Heredity **68** (1992) 337–344

Received 15 April 1991

The alcohol dehydrogenase polymorphism in natural populations of *Drosophila melanogaster*: ADH activity variation restriction site polymorphism and the *Adh* cline

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Alcohol dehydrogenase activity has been measured in 186 iso-second chromosome lines — 104 from seven Australian populations and 82 from six Chinese populations. Restriction endonuclease variation in the *Adh* gene region in these lines has previously been described (Jiang & Gibson, 1991). The mean ADH activity of Adh^F and Adh^S lines was significantly higher in the Chinese samples than in the Australian samples. In each population on both continents the mean activity of the Adh^F lines is significantly higher than that of the Adh^S lines. Six lines homozygous for a thermostability variant, Adh^{FChD} (detected in four of the Chinese populations), had intermediate levels of ADH activity and protein amount. In a subset of the lines with the highest and lowest levels of ADH, there was a correlation of 0.69 between ADH activity and ADH CRM. None of the restriction site variants was consistently associated with the amount of ADH activity. Associations between *Bam*HI (−7.2), the *Adh* polymorphism and ADH activity suggest that there are modifiers of ADH 5′ to the gene. The deletion (0.2) at position −2.8 on the restriction map (Jiang & Gibson, 1991) was associated with increased levels of ADH activity in Adh^S lines from China. Two unique insertions in the gene region were associated with low activity in Adh^F lines and a null activity allele had a deletion removing most of exon 2. A single line with a duplication of a part of the *Adh* coding region and of the 5′ regulatory section had relatively high ADH activity. Considering all the data, the main factor affecting ADH activity levels in populations is the frequency of Adh^F.

Keywords: alcohol dehydrogenase, *D. melanogaster*, enzyme activity, polymorphism.

Introduction

The enzyme alcohol dehydrogenase (ADH) is relatively abundant in *Drosophila melanogaster*, comprising 1 per cent of the soluble protein. In adult flies ADH is expressed in the hindgut, rectum, fat body, Malpighian tubules and structures derived from the male genital disc, and in larvae in the fat body, midgut and Malpighian tubules (Fischer & Maniatis, 1986). The ADH enzyme of *Drosophila melanogaster* has a role in alcohol detoxification, as flies homozygous for *Adh* null activity alleles do not survive exposure to ethanol levels above 6 per cent (Grell *et al.*, 1968; Freeth *et al.*, 1986; Van Delden & Kamping, 1988), although the relationship between fitness and variation within the normal range of activity remains controversial (for regions see Van Delden, 1982; Gibson & Wilks, 1988).

Within populations of *D. melanogaster*, considerable variation in ADH activity is caused by genetic and environmental (mainly nutritional) factors. Anderson & Gibson (1985) assessed ADH levels in newly caught single flies and noted a greater than sixfold difference in ADH activity and ADH CRM between flies with the lowest and highest activities. A factor contributing to this variation is the world-wide polymorphism at the *Adh* locus for two allozymes, ADH-S and ADH-F

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(Lewis & Gibson, 1978; Laurie-Ahlberg *et al.*, 1980; Anderson & Gibson, 1985). ADH enzyme activity per fly is 2–3 times higher in flies homozygous for Adh^F alleles than in Adh^S homozygotes and this is largely due to differences in ADH protein amounts (Gibson, 1972; Birley & Marson, 1991; Maroni *et al.*, 1982) but the higher catalytic efficiency of ADH-F (Winberg *et al.*, 1985) also has an effect. ADH activity and tissue distribution are directly affected by genetic variants at the structural gene locus, and are regulated by *cis* and *trans* acting elements (Goldberg *et al.*, 1983; Posakony *et al.*, 1985; Fischer & Maniatis, 1986; Corbin & Maniatis, 1990). Clarke & Whitehead (1984) have argued that the structural and regulatory contributions to ADH variation are likely to have co-evolved as a linked group of selectively interacting factors. If this has occurred then non-random associations between regulatory and structural factors might be reflected in patterns of restriction endonuclease variation and ADH activity levels. There is evidence that some restriction fragment length polymorphisms in the *Adh* region are associated with ADH activity levels (Birley, 1984; Aquadro *et al.*, 1986; Schott *et al.*, 1988).

