Alcohol dehydrogenase and sn-glycerol-3-phosphate dehydrogenase null activity alleles in natural populations of *Drosophila melanogaster*

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Alcohol dehydrogenase (*Adh*) null activity alleles have been detected in each of 2 years in Australian populations of *D. melanogaster* at frequencies up to 3.9 per cent, but with an average of 1.3 per cent in 1983 and 0.7 per cent in 1984. In the same populations the frequency of sn-glycerol-3-phosphate dehydrogenase (*Gpdh*) null alleles was as high as 2.7 per cent with an average of 1.6 per cent in 1983 and 0.5 per cent in 1984. These values compare with previously reported frequencies of 0.09 per cent for *Adh* and 0.8 per cent for *Gpdh* in a North Carolina (U.S.A.) and a London (U.K.) population. Of the 12 second chromosomes bearing *Adh* null alleles, four were homozygous lethal, but all were viable and fertile in combination with *Df(2L)64j*.

INTRODUCTION

It is generally assumed that homozygotes and heterozygotes for enzyme variants can be unambiguously discriminated after electrophoresis; that the phenotypes of apparent homozygotes represent two copies of the same allele, and that null activity alleles at allozyme loci are sufficiently rare to be disregarded.

Nevertheless, the presence of null activity alleles has been postulated to explain the homozygote excesses, often found in marine organisms (Ayala *et al.*, 1974; Boyer, 1974; Gartner-Kepkay *et al.*, 1980). Skibinski *et al.*, (1983) estimated that null allele frequencies would have to reach 10 per cent to account for the observed excess of apparent homozygotes at some loci. Koehn and Mitton, (1972) and Boyer, (1974) found a few *Mytilus edulis* individuals with no detectable leucine amino-peptidase activity in samples with a significant homozygote excess, which may be homozygotes for a null activity allele or represent cases of physiological inactivation of the enzyme.

Data from three recent surveys carried out specifically to identify null activity alleles also support the assumption that such alleles are rare. Mean frequencies of nulls for 20 autosomal loci in *Drosophila melanogaster* were estimated to be 0.25 per cent for a Raleigh, North Carolina and 0.23 per cent for a London, England population (Voelker *et al.*, 1980 and Langley *et al.*, 1981). In

two *Pinus* species null activity alleles were found at three of 27 loci in *Pinus resinosa* and at 8 of 29 loci in *P. ponderosa* giving mean frequencies of 0.28 per cent and 0.31 per cent respectively (Allendorf *et al.*, 1981).

The distributions of null activity alleles in both the *D. melanogaster* populations were consistent with a mutation-selection balance mechanism (Langley *et al.*, 1981). Mukai and Cockerham, (1977) estimated the rate of spontaneous mutation to null activity alleles to be 1.03×10^{-5} over five loci and in a later analysis of the same material Voelker, Schaffer, and Mukai, (1980) estimated an average rate of 3.86×10^{-6} over seven loci. This is lower than their estimate of 1.28×10^{-6} for new electrophoretic mobility variants which were obtained from the same study.

In this paper we describe an unexpectedly high frequency of null activity alleles at the alcohol dehydrogenase (EC 1.1.1.1) locus (*Adh*) and at the sn-glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) locus (*Gpdh*) in Australian populations of *D. melanogaster*. The average frequencies of *Adh* null alleles is about ten times higher than those reported by Voelker *et al.*, (1980).

MATERIAL AND METHODS

Populations of *D. melanogaster* were sampled in May, 1983 in Tasmania. Two sites in the south of

the island, Cygnet and Huonville (I), are apple processing factories about 10 km apart and two sites 200 km to the north, Tamar Valley and Pipers Brook, are wineries (30 km apart). To test the generality of the results flies were also collected in July 1983 from a banana plantation at Coffs Harbour, N.S.W. In May 1984 further collections were made at Cygnet and Tamar and at a site 3 km from Huonville where apple processing waste had been dumped for cattle fodder (Huonville II). The collection sites spanned 13 degrees of latitude on a north-south axis.

