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The alcohol dehydrogenase polymorphism in natural populations of *Drosophila melanogaster*: restriction map variation in the region of the *Adh* locus in populations from two hemispheres

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Restriction endonuclease variation in the 12 kb region surrounding the *Adh* locus was measured in seven Australian and six Chinese populations of *Drosophila melanogaster*. There is a higher level of nucleotide-substitution variation in the Australian populations than in the Chinese, which is possibly a reflection of their origins. None of the restriction site polymorphisms, nor any of the insertions, showed a significant association with latitude. A 0·2 kb deletion varied with latitude in the Chinese populations. In accordance with previous studies, a majority of the insertions were located in a region 1.5–3.5 kb 3′ from the *Adh* coding region, and a majority of the deletions were at a site 3 kb 5′ to the *Adh* coding region. Two of the insertions shared homologies with known mobile elements. Overall, the data suggest that restriction endonuclease variation in the *Adh* region is not related to the cline in Adh^S frequencies.

Keywords: alcohol dehydrogenase, *D. melanogaster*, polymorphism, restriction enzymes.

Introduction

Allozyme variation at the alcohol dehydrogenase locus in *Drosophila melanogaster* is geographically differentiated world-wide in a remarkable pattern, which is unique amongst enzyme polymorphisms in any organism. Most populations of *D. melanogaster* contain two electrophoretically distinguishable alleles, Adh^F and Adh^S, with the population frequency of Adh^S decreasing with distance from the equator. This pattern was first described for North American population samples (Johnson & Schaffer, 1973) but later studies revealed very similar distributions in Australasia, Europe, Africa and Asia (Wilks *et al.*, 1980; Oakeshott *et al.*, 1982; David, 1982; David *et al.*, 1989; Singh *et al.*, 1982; Jiang *et al.*, 1989). The clinal distribution of alleles at the *Adh* locus has thus been observed in both hemispheres and on all continents for which data are available — there are no published data from the subcontinent of South America.

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Lewontin (1974), Endler (1977) and others have cautioned that observed geographical distributions of allele frequencies do not by themselves allow for discrimination between hypotheses about the causes leading to particular patterns. The *Adh* cline in *D. melanogaster* is no exception to this stricture and, although selective and stochastic processes have been postulated to account for the geographical distribution, the causal mechanism(s) remains unknown.

The available data from studies on natural populations do not provide any compelling evidence for selective factors acting on any aspect of phenotypic variation to which the *Adh* locus is known to contribute (Gibson *et al.*, 1980; Van Delden, 1983; Gibson & Wilks, 1988). Nevertheless, data from experimentally maintained transplanted populations appear to show that *Adh* frequencies change in the direction predicted by the cline (Oakeshott *et al.*, 1988). The possibility remains that any selection maintaining the *Adh* cline might be directed towards regions of DNA linked to the *Adh* locus, rather than to the products of the locus.

It has been clearly shown in relation to this possibility, both in populations from the Eastern United

States (Voelker *et al.*, 1978) and from Australia (Knibb, 1983), that the *Adh* cline is not caused by gametic disequilibrium between the inversion *In(2L)t* (Mettler *et al.*, 1977) and the *Adh* locus, which is located just outside the proximal breakpoint (Woodruff & Ashburner, 1979). A further class of variation which might be relevant to the *Adh* cline, and which can now be examined, is that revealed in the molecular landscape of the *Adh* locus by restriction endonucleases. Investigations using restriction enzymes that recognize six-base pairs (Langley *et al.*, 1982; Birley, 1984; Cross & Birley, 1986; Aquadro *et al.*, 1986 and Jiang *et al.*, 1988) and four-base pairs (Kreitman & Aquadé 1986a,b and Simmons *et al.*, 1989) have revealed considerable variation in the *Adh* region. The studies of Kreitman & Aquadé (1986a,b), which showed there was no differentiation between an east-coast and a west-coast North American sample, and that of Simmons *et al.* (1989) on three populations from the east coast of the United States, are the only systematic analyses of restriction endonuclease variation in relation to the *Adh* cline. To supplement this information, we have investigated whether any restriction endonuclease variation in a 12 kb region encompassing the *Adh* locus parallels the latitudinal variation in Adh^F frequency, and whether there are any similarities in populations at the same distance from the equator in China and Australia. This paper describes the restriction endonuclease variation and later we will examine the effect of the variation on ADH activity.

Materials and methods

The geographical locations of the seven Australian and six Chinese natural populations from which the *D. melanogaster* used in the present study were sampled are shown in Fig. 1. Details of the Chinese populations have been given previously (Jiang *et al.*, 1989); the Australian samples were collected in 1986 and 1987 and, like the Chinese material, were maintained in the laboratory with between 75 and 125 single female lines from each population.

A single male from each of a number of single female lines was mated with a virgin female from a second–third chromosome translocation stock, *T (2;3) ap^{Xa}*, which contains the second chromosome balancer *CyO*, and the third chromosome balancer *TM6* (Lindsley & Grell, 1968). A single *Cy* male from the progeny was backcrossed to the translocation stock females, and the *Cy* offspring were mated together to

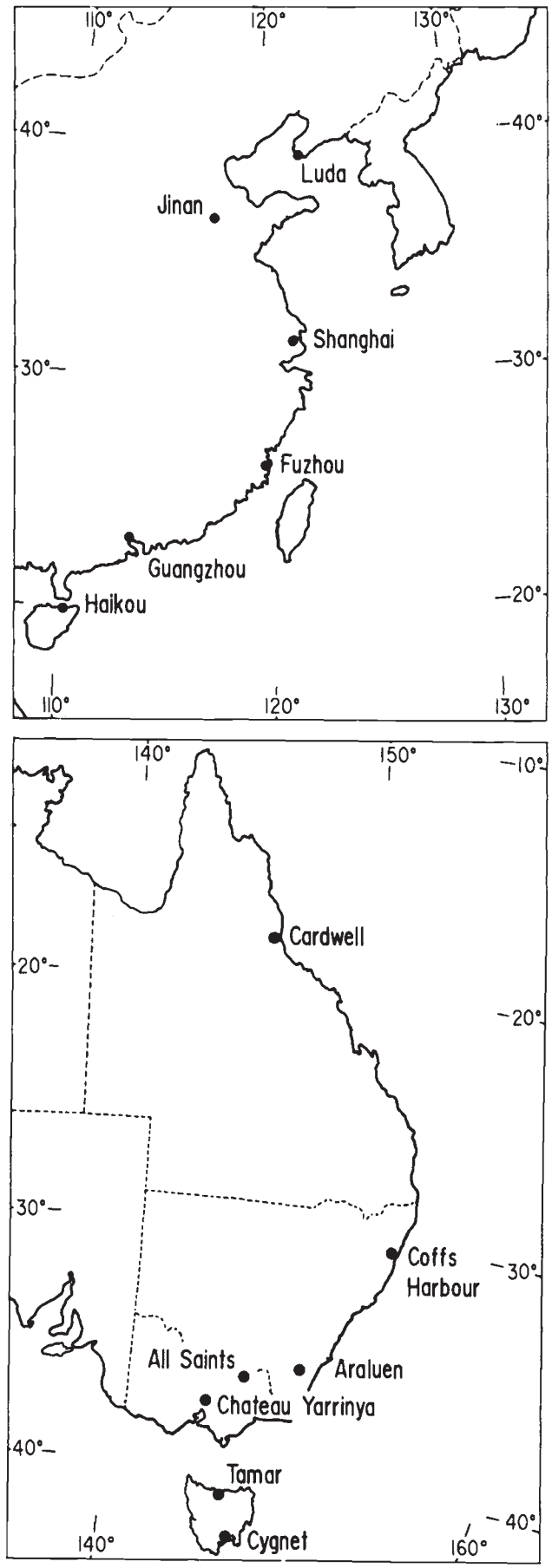

Fig. 1 The location of the Chinese and Australian populations of *D. melanogaster*.