We have recently described restriction endonuclease variation in the 12-kb region surrounding the *Adh* locus in 13 populations of *D. melanogaster* from Australia and China (Jiang & Gibson, 1991). The populations were chosen because they spanned the latitudinal cline in the Adh^S allele — this allele decreases in frequency with distance from the equator (Wilks *et al.*, 1980; Jiang *et al.*, 1989). The second chromosomes that were isolated and made homozygous by Jiang & Gibson (1991) were also assayed for their effects on ADH activity, and these data are now related to the restriction endonuclease variation which was present in the populations. The aims were (1) to assess intra-population variation in ADH activity, (2) to test whether populations at similar latitudes have similar activity levels, (3) to determine whether the difference in activity beween Adh^F and Adh^S alleles is consistent in populations from the northern and southern hemispheres, and (4) to test whether there are consistent non-random associations between restriction endonuclease variants and ADH activity.

Materials and methods

The *D. melanogaster* lines assayed for ADH activity were the same lines previously used for restriction mapping (Jiang & Gibson, 1991), except for two lines from the Guangzhou population which had been lost. There were 104 lines from seven Australian populations and 82 lines from the six Chinese populations [see map in Jiang & Gibson (1991) for the geographical locations of the populations]. Each line was homozygous for a wild-type second chromosome. The third chromosomes in the lines were randomly derived from the wild-type stock and the X chromosome extractions (Jiang & Gibson, 1991).

ADH was assayed in each line using flies from two replicate cultures. To provide similar progeny densities each culture was set up with 20 pairs of flies in 250-ml plastic containers with standard culture medium (Gibson & Wilks, 1988). Two sets of male flies from the progeny of each culture were aged for 5–7 days, weighed and then homogenized in cold 100 mM sodium phosphate buffer (pH 7.5) to a final concentration of 10 mg live weight/ml. The homogenates were centrifuged at 10,000 r.p.m. (Sorvall SM-24 rotor) for 30 min and the supernatants were kept on ice until assayed for ADH activity, as previously described (Gibson & Wilks, 1988). Two other lines, one homozygous for Adh^F and one for Adh^S, were cultured and assayed in the same way and used as controls on each occasion that assays were made. Ethanol and 2-propanol were each used as substrates so that the activity ratio (activity with 2-propanol to activity with ethanol) could be calculated (Gibson *et al.*, 1980). The heat stability of the ADH in the crude extracts was tested by keeping aliquots of the extracts in a 40°C water bath for 5 min prior to their assay.

The amount of ADH protein was investigated in 52 of the lines using radial immunodiffusion and polyclonal antisera prepared in rabbits (Lewis & Gibson, 1978). These lines were chosen because they were the ones with either the highest or lowest ADH activities in each population. Extracts of the control Adh^F and Adh^S lines were used to prepare a standard series of antigen concentrations and the protein concentrations in the samples were determined by comparison with these standards.

Results

Of the 186 lines assayed, one lacked ADH activity and CRM, and this line has been excluded from the statistical analyses. We have shown (J. B. Gibson *et al.*, unpublished data) that this *Adh* allele (*nACH105*) has a deletion of 438 bp, which includes most of exon 2, and is responsible for the lack of activity.

The lines from both continents form two groups with respect to ADH activity, with Adh^F lines having, on average, more than twice the activity of Adh^S lines (Table 1), although there is some overlap (Fig. 1). The levels of ADH activity in the Adh^F lines vary from 35.4 to 351.9 in the Australian populations and from 130.7 to 365.7 in the Chinese populations (Fig. 1). The levels in activity for the Adh^S lines range from 36.1 to 179.5

