The probe was stripped from the membrane with 0·2 N NaOH for 30 min at 45 °C. Scintillation cocktail was added and samples were counted on an LKB model 1209 liquid scintillation counter (Pharmacia LKB Biotechnology, Gaithersburg, MD). Relative hybridization is defined as the ratio of the mock heterozygote Adh^{jockey} or $Adh^{wild\text{-}type}$ cpm to the Adh^{nLA248} cpm.

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(viii) *Statistical analyses*

Data from the enzyme activity measurements were analysed for significant differences between the Adh^{jockey} and $Adh^{wild\text{-}type}$ strains with the SuperANOVA statistics program (Abacus Concepts, Berkeley, CA) and the SAS program package (SAS Institute, Cary, NC). Nested analysis of variance (ANOVA) tests were conducted between type (Adh^{jockey} and $Adh^{wild\text{-}type}$), on lines within type ($Adh^{jockey}1$, $Adh^{jockey}2$, $Adh^{jockey}3$; and $Adh^{wild\text{-}type}1$, $Adh^{wild\text{-}type}2$, $Adh^{wild\text{-}type}3$), and individual groups within line to account for any measurement error.

Relative hybridizations of mRNA transcripts for mock heterozygote groups were analysed for significant differences using the SuperANOVA program software. Nested ANOVA tests were conducted between type ($Adh^{jockey}/Adh^{nLA248}$, $Adh^{wild\text{-}type}/Adh^{nLA248}$), and on lines within type ($Adh^{jockey}1/Adh^{nLA248}$, $Adh^{jockey}2/Adh^{nLA248}$, $Adh^{jockey}3/Adh^{nLA248}$; and $Adh^{wild\text{-}type}1/Adh^{nLA248}$, $Adh^{wild\text{-}type}2/Adh^{nLA248}$).

3. RESULTS

(i) *ADH activity measurements*

To measure the effects of this *cis* insertion mutation, we compared the ADH enzyme activity levels from groups of adult males of the two isogenic strains, Adh^{jockey} and $Adh^{wild\text{-}type}$. Table 1 shows the corrected mean activities (mOD/min per mg fly) of the adult

Table 1. *Adult Adh^{jockey} and $Adh^{wild\text{-}type}$ enzyme activity*

(*a*) *Adult ADH activities*

	Adh^{jockey}	$Adh^{wild\text{-}type}$
Mean ± SE	0·062 ± 0·003*	0·093 ± 0·006
Mean ± SE	0·059 ± 0·003*	0·079 ± 0·002

(*b*) *Adult ADH activity ANOVA table*

Source	d.f.	SS	MS	*F* ratio	*P* value
Block	1	0·00030533	0·00030533	0·040	> 0·500
Type (block)	2	0·01527586	0·00763793	11·11	< 0·025
Line (type)	4	0·00274990	0·00068750	2·50	> 0·050
Indiv (line)	42	0·01154021	0·00027477	1·39	> 0·100
Error	49	0·00971680	0·00019830		

(*a*) Mean adult Adh^{jockey} and $Adh^{wild\text{-}type}$ ADH enzyme activities. Units are mOD/min per mg fly. *Significant at $P < 0{\cdot}05$.
(*b*) Nested analysis of variance table. 'Type' denotes Adh^{jockey} and $Adh^{wild\text{-}type}$ strains. 'Line' indicates separate Adh^{jockey} and $Adh^{wild\text{-}type}$ lines. Adh^{jockey} enzyme activity is significantly reduced from the wild-type.

Table 2. *Larval Adh^{jockey} and $Adh^{wild\text{-}type}$ enzyme activity*

(*a*) *Mean Larval ADH activities*

	Adh^{jockey}	$Adh^{wild\text{-}type}$
Mean ± SE	74·61 ± 1·08	82·51 ± 1·48
Mean ± SE	77·27 ± 1·27	73·13 ± 1·31

(*b*) *Larval ADH activity ANOVA table*

Source	d.f.	SS	MS	*F* ratio	*P* value
Block	1	1264·5355	1264·5355	0·336	> 0·500
Type (block)	2	7536·0040	3758·0020	3·334	> 0·100
Line (type)	4	4521·1595	1130·2898	1·190	> 0·250
Indiv (line)	277	263020·095	949·5310	18·266	< 0·005
Error	560	29110·2040	51·9825		

(*a*) Mean larval Adh^{jockey} and $Adh^{wild\text{-}type}$ ADH activity. Units are mOD/min per mg total protein.
(*b*) Nested analysis of variance table for larval ADH activity values. 'Type' denotes Adh^{jockey} and $Adh^{wild\text{-}type}$ strains. 'Line' indicates separate Adh^{jockey} and $Adh^{wild\text{-}type}$ lines. There are no differences between Adh^{jockey} and $Adh^{wild\text{-}type}$.