produce a line homozygous for a single second chromosome. Any homozygous lethal chromosomes were maintained heterozygous with *CyO*. About 15 second chromosomes were extracted from each of the 13 populations, to give a total of 104 chromosomes from the Australian populations and 90 from the Chinese populations.

DNA was extracted from adult flies from the 194 lines either by the two methods previously described (Jiang *et al.*, 1988) or by the following short method. Fifty adult flies were homogenized in 50 μl cold TE buffer (100 mM Tris pH 9.0, 100 mM EDTA), then 500 μl of 2 per cent SDS were added to the homogenate which was then incubated at 65°C for 1 h. After the homogenate had cooled, 120 μl of 8 M potassium acetate were added and DNA was precipitated from the supernatant with 500 μl of isopropanol. The DNA pellet was washed twice with cold 70 per cent ethanol, dried under vacuum and resuspended in 50 μl TE buffer containing 20 μg/ml boiled RNAse.

The DNA extracted from each of the single second chromosome lines was digested with eight hexanucleotide restriction endonucleases, *Bam* HI, *Eco* RI, *Hind* III, *Hpa* I, *Pst* I, *Sal* I, *Xba* I and *Xho* I, under the buffer conditions recommended by the manufacturer (Amersham). Double and triple digestions were made in order to construct restriction maps; agarose gels of 2 and 0.5 per cent were used for electrophoresis of the fragments but we were unable to obtain a reliable resolution of less than 50 bp.

The methods used to transfer the DNA fragments from agarose gels to nitrocellulose filters and the prehybridization and hybridization conditions have been described previously (Jiang *et al.*, 1988). The nitrocellulose filters were probed with the sAF-2 plasmid (Goldberg, 1980), which includes an 11-kb insert spanning the region of the *Adh* gene, labelled with [α-^{32}P]-*d*CTP by nick translocation (Rigby *et al.*, 1977). Autoradiography was performed at −70°C on Kodak film (*XRP*-1) using intensifying screens.

The sizes of the restriction fragments which hybridized to the labelled probe were determined graphically from a semi-logarithmical plot of the sizes of λ DNA fragments digested with *Hind* III.

A number of the insertions found in the Australian and Chinese populations were cloned and probed with sequences from known mobile elements to test for homologies. Ten single second chromosome lines, each of which had been shown to contain a different sized insertion (one line, H2, contained two insertions), had their DNA digested with *Bgl* II and the fragments ligated to λ EMBL3 arms with *Bam* HI ends (Frischauf *et al.*, 1983). Recombinant molecules were packaged and the 10 libraries, in *Escherichia coli* strain LE392, were screened by plaque hybridization using the sAC1 clone as a probe (which contains a 4.7 kb *Eco* RI fragment encompassing the *Adh* gene) labelled with [α-^{32}P]-*d*CTP. Positive clones were plaque-purified and DNA from each was transferred to nitrocellulose membranes which were then probed with ^{32}P-labelled clones of mobile elements. The cloned mobile elements used as probes were: *pπ25.1, a Bam* HI fragment containing a complete 2.9 kb P element cloned into the pBR 322 vector (O'Hare & Rubin, 1983); *Copia*, a *Bam* HI fragment containing a 5.0 kb copia element in pBR 322 (Rubin *et al.* 1981); *B104B*, an *Eco* RI fragment containing a 2.8 kb *B104B*, which is part of the *B104* element, in the PACYC184 vector (Scherer *et al.*, 1982); *p1407*, a *Sal* I fragment containing a 5.4 kb *I* factor in the PAT153 vector (Boucheton *et al.*, 1984); *cDm412*, a *Hind* III fragment containing a 7 kb 412 *copia*-like element in pBR322 (Finnegan *et al.*, 1978; *pPW297*, a *Hind* III fragment containing a 6.5 kb 297 copia-like element in pBR322 (Finnegan *et al.*, 1978; Rubin *et al.*, 1982); *pDMI-101*, an *Eco* RI fragment containing a 4.3 kb *F* element in pBR322 (Dawid *et al.*, 1981, and I. B. Dawid personal communication).

Results

The restriction endonuclease variation observed in the region of the *Adh* gene, using sAF-2 as a probe, in seven Australian and six Chinese populations, is listed in Table 1. Some of the data for the two Tasmanian populations have already been published (Jiang *et al.*, 1988), but they are included here for completeness. To facilitate comparisons with previous work on the *Adh* gene region, the *Bam* HI site in the second exon of the *Adh* transcription unit is given the co-ordinate of 0.0.

Overall in the seven Australian populations, 13 polymorphic restriction sites, seven insertions and one deletion were observed (Fig. 2a). In the Chinese populations there were 12 polymorphic restriction sites, five insertions and one deletion (Fig. 2b). Ten of the polymorphic restriction sites were shared by populations on both continents but only three of the 11 different structural changes were shared. A 0.2 kb deletion and a 0.4 kb insertion were present in most of the populations but each of the insertions larger than 1 kb was unique to a particular population, although sometimes found in more than one single chromosome line. One chromosome extracted from the Tamar population contained a complex duplication of parts of the *Adh* coding region (Jiang *et al.*, 1988).