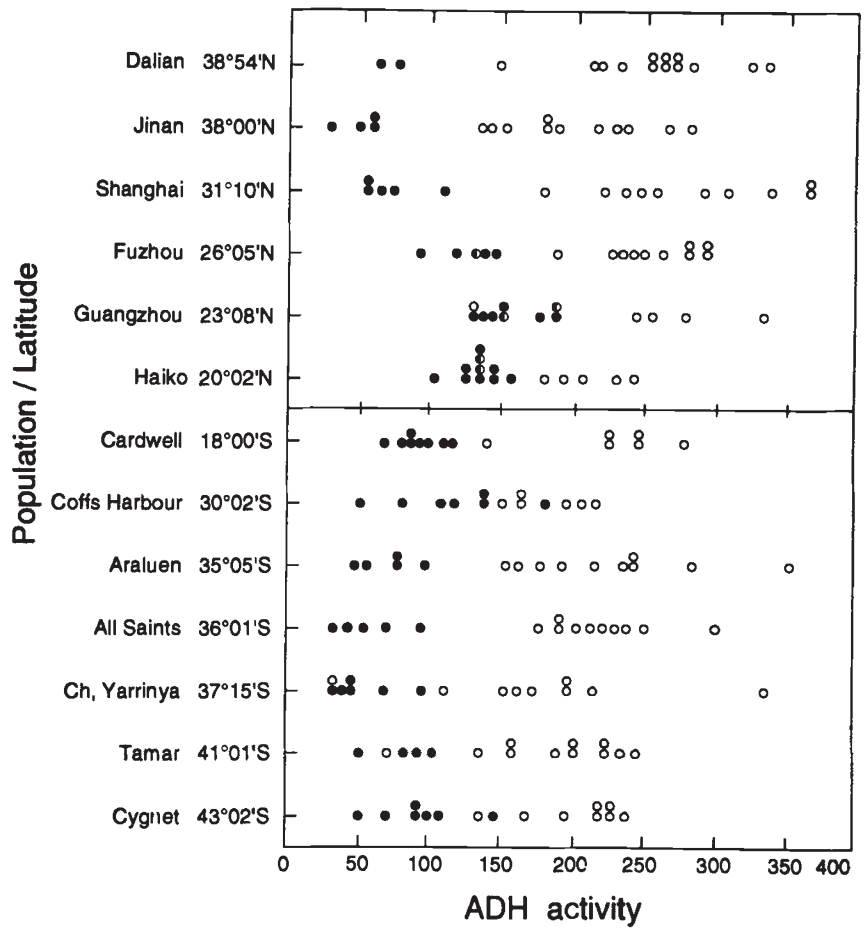

Fig. 1 The ADH activity of each isochromosome line in the Chinese and Australian populations sampled. The latitude of each population is indicated. (○) Adh^F lines, (●) Adh^S lines.

Table 1 Mean ADH-F and ADH-S activities in Australian and Chinese populations. Standard errors are given in parentheses. *n* is the number of samples

Australia						China					
Population	Latitude	ADH-F	*n*	ADH-S	*n*	Population	Latitude	ADH-F	*n*	ADH-S	*n*
Cygnet	43°09′S	200.0(12.7)	8	91.5(9.5)	7						
Tamar	41°02′S	194.7(11.1)	10	78.4(7.9)	5						
						Dalian	38°54′N	255.9(13.1)	13	69.0(7.0)	2
Chateau Yarrinya	37°36′S	174.9(26.8)	9	55.6(9.5)	6						
						Jinan	37°N	190.5(14.8)	10	50.3(7.0)	4
All Saints	36°03′S	204.3(18.9)	11	63.5(12.6)	4						
Araluen	35°39′S	225.6(20.4)	10	71.2(18.9)	5						
						Shanghai	31°10′N	280.8(19.9)	10	74.0(9.6)	5
Coffs Harbour	30°18′S	180.0(10.6)	6	116.5(15.8)	7						
						Fuzhou	26°05′N	246.0(7.4)	9	135.0(15.8)	5
						Guangzhou	23°08′N	220.9(48.8)	6	146.6(10.5)	5
						Haikou	20°20′N	185.6(15.2)	6	128.4(6.1)	7
Cardwell	18°16′S	225.9(18.8)	6	94.5(5.2)	9						
Overall populations		200.2(7.4)	60	87.4(5.1)	43			243.4(7.8)	54	111.9(8.6)	28

(Australian populations) and 30.3 to 190 (Chinese populations) (Fig. 1). The average ADH activity is higher in the Chinese than in the Australian population samples (Table 1), both overall ($t=4.0$, $P<0.001$) and for Adh^F ($t=4.0$, $P<0.001$) and Adh^S lines ($t=2.8$, $P<0.01$).