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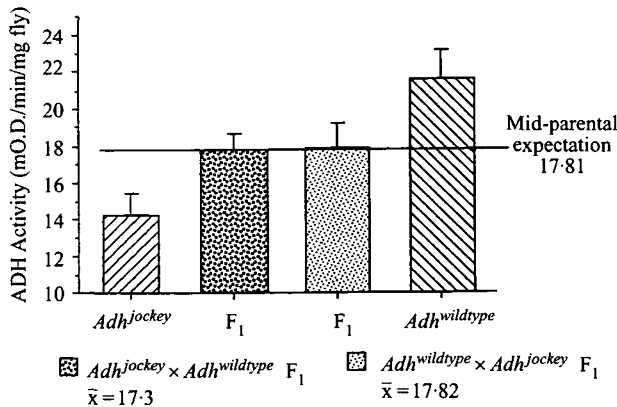

Fig. 2. F_1 ADH activity levels in comparision with the parental types (Adh^{jockey} and $Adh^{wild\text{-}type}$). The mid-parental expectation is the average of the Adh^{jockey} and $Adh^{wild\text{-}type}$ enzyme activities. Deviations from the mid-parental expectation indicate non-additive genetic variance. Reciprocal crosses of Adh^{jockey} and $Adh^{wild\text{-}type}$ yielded F_1 that were not significantly different from the mid-parental value. The Adh^{jockey} allele interacts with the wild-type in an additive manner. Any difference between Adh^{jockey} and $Adh^{wild\text{-}type}$ is associated only with the presence of the insertion allele. Error bars represent the standard error of the mean.

Adh^{jockey} and $Adh^{wild\text{-}type}$ lines. The mean Adh^{jockey} value is significantly different from the mean $Adh^{wild\text{-}type}$ value ($P < 0{\cdot}025$). There were no significant differences between experimental blocks, lines within type, or individual groups within line.

Activity levels from groups of early third instar larvae from Adh^{jockey} and $Adh^{wild\text{-}type}$ strains were measured and corrected mean activity levels (mOD/min per mg total protein) of each are shown in Table 2. There were no significant differences between Adh^{jockey} and $Adh^{wild\text{-}type}$ mean activities. Also, there were no significant differences between experimental blocks or lines within type. Individual larval groups within line showed a significant difference indicating possible measurement error.

The activity levels of the F_1 progeny from reciprocal crosses of Adh^{jockey} and $Adh^{wild\text{-}type}$ strains were measured and, as shown in Fig. 2, corrected mean activity levels (mOD/min per mg of fly) of the progeny are not significantly different from each other. The mid-parental expectation, 17·81 activity units, is the mean of the two parental activity values of 14·26 units (Adh^{jockey}) and 21·36 units ($Adh^{wild\text{-}type}$). Standard errors of the mean are 1·19, Adh^{jockey}; 0·85, F_1 $Adh^{jockey} \times Adh^{wild\text{-}type}$; 1·36, F_1 $Adh^{wild\text{-}type} \times Adh^{jockey}$; and 1·43, $Adh^{wild\text{-}type}$ (Fig. 2). The F_1 values from both reciprocal crosses show no significant differences from the mid-parental expectation using Student's *t*-test. These results indicate that no other genetic factors are likely to explain the expression differences of the *Adh* gene and that the two alleles are interacting in an additive genetic fashion.

(ii) *Relative hybridization of mRNA transcript levels*

To ascertain the basis of decreased enzyme activity, relative hybridizations of *Adh* mRNA from mock heterozygotes of $Adh^{nLA248}/(Adh^{jockey}, Adh^{wild\text{-}type})$ were measured by scintillation counting. The c.p.m. (radioactive counts per minute) values of experimental strain mRNA (Adh^{jockey} or $Adh^{wild\text{-}type}$) were measured and divided by the c.p.m. values of the internal control Adh^{nLA248} mRNA. There was a significant difference in mean mRNA levels (Table 3) between $Adh^{jockey}/Adh^{nLA248}$ and $Adh^{wild\text{-}type}/Adh^{nLA248}$ using ANOVA at $P < 0{\cdot}015$.