The populations at Cygnet and at Huonville I and II were breeding on decomposing apple pulp and those at Coffs Harbour on avocados and bananas. The Tamar and Pipers Brook collections were made over piles of discarded grape pressings.

At all sites adult flies were net swept and transported on standard laboratory media. The populations of adult flies were very large at most sites.

From each of the 1983 samples between 66 and 187 fertilised females were set up singly in vial cultures. From each culture the female parent and ten progeny were classified after electrophoresis (see below) for ADH and GPDH allozymes. The Cygnet samples revealed cultures in which the distribution of ADH or GPDH phenotypes among the progeny could be explained only by invoking the presence of a null activity allele. Similar cultures were found in the Tamar, Pipers Brook and Coffs Harbour samples. The types of culture suggesting null activity alleles are shown in table 1 and in no case can the anomalies be explained by double matings. From nine of the anomalous cultures *Adh* null activity alleles were isolated using *CyO* as a second chromosome balancer (Lindsley and Grell, 1968), or by inbreeding from appropriate single pair cultures. If the anomalous cultures produced *Adh^S/n* male flies these were mated to *CyO* heterozygotes (homozygous *Adh^F*). From the

progeny single pair matings were set up, *CyO/n* × *CyO/n* matings were identified by electrophoresis and *n/n* homozygotes were obtained.

The 1984 Tasmanian collections were used to test the 1983 results with the null assay technique described by Voelker *et al.*, (1980). To reduce the possibility of dysgenic phenomena (Engels, 1981) two homozygous tester stocks, *Gpdh^F*, *Adh^F* and *Gpdh^S*, *Adh^S* were constructed using flies from the 1983 Cygnet samples. From each of the Tasmanian populations sampled in 1984, between 43 and 323 wild caught males were singly mated to females of both tester stocks and the two progenies cultured separately. The male parents were typed for ADH and GPDH phenotypes, and cultures from males heterozygous at both loci were discarded. From each set of progeny from the heterogametic matings ten flies were separately typed for both enzymes. Following Langley *et al.*, (1981) the failure of the wild caught allozyme to show normal activity as a homodimer in a heterozygote was used to designate a putative null.

Of the seven *Adh* and five *Gpdh* putative null activity alleles identified in these crosses all were successfully isolated using *CyO* as balancer in single pair crosses of the type *CyO/n* × *CyO/n*.

To identify null activity alleles we followed the labelling protocol of Burkhart *et al.*, (1984) to specify the population and line from which the allele was extracted (table 5). Thus *Adh^{nAC14}* designates a null extracted from the 14th single female line of the Australian Cygnet population.

ADH and GPDH phenotypes were scored by electrophoresis of single fly homogenates on cellulose-acetate strips as described by Lewis and Gibson, (1978). The allele *Adh^{FCHD}* was not scored as it is very rare in the Tasmanian populations (Anderson and Gibson, 1985).

The relative viabilities of flies homozygous for chromosomes carrying *Adh* null activity alleles

Table 1 Expected electrophoretic phenotypes for the progeny of females apparently homozygous for a fast (F) or slow (S) mobility variant. (FS = heterozygote pattern; N = no activity)

Electrophoretic phenotype of female parent	Progeny classes with electrophoretic phenotypes		
	Null allele present	Null allele absent	Null allele undetectable
"Fast" [FF or Fn]	(i) S and FS	(i) all FS	(i) all F
	(ii) F, S and FS		(ii) F and FS
	(iii) F, S, FS and N		
	(iv) F and N		
"Slow" [SS or Sn]	(i) F and FS	(i) all FS	(i) all S
	(ii) F, S and FS		(ii) S and FS
	(iii) F, S, FS and N		
	(iv) S and N		

were estimated from the proportions of wild type progeny in $Cy/n_1 \times Cy/n_1$ crosses. The viability of the extracted chromosomes as hemizygotes with *Df(2L)64j* (Lindsley and Grell, 1968) was tested in the progeny of crosses between either n_1/n_1 or CyO/n_1 and $CyO/Df(2L)64j$.