There was no significant correlation between the weights of the male flies to make the extracts and ADH activity (cf. Clarke *et al.*, 1979). The mean body weights of these samples did not differ between the Australian lines (13.45 ± 1.26 mg/20 flies) and the Chinese lines (13.85 ± 1.48 mg/20 flies), nor did they vary with latitude.

In each of the Australian and the Chinese populations the mean ADH activity of the Adh^F lines is significantly higher than that of the Adh^S lines (Table 1). The highest mean ADH activity of Adh^F lines occurred in the Shanghai population and this level was significantly higher than that of two Chinese populations (Jinan, $t_{18}=3.01$, $P<0.01$ and Haikou, $t_{15}=3.48$, $P<0.005$) and two Australian populations (Chateau Yarrinya, $t_{17}=3.2$, $P<0.01$ and Coffs Harbour, $t_{14}=3.01$, $P<0.01$). Overall the variation in mean ADH activities in Adh^F lines is not related to latitude in either hemisphere. However, the ADH activity in Adh^S lines in the Chinese, but not the Australian, populations is significantly negatively correlated with latitude ($r=-0.89$, $P<0.01$). The mean ADH activities in the three most southern populations (Haikou, Guangzhou and Fuzhou) are each significantly higher than in Shanghai ($t_{10}=5.58$, $P<0.01$; $t_8=6.03$, $P<0.001$; $t_8=3.3$, $P<0.02$ respectively), in Jinan

Table 2 Mean ADH activity, ADH protein amount, and the correlation coefficient between them in Adh^{FChD} lines and in Adh^F and Adh^S lines with the highest or lowest activity in each population sampled. Standard errors are given in parentheses

Genotype class	Number of strains	Mean ADH activity	Mean ADH protein amount	Correlation coefficient
Adh^F	27	206.0(16.4)	74.5(3.9)	0.73**
Adh^S	25	90.2(8.7)	56.1(5.1)	0.69**
Adh^{FChD}	6	144.4(9.3)	64.0(1.6)	−0.53(ns)
Overall	58	149.7(11.2)	67.4(2.7)	0.69**

**$P<0.01$.

Table 3 ADH activity in lines that differ for the presence or absence of particular restriction endonuclease site variants. Data is only shown for lines where at least one comparison is significant and there are sufficient data (see Materials and Methods). The number of lines of each electrophoretic class is indicated and k is the number of lines with a particular restriction site. Standard errors are given in parentheses. + and − denote the presence or absence of the variant respectively

		Australia						China					
		Adh^F ($n=56$)			Adh^S ($n=46$)			Adh^F ($n=56$)			Adh^S ($n=28$)		
Restriction site		k	+	−	k	+	−	k	+	−	k	+	−
*Bam*HI	(−7.2)	15	175.5* (13.0)	210.0 (8.5)	35	87.9 (5.2)	85.8 (15.2)				11	87.5** (14.7)	127.5 (8.6)
*Hind*III	(−3.7)	6	155.5** (29.5)	206.1 (7.1)									
*Hind*III	(−3.0)	12	191.1 (10.5)	203.4 (8.9)	12	88.5 (11.7)	87.0 (5.6)				5	140.0* (10.1)	105.0 (9.9)
*Pst*I	(1.3)				9	60.4** (6.7)	93.9 (5.7)				7	121.6 (17.2)	108.6 (10.1)
*Eco*RI	(9.0)	9	240.9* (16.0)	192.0 (7.8)	10	84.2 (6.8)	88.4 (6.3)	7	239.9 (21.7)	244.6 (8.5)	5	136.3 (5.7)	106.4 (10.2)

*$P<0.05$, **$P<0.01$.

($t_g = 8.74$, $P < 0.01$; $t_7 = 9.27$, $P < 0.001$; $t_7 = 5.09$, $P < 0.01$) and in Dalian ($t_7 = 5.15$, $P < 0.001$; $t_5 = 5.48$, $P < 0.005$; $t_5 = 2.68$, $P < 0.05$).