Estimates of nucleotide substitution variation were derived using equations for the proportion of polymorphic nucleotides (Ewens *et al.*, 1981; Hudson, 1982), the heterozygosity per nucleotide pair, θ (Ewens

Table 1a Restriction endonuclease variation in the *Adh* gene region of the Australian natural populations of *Drosophila melanogaster*. I = insertion, D = deletion. Restriction sites are *Bam* HI (B), *Eco* RI (E), *Hin*d III (H), *Hpa* I (Hp), *Pst* I (P), *Xba* I (B), *Xho* I (Xh). The size of the insertions and deletions and the position of the restriction sites are indicated in parentheses. The *Adh* allotype is indicated with $F(Adh^F)$, $S(Adh^S)$ and $ChD(Adh^{FChD})$. The populations are Cygnet (C), Tamar (T), Chateau Yarrinya (CY), All Saints (AS), Araluen (Ar), Coffs Harbour (CH) and Cardwell (Cd)

	X (−8.0)	X (−7.4)	B (−7.2)	I (0.28)	Hp (−6.9)	X (−5.3)	H (−3.7)	H (−3.0)	D (0.2)	I (5.0)		P (1.1)	Xh (1.2)	P (1.3)	E (1.4)	I (0.4)	I (0.7)	I (3.0)	I (4.5)	I (1.0)	B (3.9)	E (9.0)
C 3	-	-	+	+	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
C 5	-	-	+	-	-	-	-	+	+	-	F	-	+	-	-	-	-	-	-	-	-	-
C 8	-	-	-	-	-	-	-	-	-	-	S	-	-	+	-	-	-	-	-	-	-	-
C 11	-	-	+	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
C 12	-	-	+	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
C 20	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
C 25	-	-	+	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
C 28	-	-	-	-	+	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
C 29	-	-	-	-	-	-	-	+	+	-	F	-	+	-	-	-	-	-	-	-	-	-
C 33	-	-	-	-	-	-	-	-	+	-	F	-	+	-	-	+	-	-	-	-	-	-
C 35	-	-	-	-	-	-	-	+	+	-	F	-	+	-	-	-	-	-	-	-	-	-
C 45	-	-	-	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
C 51	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	+
C 61	-	-	+	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-
C 72	-	-	-	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
T 1	-	-	-	-	+	-	-	-	-	-	S	-	+	+	-	+	-	-	-	-	+	-
T 12	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
T 17	-	-	+	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	+	-
T 19	-	-	+	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-
T 29	-	-	-	+	+	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
T 30	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	+
T 32	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
T 33	-	-	+	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	+
T 44	-	-	-	-	-	-	-	-	-	-	F	+	+	-	-	+	-	-	-	-	-	-
T 50	-	-	+	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
T 54	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
T 56	-	-	+	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
T 60	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
T 73	-	-	+	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
CY 1	-	+	+	-	-	-	-	+	-	-	S	-	+	+	-	+	-	-	-	-	-	-
CY 2	+	-	+	-	-	-	-	+	-	-	S	-	-	+	-	-	-	-	-	+	-	-
CY 3	-	-	+	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
CY 4	+	-	+	-	-	-	-	+	-	-	S	-	-	+	-	-	-	-	-	+	-	-
CY 5	-	-	+	-	-	-	-	+	-	-	S	-	-	-	-	-	-	-	-	-	-	-
CY 6	-	+	+	-	-	-	-	-	-	-	S	-	+	+	-	+	-	-	-	-	-	-
CY 7	-	-	-	-	-	-	-	+	-	-	F	-	+	-	-	-	-	-	-	-	-	-
CY 8	-	-	-	-	-	-	-	-	-	-	F	-	-	-	-	-	-	-	-	-	-	-
CY 9	-	-	+	-	-	-	-	+	-	-	F	-	+	-	-	-	-	-	-	-	-	-
CY 10	-	-	-	-	-	-	+	-	+	-	F	-	+	-	-	-	-	-	-	-	-	-
CY 11	-	+	-	-	-	-	+	-	-	-	F	-	+	+	-	+	-	-	-	-	-	-
CY 12	-	-	+	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
CY 13	-	-	+	-	-	-	+	-	+	-	F	-	-	-	-	-	-	-	-	-	-	-
CY 14	-	-	-	-	-	-	+	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
CY 15	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
AS 1	-	+	+	+	-	-	-	-	+	-	S	-	+	+	-	+	-	-	-	-	-	-
AS 2	-	-	+	-	-	-	+	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
AS 3	-	+	+	-	-	+	-	-	-	-	S	-	+	+	-	+	-	-	-	-	-	-
AS 4	-	-	+	-	-	-	-	-	-	-	F	+	+	-	-	-	-	-	-	-	-	-
AS 5	-	+	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
AS 6	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
AS 7	-	-	+	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-
AS 8	-	-	-	-	-	-	-	+	+	-	F	-	+	-	-	-	-	-	-	-	-	-
AS 9	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
AS 10	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
AS 11	-	+	-	-	-	-	-	-	-	-	F	+	+	-	-	-	-	-	-	-	-	-
AS 12	-	-	+	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
AS 13	-	-	-	-	-	-	-	-	-	+	F	-	+	-	-	-	-	-	-	-	-	-
AS 14	-	-	-	-	-	-	+	-	+	-	F	-	+	-	-	-	-	-	-	-	-	+
AS 15	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
Ar 1	-	-	+	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-
Ar 2	-	-	+	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	+
Ar 3	-	-	+	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	+
Ar 4	-	-	-	-	-	-	-	-	-	-	S	+	+	-	-	+	-	-	-	-	-	-
Ar 5	-	-	+	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-
Ar 6	-	-	-	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
Ar 7	-	-	+	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	+
Ar 8	-	-	-	-	-	-	-	-	-	-	F	-	+	+	-	+	-	-	-	-	-	-

Table 1a *Continued*

	X (−8.0)	X (−7.4)	B (−7.2)	I (0.28)	Hp (−6.9)	X (−5.3)	H (−3.7)	H (−3.0)	D (0.2)	I (5.0)		P (1.1)	Xh (1.2)	P (1.3)	E (1.4)	I (0.4)	I (0.7)	I (3.0)	I (4.5)	I (1.0)	B (3.9)	E (9.0)
Ar 9	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	+
Ar 10	-	-	-	-	-	-	-	+	+	-	F	-	+	-	-	-	-	-	-	-	-	-
Ar 11	-	-	-	-	-	-	-	+	+	-	F	-	+	-	-	-	-	-	-	-	-	-
Ar 12	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
Ar 13	-	-	-	-	-	-	-	-	-	-	F	+	+	-	-	-	-	+	-	-	-	-
Ar 14	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	+
Ar 15	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
CH 1	-	-	-	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	+
CH 2	-	-	+	-	+	-	-	+	-	-	S	+	+	-	-	+	-	-	-	-	+	-
CH 3	-	-	+	-	-	-	-	+	-	-	S	-	-	-	-	-	-	-	-	-	-	-
CH 4	-	-	+	-	+	-	-	-	-	-	S	+	+	-	-	+	-	-	-	-	+	-
CH 5	-	-	-	-	-	-	-	+	-	-	F	-	+	-	-	-	-	-	-	-	-	-
CH 6	-	-	-	-	-	-	-	-	+	-	F	-	+	-	-	-	-	-	-	-	-	-
CH 7	-	-	+	-	-	-	-	+	+	-	F	-	+	-	-	-	-	-	-	-	-	-
CH 8	-	-	+	-	-	-	-	+	-	-	S	-	+	-	-	-	-	-	-	-	+	-
CH 9	-	-	+	-	-	-	-	+	-	-	F	-	+	-	-	-	-	-	-	-	-	-
CH 10	-	-	+	-	-	-	-	+	-	-	S	+	+	-	-	-	-	-	-	-	-	-
CH 11	-	-	+	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
CH 12	-	-	+	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
CH 13	-	-	+	-	-	-	-	-	-	-	F	-	+	+	-	-	-	-	-	-	-	-
CH 14	-	-	-	-	-	-	-	-	+	-	null	+	+	-	+	-	-	-	-	-	-	+
Cd 1	-	-	-	-	-	-	+	-	+	-	S	-	-	-	-	-	-	-	-	-	-	-
Cd 2	-	-	+	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-
Cd 3	-	-	+	-	-	-	-	-	-	-	S	+	+	-	-	+	-	-	-	-	-	-
Cd 4	-	-	+	-	-	-	-	+	-	-	S	-	+	-	-	-	-	-	-	-	-	+
Cd 5	-	-	+	-	-	-	-	+	-	-	S	-	-	+	-	-	+	-	-	-	-	-
Cd 6	-	-	+	-	+	-	+	-	+	-	S	-	+	-	-	-	-	-	-	-	-	-
Cd 7	-	-	+	-	-	-	-	+	-	-	S	-	-	-	-	-	-	-	-	-	-	-
Cd 8	-	-	+	-	-	-	-	+	-	-	S	-	-	-	-	-	-	-	-	-	-	+
Cd 9	-	-	-	-	-	-	-	+	-	-	S	-	-	-	-	-	-	-	-	-	-	-
Cd 10	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
Cd 11	-	-	-	-	-	-	+	+	-	-	F	-	-	-	-	-	-	-	-	-	-	-
Cd 12	-	-	+	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
Cd 13	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	-	-	-	-
Cd 15	-	-	-	-	+	-	-	-	-	-	F	-	+	+	-	-	-	-	-	+	+	+
Cd 16	-	-	-	-	-	-	-	-	-	-	S	-	+	-	-	-	-	-	-	-	-	-