Putative *Adh* null homozygotes were subjected to 4 min exposure to 5 per cent pentenol vapour (Sofer and Hatkoff, 1972). This is lethal to flies with any ADH activity. Wild caught flies from the 1984 Huonville II sample were also screened with this test within one day of collection.

RESULTS

The number of anomalous cultures in the 1983 samples and the null activity allele frequencies derived from these are given in table 2. These frequencies are likely to be under-estimates as they are calculated on the assumption that an anomalous culture contains only a single null allele and a proportion of nulls are likely to be undetected by the technique used (see table 1).

The highest null frequency was 3.9 per cent at the *Adh* locus in Cygnet but none were detected in Huonville I, 10 km north of Cygnet. There was no significant heterogeneity in *Adh* null frequencies between the four Tasmanian populations ($X^2_3 = 5.46$, $p > 0.05$) nor between them and Coffs Harbour ($X^2_1 = 0.67$, $p > 0.05$). The frequency of *Gpdh* null alleles was similar to that of *Adh* in the Tasmanian populations sampled ($X^2_1 = 0.05$, $p > 0.05$) and in Coffs Harbour ($X^2_1 = 0.002$, $p > 0.05$).

Nine *Adh* null alleles were isolated from the 1983 material (seven from Cygnet, one from Tamar and one from Pipers Brook). Apart from three of the Cygnet nulls which were on homozygous lethal chromosomes, all of these nulls lacked ADH activity on cellulose acetate membranes as homozygotes and survived 4 mins exposure to pentenol vapour.

Frequencies of null activity alleles at the *Adh* and *Gpdh* loci for the Tasmanian populations sampled in 1984 are given in table 3. In comparison with the previous year, the frequency of *Adh* null alleles in Cygnet was lower ($X^2_1 = 4.58$, $p < 0.05$) but at Tamar similar in each year ($X^2_1 = 0.07$, $p > 0.05$). The frequency at Huonville II was found to be 3.2 per cent even though only 94 alleles were tested but, overall, there was no significant heterogeneity between the three Tasmanian populations ($X^2_2 = 4.93$, $p > 0.05$). *Gpdh* nulls were again present at similar frequencies to those in 1983 in the Cygnet ($X^2_1 = 0.39$, $p > 0.05$) and the Tamar ($X^2_1 = 0.07$, $p > 0.05$) populations.

The 1983 data, suggested that in the southern Tasmanian populations *Adh* null alleles might be at frequencies as high as 4 per cent, so it was worthwhile attempting to test whether homozygotes for null alleles were present. Sufficient flies for this purpose were only collected at the Huonville II site where 2.5 gms of adult flies (estimated 5234 *D. melanogaster*) were netted and exposed to pentenol vapour within 24 hours of capture. None of the flies survived. The *Adh* null allele frequency at Huonville II was 3.2 per cent so that

Table 2 Null allele frequencies at the *Adh* and *Gpdh* loci for the populations sampled in 1983

Population sampled	Lat °South	Long °East	Adh				Gpdh			
			Single female lines	Homozygous lines	Lines with evidence of null allele	Null allele frequency %	Single female lines	Homozygous lines	Lines with evidence of null allele	Null allele frequency %
Cygnet (TAS)	43.1	147.0	78	44	6	3.9	75	38	4	2.7
Huonville I (TAS)	43.0	147.0	79	36	0	0	N.T.	—	—	—
Tamar (TAS)	41.2	146.5	69	43	1	0.7	66	34	1	0.8
Pipers Brook (TAS)	41.1	147.1	81	41	3	1.9	N.T.	—	—	—
Coffs Harbour (N.S.W.)	30.2	153.1	187	84	3	0.8	84	50	2	1.2
Average null allele frequencies						1.3		1.6		

Table 3 Null allele frequencies at the *Adh* and *Gpdh* loci for the populations sampled in 1984

Population sampled	<i>Adh</i>		<i>Gpdh</i>	
	Males tested	Null allele frequency %	Males tested	Null allele frequency %
Cygnnet (TAS)	120	0.4	119	1.3
Huonville II (TAS)	47	3.2	43	1.2
Tamar (TAS)	323	0.5	304	0.2
Average null allele frequency		0.7		0.5

five homozygous null flies were expected on Hardy Weinberg predictions ($X^2 = 3.20$, $p > 0.05$).