As the samples had been scored for thermostability at 40°C and for activity ratio it was possible to obtain data for a third *Adh* allele, Adh^{FChD}, which segregates in some natural populations (Wilks *et al.*, 1980; Gibson *et al.*, 1982; Jiang *et al.*, 1989; Gibson *et al.*, 1990). This allele had been detected in four of the Chinese populations — Haikou, Guangzhou, Fuzhou and Jinan — and six lines containing the allele were assayed. The mean ADH activity in these six lines homozygous for Adh^{FChD} (144.4 ± 9.3) was significantly higher than in the Adh^S lines and significantly lower than in the Adh^F lines (Table 3).

The amount of ADH protein was measured in the six lines homozygous for Adh^{FChD} and also in the 27 Adh^F lines and 25 Adh^S lines were chosen because they had the highest or lowest activities in each population. Over all 58 lines there was a significant correlation between ADH activity and ADH protein amount, and the correlations were also significant and of similar magnitude for Adh^F and Adh^S lines, but not for Adh^{FChD} lines (Table 2). On average the Adh^F lines have significantly more ADH CRM than do ADh^S lines ($P < 0.01$). The amount of ADH CRM in the Adh^{FChD} lines was intermediate between, but not significantly different from, that of Adh^F and Adh^S lines (Table 2).

ADH activities were compared between lines which differed in the presence or absence of specific restriction endonuclease variants (variants with frequencies less than 10 per cent were excluded). In the Australian and Chinese populations, the restriction site variation in the *Adh* region is shown in Fig. 2 in Jiang & Gibson (1991). Fifteen restriction endonuclease recognition site polymorphisms were found, and, of these, five (*Bam*HI (−7.2), *Hind*III (−3.7), *Hind*III (−3.0), *Pst*I (1.3) and *Eco*RI (9.0) are associated (in at least one population) with significant differences in ADH activity (Table 3). None of these polymorphisms has a consistent effect on ADH activity in populations on either continent (Table 3), although there are some intriguing associations when only the lines with the highest and lowest ADH activities in each population are considered. For example, in the Chinese populations only two of the 11 high-activity Adh^S lines have *Bam*HI (−7.2) even though the frequency of this variant in Adh^S alleles is 0.4. Amongst the Adh^F lines the frequency of *Bam*HI (−7.2) is 0.26 and five of the 14 low-activity Adh^F lines in Australian populations have this site and in one of them the activity was 35.4 (cf. average Australian Adh^F lines 200.2 ± 7.4). There were no detectable insertions or deletions in the *Adh* region in this line.

The majority of the insertions and deletions had only slight effects on ADH activity and none of the effects was consistent in populations from both continents. In the Chinese populations Adh^S lines with the 200 bp deletion located at −2.8 (Jiang & Gibson, 1991) were associated with higher ADH activity (147.7 ± 7.1) than lines without the deletion (80.6 ± 8.8). This deletion, which was present in all populations except Tamar, decreased in frequency with increasing latitude in the Chinese populations (Jiang &