Table 3. *Adult Adh^{jockey} and $Adh^{wild\text{-}type}$ mRNA levels relative to Adh^{nLA248}*

(*a*) *Adult mRNA transcript levels*

	Adh^{jockey}	$Adh^{wild\text{-}type}$
Mean	0·947*	1·231
SD	0·091	0·071
SE	0·037	0·035

(*b*) *Adult mRNA transcript ANOVA table*

Source	d.f.	SS	MS	*F* ratio	*P* value
Type	1	0·192	0·192	32·0496	< 0·015
Line (type)	3	0·018	0·006	0·7852	> 0·551
Error	5	0·038	0·008		

(*a*) Mean adult Adh^{jockey} and $Adh^{wild\text{-}type}$ mRNA transcript levels relative to Adh^{nLA248} mRNA values (ratio of experimental radioactive c.p.m. to Adh^{nLA248} c.p.m.). *Significant at $P < 0{\cdot}015$.
(*b*) Nested analysis of variance table for adult Adh^{jockey} and $Adh^{wild\text{-}type}$ mRNA levels. Adh^{jockey} mRNA levels are significantly reduced from the wild-type.

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4. DISCUSSION

(i) *Separation of the mutant and ancestral alleles into isogenic lines*

The insertion mutation occurred in a line of *D. melanogaster* that had been maintained isogenically, and the mutant allele was segregating with its ancestral wild-type form in the common background. Crosses were designed and carried out to separate the two alleles (Adh^{jockey} and $Adh^{wild\text{-}type}$) into two homozygous isogenic lines. The results of these genetic crosses were two strains that differ only in the presence or absence of a *jockey* insertion near the *Adh* gene. The genetic background is identical in the two strains and is the same as the genetic background of the original third chromosome substitution line. These two strains allow a systematic study of the effects of a specific insertion mutation on a well-known gene–enzyme system, namely *Adh*.

(ii) *Adult and larval ADH activity assays*

The effect of the *jockey* insertion on the expression of the *Adh* gene was assayed by measuring the ADH activity levels of both Adh^{jockey} and $Adh^{wild\text{-}type}$ isogenic lines. The Adh^{jockey} allele appears to be associated with a significant decrease in adult ADH activity levels. The larval ADH activity levels are unaffected by the presence of the insertion. To verify that the activity differences observed were truly related only to the presence of the *jockey* insertion, crosses were performed to generate $Adh^{jockey}/Adh^{wild\text{-}type}$ F_1 progeny. If there are no other genetic factors involved in Adh^{jockey} expression, then the ADH activity of the F_1 progeny of Adh^{jockey} and $Adh^{wild\text{-}type}$ flies will be additive and approximate an average of the two parental activities. F_1 activity levels that are skewed towards one parental type indicate that other maternal or paternal factors are involved. We performed reciprocal Adh^{jockey} and $Adh^{wild\text{-}type}$ crosses and compared the F_1 ADH activity levels with those of the parental types. Our results indicate that the effect of the Adh^{jockey} allele is genetically additive and there are no other genetic factors controlling *Adh* expression that are different between the two strains.

(iii) *Adh* transcript levels

We measured the relative hybridization of Adh^{jockey} and $Adh^{wild\text{-}type}$ to Adh^{nLA248} adult *Adh* transcripts and the results indicate that there is a significant difference in adult *Adh* mRNA transcript levels between the two types. These results parallel those for the ADH enzyme activity. The adult mRNA transcript level of homozygous Adh^{jockey} lines is approximately 1·3 fold lower than that of the $Adh^{wild\text{-}type}$ lines. This difference translates into a similar difference in adult ADH activity levels – a 1·4-fold decrease in activity in the Adh^{jockey} lines. These results indicate that the presence of the *jockey* insertion near the *Adh* gene alters transcriptional regulation of the gene, which in turn correlates with reduced ADH activity.

In most cases the effects of TE-induced mutation on gene expression can be detected only if the effect is large. Unrelatedness of alleles and genetic background (differences in *cis* and *trans* factors) may cause a high background noise that drowns out the subtle effects of the mutations. The strength of this $Adh^{jockey}/Adh^{wild\text{-}type}$ comparison is that $Adh^{wild\text{-}type}$ is the direct ancestral allele of Adh^{jockey} and not only is the genetic background of the two strains identical, it is the original background in which the mutation occurred.

Therefore, this study factors out heterogeneity of *cis* and *trans* factors and allows the subtle effects of TE-induced mutations on gene expression to be detected. Previous work on *Adh* has indicated that DNA regions 5′ to the *Adh* adult enhancer are important for larval expression of the gene (Corbin & Maniatis, 1990) and that a 3·2 kb fragment encompassing the *Adh* adult enhancer, both promoters and the structural gene was all that was necessary for wild-type adult expression (Posakony *et al.*, 1986). Our results indicate that a TE insertion outside the area defined for adult expression and located within a putative larval enhancer is associated with an alteration in adult gene regulation but has no effect on larval gene expression.

A likely mechanism for this alteration is that the presence of the *jockey* element causes a conformational change in DNA structure which may interfere with the ability of the *Adh* adult enhancer element (located just downstream of the insertion) to interact with *trans*-acting factors or with the distal promoter.

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