Sixteen chromosomes bearing *Adh* null activity alleles were isolated from the 1983 and 1984 Tasmanian samples. Table 4 lists the relative viabilities of twelve of these, together with data for six other second chromosomes bearing active *Adh* alleles extracted at the same time.

Table 4 Relative viabilities, compared with *CyO*, of the *Adh* null bearing second chromosomes extracted from the Tasmanian populations. Also included are chromosomes with active alleles (+) extracted at the same time

Year sampled	Population	Chromosome	Relative Viability
1983	Cygnnet	AC14	N.T.
1983	Cygnnet	AC22	0.81‡
1983	Cygnnet	AC41	Lethal
1983	Cygnnet	AC53	N.T.
1983	Cygnnet	AC60	Lethal
1983	Cygnnet	AC62	Lethal
1983	Cygnnet	AC80	0.69‡
1983	Cygnnet	AC28†	0.47‡
1983	Cygnnet	AC34†	0.68*
1983	Tamar	AT33	N.T.
1983	Pipers Brook	AP81	N.T.
1984	Cygnnet	AC95	0.46‡
1984	Huonville II	AH36	Lethal
1984	Huonville II	AH41	0.55‡
1984	Huonville II	AH108	1.03
1984	Huonville II	AHC1†	0.88
1984	Huonville II	AHC2†	0.94
1984	Tamar	AT240	1.05
1984	Tamar	AT265	0.82
1984	Tamar	AT340	1.01
1984	Tamar	ATC1†	0.65‡
1984	Tamar	ATC2†	1.02

* $p < 0.01$, ‡ $p < 0.001$.

Four of the *Adh* null bearing chromosomes were homozygous lethal and two of these (AC41 and AC60 from Cygnnet) were lethal in combination. The lethality associated with AH36 from Huonville II was separable from the *Adh* locus by recombination and this null allele can now be maintained as a homozygote.

Of the eight homozygous viable null bearing chromosomes four were significantly less viable as homozygotes than when heterozygous with *CyO*. None of six chromosomes with active *Adh* alleles were lethal, but three of them had lower viabilities as homozygotes than when heterozygous with *CyO*.

The distribution of viabilities in second chromosomes containing *Adh* null alleles is not dissimilar to that found in earlier surveys of 100 second chromosomes from each of the Cygnnet and Tamar populations, in which an average of 32 per cent homozygous lethals were detected (Gibson, unpublished results).

All of the *Adh* null allele bearing chromosomes were viable and fertile in combination with *Df(2L)64j*.

DISCUSSION

The average frequency of *Adh* and *Gpdh* null alleles in the 1983 collections from four Tasmanian populations was 1.6 per cent and 1.8 per cent respectively. Inclusion of the data for Coffs Harbour reduces these averages to 1.3 and 1.6 per cent. The frequency of *Adh* null activity alleles is fourteen times, and that of *Gpdh* twice, the average values reported by Langley *et al.*, (1981) for their Raleigh and London samples. Null allele frequencies at both loci were lower in 1984 than in the previous year, but the average *Adh* null frequency in the Tasmanian populations remained over seven times higher than reported by Langley *et al.*, (1981) who detected only one *Adh* null activity allele out of 1170 alleles screened (0.09 per cent) and ten *Gpdh* nulls out of 1200 alleles tested (0.83 per cent).