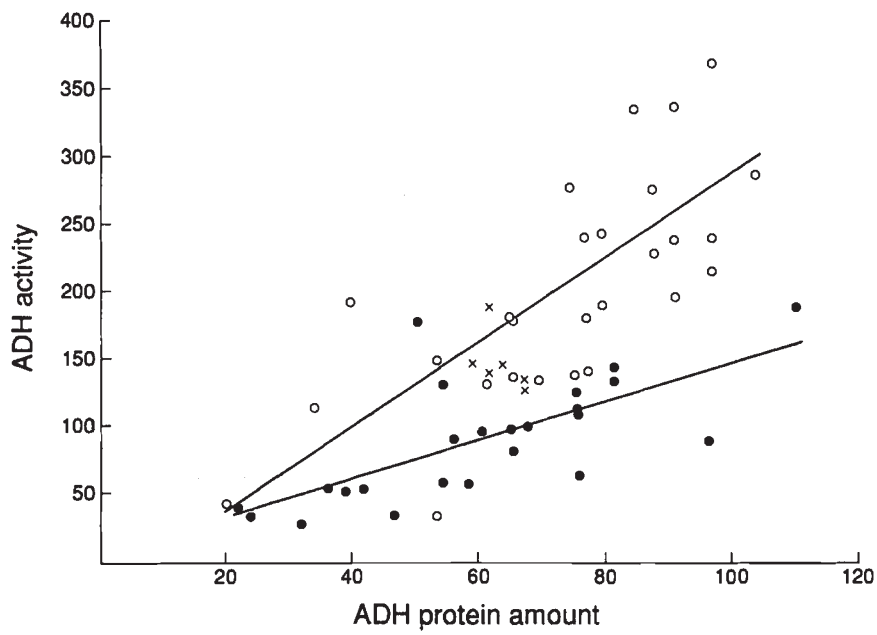

Fig. 2 The relationships between ADH activity and ADH protein amount in the iso-chromosome lines. The regression lines are drawn for the Adh^F (○) and Adh^S (●) lines. (×) Adh^{FChD} lines.

Gibson, 1991). Some of the large insertions in the *Adh* region were associated with lowered ADH activity. An All Saints Adh^F line with a 5.0 kb insertion in intron 1 had low activity (43.1), and an Adh^F line from Haikou with a 1.5 kb insertion 2 kb from the 5′ end of the coding region had ADH activity of 132.9 (in this population the mean activity of Adh^F lines = 185.6 and Adh^S lines = 128.4). The null activity allele in the Coffs Harbour population had a deletion removing most of exon 2.

One of the lines (from Tamar) was remarkable in that it contained a partial duplication of the *Adh* coding region together with a duplication of a region approximately 4 kb upstream to the coding region (Fig. 3). Detailed analysis revealed that a fragment similar in size and restriction map to the *Hpa*I–*Bam*HI fragment (approximately 932 bp) in the coding region of the *Adh* gene was present 3.7 kb, 3′ to the gene (Fig. 3). Contiguous with this insertion was a 2-kb fragment which appears to be the same as a *Bam*HI–*Pst*I segment 5′ to the gene (Fig. 3). This line had the highest ADH activity of the Tamar lines even though the duplicated copy of the gene is incomplete.

Discussion

A striking feature of these data is the consistent difference in ADH activity between Adh^F and Adh^S alleles. In each of the 13 populations there was little overlap in the ADH activities of lines homozygous for Adh^F and lines homozygous for Adh^S. Although *trans* acting modifiers of ADH activity have been shown to segregate in natural populations of *D. melanogaster* (Laurie-Ahlberg *et al.*, 1980) their effects do not blur the differences in ADH activity due to the alleles at the *Adh* locus.

Our data for sub-samples of the lines show that the amount of ADH activity per unit ADH CRM for Adh^F versus Adh^S lines is 1:0.58. This suggests that the catalytic efficiency of the ADH-S enzyme is only about two-thirds that of the ADH-F enzyme. Winberg *et al.* (1985), measuring active ADH molecules (not just immunologically identical molecules), found that the ADH-F enzyme has a catalytic-centre activity for secondary alcohols that is four times that of Adh^S. This is consistent with a stronger binding of the reduced coenzyme to ADH-S, and hence a slower dissociation of the coenzyme from the enzyme–NAD complex. With ethanol as substrate, the catalytic-centre activity of ADH-F is twice that of ADH-S. The single amino acid substitution at position 192, which distinguishes ADH-F from ADH-S, is mainly responsible for the difference in catalytic-centre activity between the two allozymes (Winberg *et al.*, 1982; Winberg *et al.*, 1985; Laurie-Ahlberg & Stam, 1987).

Relatively few lines were homozygous for the thermostable allele Adh^{FChD}, but the six lines investigated [all from southern Chinese populations where the frequency of Adh^{FChD} reaches 36 per cent (Jiang *et al.*, 1989)] were homogeneous in their properties. The Adh^{FChD} lines had levels of ADH activity and CRM intermediate between those of ADH-F and ADH-S, as had previously been found for Adh^{FChD} lines from Australia (Lewis & Gibson, 1978; Gibson *et al.*, 1980; Wilks *et al.*, 1980). Using allele-specific oligonucleotides it has been shown that samples of