Table 1b Restriction endonuclease variation in the *Adh* gene region of Chinese natural populations of *D. melanogaster*. All abbreviations are the same as in Table 1a. The populations are Haikou (H), Guangzhou (G), Fuzhou (F), Shanghai (S), Jinan (J) and Dalian (D)

	E (−8.6)	X (−7.4)	B (−7.2)	I (0.35)	Hp (−6.9)	X (−5.3)	H (−3.7)	H (−3.0)	D (0.2)	I (1.5)		P (1.1)	Xh (1.2)	P (1.3)	I (0.48)	I (0.4)	I (0.7)	E (2.5)	E (9.0)
H 1	-	-	-	-	-	-	-	-	+	-	F	-	+	-	-	-	-	-	+
H 2	-	-	-	+	-	+	-	-	+	-	ChD	-	+	-	-	+	-	+	-
H 3	-	-	-	-	-	-	-	-	+	-	ChD	-	+	-	-	-	-	-	-
H 4	-	-	-	-	-	-	-	-	+	+	F	-	+	+	-	-	-	-	-
H 5	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	+	-	-	-
H 6	-	-	-	-	-	-	-	-	+	-	F	-	+	-	-	-	-	-	-
H 7	-	-	-	-	-	-	-	-	+	-	F	-	+	-	-	-	-	-	-
H 8	-	-	-	-	-	-	-	-	+	-	F	-	+	-	-	-	-	-	+
H 9	-	-	-	-	-	-	-	-	+	-	S	-	+	-	-	-	-	-	-
H 10	-	-	-	-	-	-	-	+	+	-	S	-	+	+	-	+	-	-	+
H 11	-	-	-	-	-	-	-	-	-	-	S	-	+	+	-	+	-	-	-
H 12	-	-	-	-	-	-	-	+	+	-	S	-	+	+	-	+	-	-	+
H 13	-	-	-	-	+	-	-	+	+	-	S	-	+	+	-	+	-	-	+
H 14	-	-	-	-	-	-	-	-	+	-	S	-	+	-	-	-	-	-	+
H 15	-	-	-	-	-	-	-	+	+	-	S	-	+	-	-	-	-	-	+
G 1	-	-	-	-	-	-	-	-	-	-	F	-	+	-	-	-	-	-	+
G 2	-	-	-	-	-	-	-	-	+	-	ChD	-	+	-	-	-	-	-	-
G 3	-	-	-	-	-	-	-	+	+	-	F	-	+	-	-	-	-	-	+
G 4	-	-	-	-	-	-	-	+	+	-	F	-	+	-	-	-	-	-	-
G 5	-	-	-	-	-	-	-	+	+	-	F	-	+	-	-	-	-	-	-
G 6	-	-	-	-	-	-	-	-	+	-	ChD	-	+	-	-	-	-	-	-
G 7	-	-	+	-	-	-	-	+	+	-	F	-	+	-	-	-	-	-	-

Table 1b *Continued*

	E (−8.6)	X (−7.4)	B (−7.2)	I (0.35)	Hp (−6.9)	X (−5.3)	H (−3.7)	H (−3.0)	D (0.2)	I (1.5)		P (1.1)	Xh (1.2)	P (1.3)	I (0.48)	I (0.4)	I (0.7)	E (2.5)	E (9.0)
G 8	−	−	−	−	−	−	−	+	+	−	F	−	+	−	−	−	−	−	−
G 9	−	−	+	−	−	−	−	−	+	−	S	−	+	−	−	−	−	−	−
G 10	−	−	+	−	−	−	−	−	+	−	S	−	+	−	−	−	−	−	−
G 11	−	−	−	−	−	−	−	+	+	−	S	−	+	+	−	+	−	−	−
G 12	−	−	+	−	−	−	−	−	−	−	S	−	+	−	−	−	−	−	−
G 13	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
G 14	−	−	−	−	−	−	−	−	+	−	S	−	+	−	−	−	−	−	+
G 15	−	−	−	−	−	−	−	−	+	−	S	−	+	−	−	−	−	−	−
F 1	−	−	+	−	−	−	−	−	−	−	S	−	+	−	−	−	−	−	−
F 2	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
F 3	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
F 5	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
F 7	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
F 10	−	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	−
F 11	−	−	−	−	−	−	−	−	+	−	S	−	+	−	−	−	−	−	−
F 14	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
F 16	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
F 21	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
F 22	−	−	−	−	−	−	−	−	+	−	ChD	−	+	−	+	−	−	−	−
F 23	−	−	−	−	−	−	+	+	−	−	F	−	+	−	−	−	−	−	−
F 28	−	−	−	−	−	−	−	−	+	−	S	−	+	−	−	−	−	−	−
F 33	−	−	−	−	−	−	+	−	−	−	S	−	+	−	−	−	−	−	−
F 39	−	−	−	−	−	−	−	−	+	−	S	−	+	−	−	−	−	−	−
S 2	−	−	+	−	−	−	−	−	−	−	S	−	+	−	−	−	−	−	−
S 3	−	−	+	−	−	−	−	−	−	−	S	−	+	−	−	−	−	−	−
S 4	−	+	−	−	−	−	−	−	−	−	F	−	+	−	+	−	−	−	−
S 5	−	−	−	−	−	−	−	+	−	−	F	−	+	−	−	−	−	−	−
S 6	−	+	−	−	−	−	−	−	+	−	F	+	+	−	−	+	−	−	−
S 7	−	+	−	−	−	−	−	−	+	−	F	+	+	−	−	+	−	−	−
S 13	+	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
S 18	−	+	−	−	−	−	−	−	+	−	F	+	+	−	−	+	−	−	−
S 24	−	−	+	−	−	−	−	−	−	−	S	−	+	−	−	−	−	−	−
S 26	+	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
S 27	−	+	−	−	−	−	−	−	−	−	S	−	+	+	+	−	−	−	−
S 28	−	−	−	−	−	−	−	−	−	−	S	−	+	−	−	−	−	−	−
S 32	−	+	−	−	−	−	−	−	+	−	F	+	+	−	+	−	−	−	−
S 34	−	−	−	−	−	−	−	+	−	−	F	−	+	−	−	−	−	−	−
S 39	−	−	−	−	−	−	+	−	−	−	F	+	+	−	−	+	−	−	−
J 1	−	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	−
J 3	−	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	−
J 4	−	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	−
J 6	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
J 7	−	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	−
J 8	−	−	−	−	−	−	−	−	−	−	F	−	+	+	−	−	+	−	−
J 11	−	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	−
J 12	−	−	−	−	−	−	−	−	+	−	F	−	+	+	−	−	−	−	−
J 14	−	−	+	−	−	−	−	−	−	−	S	−	+	−	−	−	−	−	−
J 16	−	−	+	−	−	−	−	−	−	−	S	−	+	+	−	+	−	−	−
J 21	−	−	−	−	−	−	−	−	−	−	S	−	+	−	−	−	−	−	−
J 26	−	−	+	−	−	−	+	−	−	−	F	−	+	−	−	−	−	−	−
J 30	−	−	+	−	−	−	−	−	−	−	S	−	+	−	−	−	−	−	−
J 36	−	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	−
J 42	−	−	+	−	−	−	+	−	−	−	ChD	−	+	−	−	−	−	−	−
D 2	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
D 4	+	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
D 6	−	−	−	−	−	−	−	+	−	−	F	−	+	−	−	−	−	−	−
D 7	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
D 10	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
D 11	+	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
D 12	−	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	−
D 14	−	−	−	−	−	−	−	−	+	−	F	−	+	−	−	−	−	−	−
D 15	−	−	+	−	−	−	−	−	−	−	S	−	+	−	−	−	−	−	−
D 16	−	−	−	−	−	−	−	+	−	−	F	−	+	−	−	−	−	−	−
D 17	−	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	−
D 20	−	+	−	−	−	−	−	−	−	−	F	−	+	−	−	+	−	−	+
D 22	+	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	−
D 23	−	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	+
D 30	−	−	−	−	−	−	−	−	−	−	F	−	+	−	−	−	−	−	+

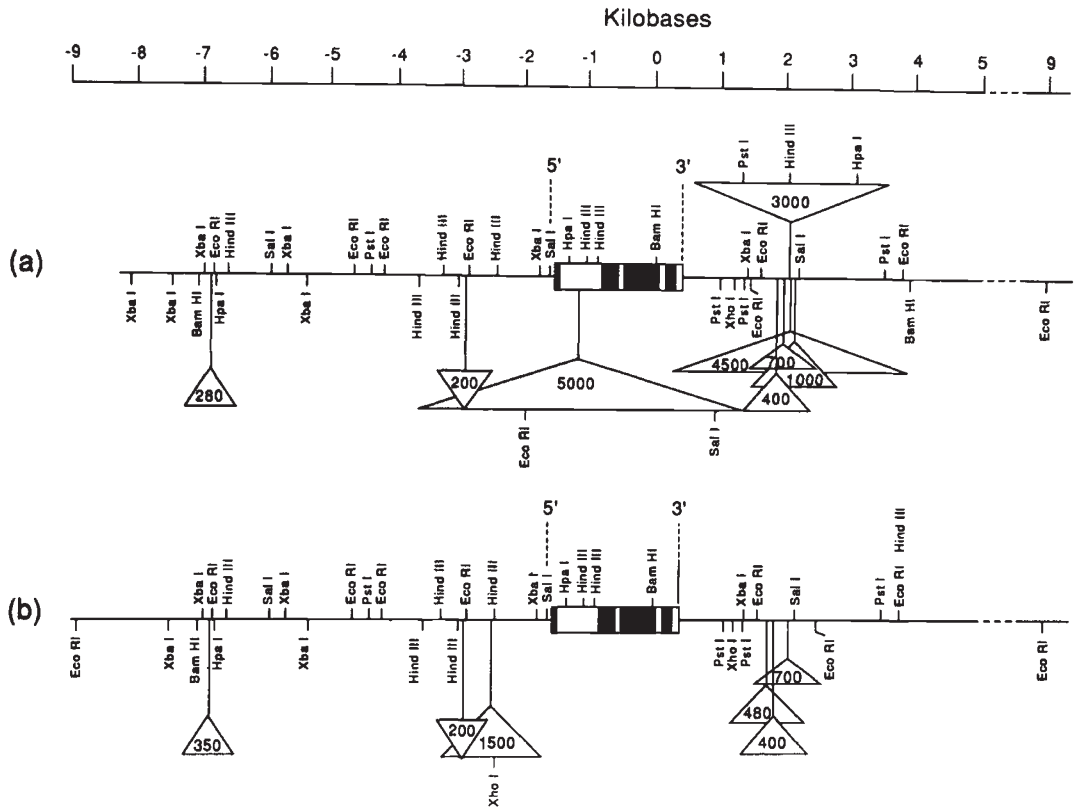

Fig. 2 Restriction endonuclease variation detected in the *Adh* region of (a) 104 chromosomes from seven Australian populations and (b) 90 chromosomes from six Chinese populations. The *Adh* transcription unit is boxed and the adult 5′ leader sequence and the three exons are shaded. Polymorphic restriction sites are indicated below the map; triangles represent insertions (points towards the map) and deletions (points away from map). Estimated sizes (in bp) of the insertions and deletions, and the restriction sites within the insertions, are indicated. The *Eco* RI (+9.0) site was outside the probed region but it could be scored unambiguously in all populations.

et al., 1981; Hudson, 1982) and the haplotype diversity (Nei & Tajima, 1981). These analyses (Table 2) show that overall there is a higher level of nucleotide-substitution variation in the Australian populations than in the Chinese populations.

Restriction map haplotype diversity (Table 3) is similar in the Australian and Chinese populations when both forms of variation (site and sequence length) are considered. The level of haplotype diversity in Adh^S-bearing chromosomes was higher than for Adh^F chromosomes in both sets of populations. The few data available for chromosomes bearing a third kind of *Adh* allele [Adh^{FChD} (all in the Chinese samples)] indicate that they are the least diverse (Table 3).

There is no evidence from these data for any consistent relationship between latitude and the estimates of variation in nucleotide-substitution or in haplotype diversity. Furthermore, none of the restriction site polymorphisms, nor any of the insertions, showed a significant association with latitude. However, the frequency of the 0.2 kb deletion, which was present in all the Chinese populations, and all but one of the Australian populations (the exception was Tamar), varied with latitude in the Chinese ($r = -0.89$, $P < 0.01$) but not in the Australian populations (Fig. 3).