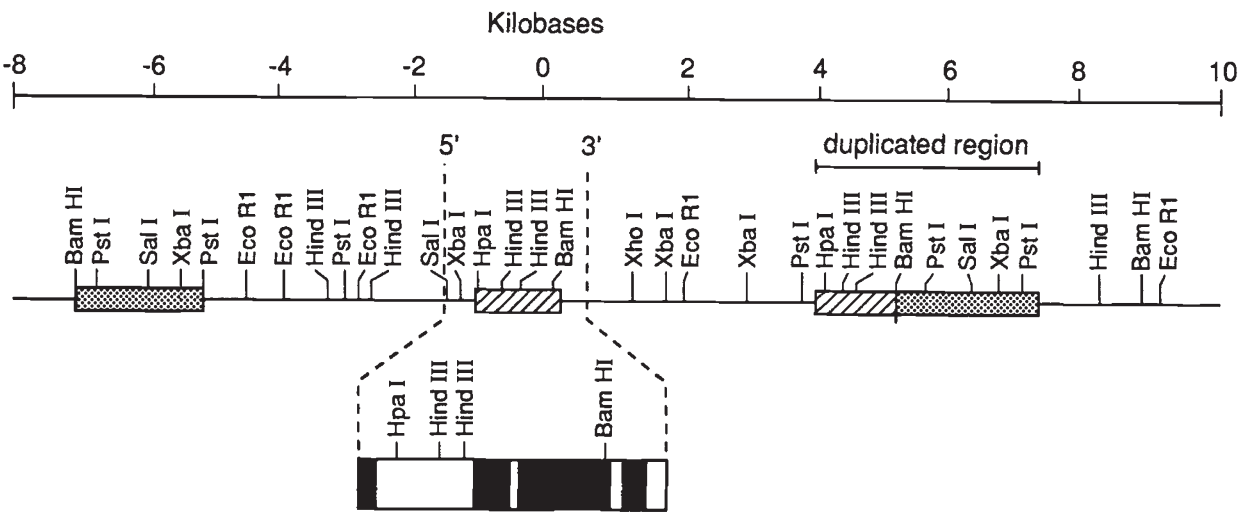

Fig. 3 The molecular landscape of the *Adh* gene region in the Tamar line with a partial duplication. The two regions duplicated are indicated, and the *Adh* transcription unit is drawn below the restriction map to show in more detail the region from which part of the duplication appears to have been derived.

Adh^{FChD} alleles from Chinese, North America and Australia natural populations all contain the triplet TCC which encodes serine at residue 214, in place of proline in ADH-F and ADH-S (Gibson *et al.*, 1990), and this substitution is probably responsible for the characteristic properties of ADH-FChD (Chambers *et al.*, 1981; Gibson *et al.*, 1990).

There were no consistent relationships over all populations between restriction endonuclease variation and ADH activity. The evidence that the majority of insertions have no consistent effects on ADH activity, suggests that the striking non-random distribution of insertions in the *Adh* gene region (Langley *et al.*, 1982; Aquadro *et al.*, 1986; Cross & Birely, 1986; Jiang & Gibson, 1991) may result from selection eliminating most of those insertions which disrupt ADH activity/allozyme relationships.

Overall, these data show that the interpopulation distribution of ADH activity is largely due to the difference in Adh^F frequency, and that the direction of the difference in activity between Adh^F and Adh^S alleles is mainly preserved regardless of the occurrence of considerable restriction endonuclease variation in the *Adh* gene region. This implies that if selection modulates ADH activity levels, it will, at least in adults, do this most readily be adjusting the frequencies of the Adh^F and Adh^S alleles in populations. Nevertheless, the marked differences in mean ADH activity between the Australian and Chinese populations, reflected similarly in the means for the Adh^F and Adh^S lines (Table 1), are probably due to differences in modifiers in the two sets of populations, perhaps because of the different geographical origins of the 'old' Chinese populations compared to the Australian populations, as previously discussed by David & Capy (1988) and Jiang & Gibson (1991).

Acknowledgements

We would like to thank Ann Wilks and Cathie Stewart-Moore for help with the manuscript. JCJ received financial support from the State Education Commission of the Peoples, Republic of China and held an Australian National University Postgraduate Scholarship.

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