Non-random associations between variants in the *Adh* region are listed in Table 4. Tests for linkage disequilibria used Fisher's exact test for a 2×2 contingency table; any variant with a frequency less than 15 or more than 85 per cent was excluded from the analysis. The most common non-random association is the one between the *Bam* HI (−7.2) site and Adh^S: this association occurred in eight of the 10 populations in which the frequency of *Bam* HI (−7.2) is greater than 15 per cent. Another common form of association is between the presence of insertions/deletions and some restriction sites, such as the non-random association between *Hind* III (−3.0) and D (0.2), which occurred in three populations.

Ten different insertions (insertion 0.28, 0.35, 0.4,

Table 2 Estimates of the proportion of polymorphic nucleotides (p), overall heterozygosity (h) (Nei & Tajima, 1981), and estimated heterozygosity per nucleotide pair (θ) (Ewens *et al.*, 1981). Standard errors (Hudson, 1982; Nei & Tajima, 1981) are given in parentheses and that for θ assumes free recombination. n is the number of second chromosomes extracted from each population

Population	Latitude	n	p	h	θ
Australia					
Cygnet	43° 09′ S	15	0.017 (0.007)	0.95 (0.01)	0.006 (0.002)
Tamar	41° 02′ S	15	0.029 (0.007)	0.86 (0.06)	0.007 (0.003)
Chateau Yarrinya	37° 36′ S	15	0.022 (0.011)	0.99 (0.01)	0.008 (0.004)
All Saints	36° 03′ S	15	0.024 (0.008)	0.95 (0.01)	0.009 (0.005)
Araluen	35° 03′ S	15	0.020 (0.008)	0.96 (0.01)	0.007 (0.003)
Coffs Harbour	30° 18′ S	14	0.024 (0.008)	1.00 (—)	0.009 (0.003)
Cardwell	18° 16′ S	15	0.024 (0.008)	0.98 (0.01)	0.009 (0.003)
Australian populations		104	0.021 (0.006)	0.96 (0.1)	0.008 (0.002)
China					
Haikou	20° 02′ N	15	0.017 (0.007)	0.94 (0.02)	0.006 (0.003)
Guangzhou	23° 08′ N	15	0.012 (0.006)	0.94 (0.02)	0.005 (0.002)
Fuzhou	26° 05′ N	15	0.009 (0.005)	0.83 (0.07)	0.004 (0.002)
Shanghai	21° 10′ N	15	0.019 (0.007)	0.89 (0.04)	0.007 (0.003)
Jinan	37° N	15	0.012 (0.006)	0.90 (0.04)	0.005 (0.002)
Dalian	38° 54′ N	15	0.015 (0.007)	0.76 (0.13)	0.005 (0.002)
Chinese populations		90	0.014 (0.004)	0.88 (0.05)	0.005 (0.001)
Overall		194	0.03 (0.003)	0.904 (0.018)	0.0065 (0.001)

Table 3 Restriction map haplotype diversity in the *Adh* region. The numbers of different haplotypes for each category are given in parentheses

Variation considered	All chromosomes	Adh^F	Adh^S	Adh^{FChD}
Australian populations*				
Restriction site	0.95 (48)	0.90 (19)	0.97 (28)	—
Sequence length	0.79 (16)	0.58 (8)	0.54 (7)	—
All variation	0.97 (59)	0.93 (28)	0.97 (30)	—
Chinese populations				
Restriction site	0.90 (28)	0.78 (14)	0.80 (10)	0.64 (4)
Sequence length	0.86 (14)	0.67 (7)	0.71 (4)	0.46 (3)
All variation	0.96 (48)	0.90 (31)	0.92 (13)	0.64 (4)

*One chromosome carried an *Adh* null allele.

0.48, 0.7, 1.0, 1.5, 3.0, 4.5 and 5.0 kb) were sub-cloned in the bacteriophage vector EMBL3 and probed with DNA sequences from each of seven known mobile elements. The results (Table 5) show that two of the cloned insertions shared homology with known mobile elements. Insertion 0.28 kb, which was present in the Cygnet, Tamar and All Saints populations, showed homology with *B104B*, which usually has approximately 100 copies in the *D. melanogaster* genome (Scherer *et al.*, 1982). A second insertion, 0.4 kb, showed homology to the mobile element *F101*, which usually has about 25 copies in the *D. melanogaster*

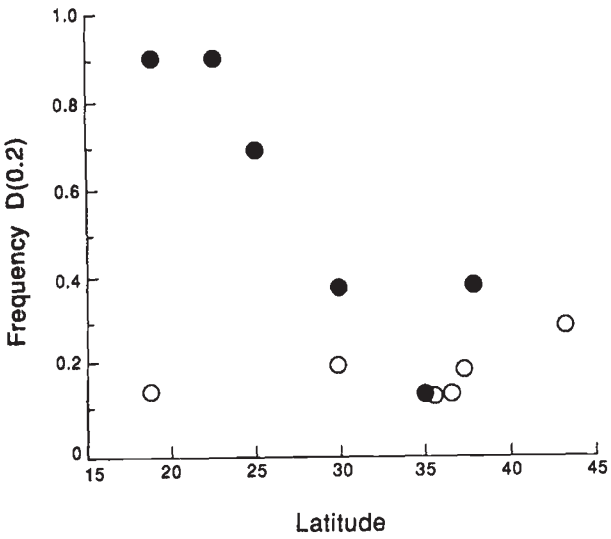

Fig. 3 Latitudinal variation in the frequency of the deletion 0.2 kb in populations from China (●) and Australia (○).

genome (Dawid *et al.*, 1981). Insertion 0.4 kb occurred in each of the Australian and Chinese populations at frequencies ranging from 0.07 to 0.47.

Discussion

The estimates of the proportion of polymorphic nucleotides, the overall heterozygosity and the heterozygosity per nucleotide pair are each higher for the Australian populations than for the Chinese populations but, in the total dataset, these values are not dissimilar to those obtained in earlier studies on populations from North America, Europe and Australia (Table 6).

The differences between the Australian and Chinese populations — particularly in ρ and θ — might be related to the history of the populations on the two continents. David & Capy (1988) have suggested that populations of *D. melanogaster* may be considered in

Table 4 Significant gametic disequilibria in the Australian and Chinese populations. The abbreviations I and D represent insertions and deletions and their sizes (kb) are given in parentheses

Populations	Significant gametic disequilibria
Australia	
Cygnet	*Hind* III(−3.0):*Adh**, *Hind* III(−3.0):D(0.2)**
	Adh:D(0.2)*
Tamar	*Bam* HI(−7.2):*Adh***, *Hpa* I(−6.9):*Bam* HI(3.9)**
	Pst I(1.1):I(0.4)**, *Xho* I(1.2):*Eco* RI(9.0)*
Chateau Yarrinya	*Hind* III(−3.0):*Hind* III(−3.7)*,
	Hind III(−3.0):D(0.2)*,
	Bam HI(−7.2):*Adh***, I(1.0):*Xba* I(−8.0)**,
	Xba I(−7.4):I(0.4)**
All Saints	*Bam* HI(−7.2):*Adh***, *Xba* I(−5.2):*Xba* I(−7.4)*,
	Xba I(−5.2):*Pst* I(1.3)*, I(0.4):*Xba* I(−7.4)**,
	I(0.4):*Xba* I(−5.2)*, I(0.4):*Pst* I(1.3)*
Araluen	*Bam* HI(−7.2):*Adh***, *Hind* III(−3.0):D(0.2)**
Coffs Harbour	*Hpa* I(−6.9):I(0.4)**, I(0.4):*Pst* I(1.1)*,
	Hpa I(−6.9):*Bam* HI(3.9)**, I(0.4):*Bam* HI(3.9)*
Cardwell	*Bam* HI(−7.2):*Adh***, *Hind* III(−3.7):D(0.2)**
China	
Haikou	D(0.2):*Eco* RI(9.0)*, *Pst* I(1.3):I(0.4)*
Guangzhou	*Bam* HI(−7.2):*Adh**
Fuzhou	None
Shanghai	*Bam* HI(−7.2):*Adh***, D(0.2):*Pst* I(1.1)*
	I(0.48):*Xba* I(−7.4)*, I(0.48):*Bam* HI(−7.2)*
	I(0.48):*Pst* I(1.3)**
Jinan	*Bam* HI(−7.2):*Adh***
Dahlian	None

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

Table 5 Cloned insertions and the results of hybridization with probes derived from known mobile elements

Second chromosome line	Insertion size (kb)	Mobile element probes†						
		ρπ25.1	Copia	B104B	P1407	412	297	F101
T29	0.28	–	–	+	–	–	–	–
CYI	0.4	–	–	–	–	–	–	+
CY2	1.0	–	–	–	–	–	–	–
AS13	5.0	–	–	–	–	–	–	–
Ar13	3.0	–	–	–	–	–	–	–
Cd5	0.7	–	–	–	–	–	–	–
Cd15	4.5	–	–	–	–	–	–	–
H2	0.35, 0.4	–	–	–	–	–	–	–
H4	1.5	–	–	–	–	–	–	–
S39	0.48	–	–	–	–	–	–	–

+ Denotes positive hybridization.
†See Materials and Methods for details.

Table 6 Estimates of the proportion of polymorphic nucleotides (ρ), haplotype diversity (h), and estimated heterozygosity per nucleotide pair (θ)

Population	ρ	h	θ
Australia			
This study	0.021	0.96	0.08
Chateau Tahbilk (Cross & Birley,1986)	0.017	0.94	0.005
North America			
Texas (Birley, 1984)	0.012	0.86	0.004
Eastern States (Aquadro *et al.*, 1986)	—	0.97	0.006
Europe			
Groningen (Cross & Birley, 1986)	0.025	0.66	0.007
China			
This study	0.014	0.88	0.005

terms of three categories which comprise 'ancestral' populations in tropical Africa (where *D. melanogaster* originated), 'ancient' populations derived from the initial colonization of Eurasia and 'new' populations introduced by *Homo sapiens*. Contemporary Chinese populations may well be a mixture of the 'ancient' and 'new', but it is likely that the *D. melanogaster*, which colonized Australia following European settlement (David & Capy, 1988; Lachaise *et al.*, 1988), had diverse origins, and this may account for the higher levels of variation in contemporary Australian populations.

The distribution of restriction endonuclease variation between the Australian populations differs from the distribution between the Chinese populations. A χ^2 analysis of the frequencies of restriction endonuclease variants between Chinese populations shows that most of the populations differ from each other and the island population of Haikou differs from each of the mainland populations (Table 7). In contrast, a similar analysis of the Australian data reveals no significant differences between the mainland populations except between Chateau Yarrinya and Coffs Harbour, which are about 2,000 km apart. All other significant differ-

Table 7 Chi-squared analysis of the frequencies of the restriction endonuclease variants compared between populations. Degrees of freedom are given in parentheses

Australian populations	Cygnet	Tamar	Chateau Yarrinya	All Saints	Araluen	Coffs Harbour	Cardwell
Cygnet	—	18.07	23.05*(12)	17.54(14)	7.4(11)	13.19(11)	12.18(14)
Tamar	—	—	40.13***(14)	14.57(13)	11.99(12)	15.28(11)	27.07(13)
Chateau Yarrinya	—	—	—	15.56(13)	20.20(12)	24.98*(14)	17.04(15)
All Saints	—	—	—	—	10.52(13)	20.45(14)	19.62(16)
Araluen	—	—	—	—	—	13.04(12)	14.98(14)
Coffs Harbour	—	—	—	—	—	—	11.02(12)

Chinese populations	Jinan	Shanghai	Fuzhou	Guangzhou	Haikou
Dahlian	19.63*(9)	19.64*(10)	10.79(7)	11.40(7)	22.89*(11)
Jinan	—	24.75**(10)	12.80*(6)	34.34**(14)	38.07***(12)
Shanghai	—	—	22.06**(9)	26.7**(10)	40.04***(15)
Fuzhou	—	—	—	11.49(6)	26.41**(11)
Guangzhou	—	—	—	—	20.88*(11)

*$P<0.05$, **$P<0.01$, ***$P<0.001$.

ences involved comparisons with the two Tasmanian populations, which themselves differ, even though Tamar is only 300 km north of Cygnet. The difference between the Cygnet and Tamar populations is interesting as it could be interpreted as evidence that the two populations, which differ markedly in Adh^F frequency (Anderson & Gibson, 1985), are more geographically isolated than any two Australian mainland populations separated by the same distance. Other than between the Tasmanian populations, the data suggest that there is less migration of *D. melanogaster* per unit distance in the eastern part of mainland China than in a similar region of Australia.

A striking component of the geographical heterogeneity between the Chinese populations is the decrease in frequency with distance from the equator of the deletion 0.2 kb. This deletion was present in all Chinese and Australian populations with the exception of Tamar. However, its frequency was relatively low in the Australian populations, and not related to latitude. This deletion is in a region of the *Adh* landscape, which has been implicated in the control of ADH expression (Goldberg *et al.*, 1983; Posakony *et al.*, 1985) and we show elsewhere that it does have an effect on ADH activity levels. Nevertheless, the lack of consistency in its distribution in Chinese and Australian populations would, at this stage, appear to mitigate against the idea that the deletion is implicated in the Adh^S cline.

A remarkable feature of the restriction endonuclease variation, which is highly consistent in the Chinese and Australian populations, concerns the non-random localization of insertions/deletions in the region of the *Adh* locus. Other than the nucleotide change responsible for the Adh^F and Adh^S alleles, there were no restriction endonuclease polymorphisms in the *Adh* coding regions, and only one change was found in an intron (one chromosome from the All Saints population had a 5.0 kb insertion in intron 1). The majority of the insertions occur in a region 1.5–3.5 kb 3′ to the coding region. The techniques used in this study were unable to resolve insertions or deletions of less than 50 bp. This level of resolution is similar to that obtained by Langley *et al.* (1982), Birley (1984) and Cross & Birley (1986), but less than that achieved by Aquadro *et al.* (1986). To summarize the results derived from the five separate studies, we have collated data on the locations of insertions/deletions of greater than 50 bp. These data (Fig. 4.) show that 51 per cent of the 85 insertions in the 336 second chromosome lines investigated in the *Adh* region were located about 1.5–2 kb 3′ from the end of the *Adh* coding region in a 0.23 kb *Eco* RI/*Sal* I fragment (85 per cent of the insertions were in the region +1.5 kb to +3.5 kb). Of the 70 deletions, 67 appeared to be at a site approximately 3 kb 5′ to the *Adh* coding region, and two were in the general region of the majority of the insertions. This clustering of insertions and deletions may arise from the susceptibilities of the two regions to the insertion of mobile elements (the DNA sequences of the two regions are not yet known, but they may contain recog-

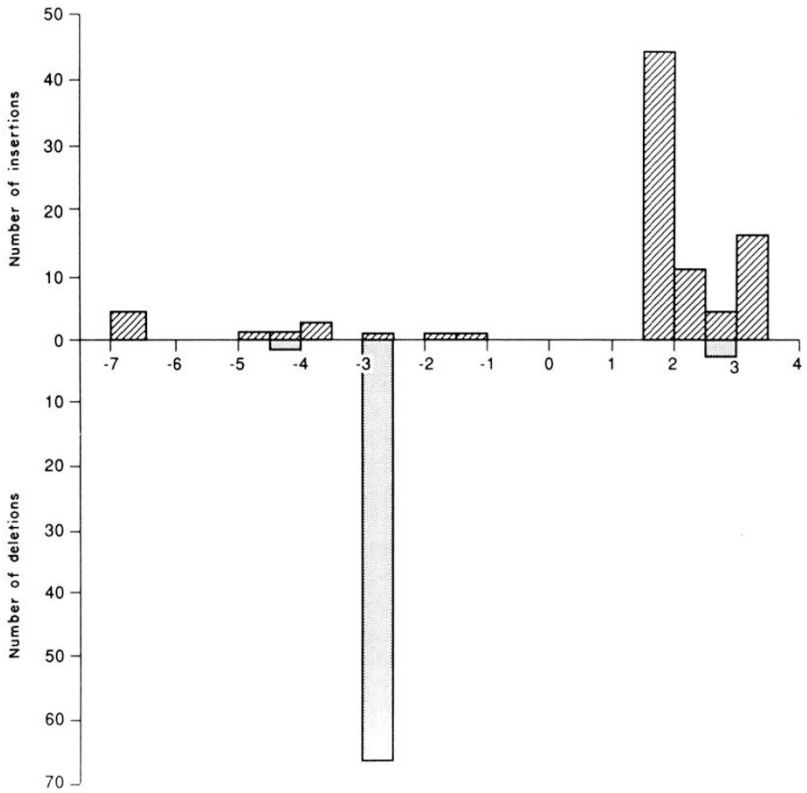

Fig. 4 The distribution of insertions and deletions (larger than 50 bp) in the 11.8 kb region of the *Adh* gene. Data have been collated from the present study and from Langley *et al.* (1982), Birley (1984), Cross & Birley (1986) and Aquadro *et al.* (1986).

nition sequences for one or more mobile elements) or it may be that insertions in the region cause no selective disadvantage.

Most of the insertions found in this and in previous studies have not been identified. The exceptions are the insertions 0.28 and 0.4 kb. The 0.28 kb insertion shared homology with the *copia*-like element *B104B*, which is usually present in about 100 copies in the genome of *D. melanogaster*. This type of insertion was found only in the Tasmanian and the All Saints populations. A typical *B104* element is 8.7 kb and is flanked by 429 bp direct terminal repeats. Finnegan *et al.* (1978) found that a 2.4 kb fragment inserted in *B104* was part of a cDM412 clone. Wensink *et al.* (1979) showed that *D. melanogaster* DNA contains clusters of short repetitive sequences (less than 1 kb) scattered over a large number (1000) of chromosome regions, and some of these clusters hybridize to the region of the cDM412 clone which contains the *B104* fragment with the 1.4 kb insertion. The 0.28 insertion may be one of these repetitive sequences. It has been estimated that the rates of insertion and excision of some of the *copia* and *copia*-like elements is less than 4×10^{-4} per element per generation (Harada *et al.*, 1990).

The other insertion that we identified (0.4 kb) was found in Australian and Chinese populations and appears similar in size and location to an insertion described by Aquadro *et al.* (1986). In our study this insertion was homologous to *F101* whilst the one found in the North American populations was homologous to cDM2161, which is an *F*-like element similar in structure to *F101* (Aquadro *et al.*, 1986). In the *Adh* region none of the insertions so far tested shared homology with *P* elements.

A further notable aspect of restriction endonuclease variation in the *Adh* region is the linkage disequilibrium between *Bam* HI (−7.2) and Adh^S. This association is a consistent finding from studies of the molecular landscape of the *Adh* region (Langley *et al.*, 1982; Aquadro *et al.*, 1986). The linkage, however, is not as tight in the Australian and Chinese data as it is in the North American populations. It has been suggested that the mutation giving rise to Adh^F occurred in an Adh^S allele on a chromosome lacking the *Bam* HI (−7.2) site and that subsequently the Adh^F allele increased in frequency in temperate regions (Cross & Birley, 1986). The disequilibrium would take a long time to decay in the absence of any selection maintaining the association (Avery & Hill, 1979). It may thus be relevant that in the 'old' Chinese populations the linkage is looser than in the Australian or North American populations (Aquadro *et al.*, 1986; Kreitman & Aquade, 1986a). There is a higher level of heterozygosity for haplotypes with Adh^S than for those with

Adh^F, and this supports the DNA sequence data which show that Adh^S is the ancestral allele (Kreitman, 1983; Ashburner *et al.*, 1984; Bodmer & Ashburner, 1984; Cohe & Moore, 1988). The few data available in the present study further show that the haplotypes with Adh^{FChD} have the lowest level of heterozygosity lending support to the argument based on amino acid and DNA sequence data (Chambers *et al.*, 1981; Collet, 1988) that Adh^{FChD} was derived from an Adh^F allele.

In conclusion, this survey of restriction endonuclease variation in populations from continents in both hemispheres has not, with the possible exception of the deletion 0.2 kb, provided any evidence (given the level of resolution) that the molecular landscape surrounding the *Adh* locus varies in a systematic way in line with the cline in Adh^S frequency. This result is in agreement with data from North America (Kreitman & Aquade, 1986a Simmons *et al.*, 1989). Nevertheless, the data indicate that the levels of variation in Australian and Chinese populations may reflect the differing histories of *D. melanogaster* populations on two continents. The evolutionary significance of the discrete pattern of insertions and deletions in the region of the *Adh* locus is more difficult to interpret, but it may reflect some form of selection that affects the genome in the region of the *Adh* locus.

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