Interpretation of the differences in null allele frequencies between the 1983 and 1984 Tasmanian population samples is confounded by the different methods used to detect null alleles in the 2 years. However, although there are too few data for a critical test they suggest that sampling variance could explain the observed heterogeneity between years, and between populations, given their relatively low frequencies. The Tasmanian populations suffer a dramatic bottleneck after May and do not

appear to breed over winter, but breeding occurs all year round at Coffs Harbour even though in winter population sizes are much reduced.

Attempts to explain the relatively high frequencies of *Adh* nulls in the Tasmanian populations must take into account the number of different null alleles, mechanisms which might enhance mutation rates, and the possibility of selective maintenance of null alleles in heterozygotes.

We are investigating the molecular characteristics of the extracted *Adh* null alleles to ascertain whether or not separately extracted nulls differ in DNA sequence and whether the same alleles are maintained over time. Our present data for *Adh* do not provide any evidence for heterogeneity as all the homozygous viable *Adh* nulls lack ADH activity, survive exposure to pentanol vapour, and do not form heterodimers with *Adh^F* or *Adh^S*. All of them are viable when heterozygous with *Df(2L)64j* and are thus unlikely to be deletions lacking the *Adh* gene.

The viability tests show that amongst 12 *Adh* null-bearing second chromosomes, three carry non-allelic recessive lethals and a further four were of lower viability as homozygotes than when heterozygous with *Cy*. The Cygnet 1983 population (which provides the largest sample of *Adh* null bearing second chromosomes for comparisons) gives some evidence for intra-population heterogeneity. Of seven Cygnet second chromosomes carrying *Adh* null alleles three are homozygous lethal and two of these are lethal in combination. Three other Cygnet second chromosomes have significantly reduced viability and a further two, although viable as homozygotes, were not tested in comparison with *CyO*. If all the Cygnet *Adh* null alleles are indeed descendants of a single mutational event, then they have remained in the population long enough for the second chromosomes to become differentiated with respect to their viabilities. It is worth noting that the average frequency of second chromosome inversions in the Tasmanian populations is very low, less than 0.2 inversions per fly (Knibb *et al.*, 1981) so that cytology was not useful in discriminating between second chromosomes.

Voelker, Schaffer, and Mukai (1980) have reported a spontaneous mutation rate to null activity alleles of 3.86×10^{-6} . The average frequency of *Adh* nulls in our 1983 study was 0.013, which would suggest a much higher mutation rate (10^{-3}) if the selection coefficient against homozygotes was 1.0 and the population frequency depended solely on mutation-selection balance.

Some mechanism in the Tasmanian populations might possibly increase the mutation rate: mutation rates as high as 10^{-4} to 10^{-3} have been reported for the X-linked singed bristle locus of *D. melanogaster* (Golubovsky *et al.*, 1977) and been attributed to male recombination (Green, 1977) and other dysgenic phenomena. There is some evidence that this is unlikely to be a cause of the relatively high frequency of *Adh* null alleles in the Tasmanian populations. Dr I. Boussy (personal communication) has found no gonadal dysgenesis in the F_1 between flies from the Cygnet population and a standard P strain (although it does exist in similar F_1 's between Coffs Harbour flies and a standard P strain) and in a survey of 30 second chromosomes, we find no male recombination in flies from Cygnet or Tamar but male recombination does exist in the Coffs Harbour population. In addition the frequency of second chromosome recessive lethals does not differ significantly between the Tasmanian and Coffs Harbour populations (Gibson, unpublished). It is also worth emphasising that the breeding programmes we used to detect null activity alleles avoided dysgenic effects. It remains possible that high mutation rates might be responsible for the high frequencies of nulls, and a molecular analysis of these alleles will help to elucidate this possibility. It will enable comparisons to be made between these naturally occurring null alleles and chemically induced *Adh* null alleles (Schwartz and Sofer, 1976; Thatcher, 1980; Benyajati *et al.*, 1983).

Given the frequencies of null alleles which they observed and using the mutation rates found by Voelker, Schaffer and Mukai, (1980), Langley *et al.*, (1981) calculate the selective disadvantage of null heterozygotes to be 0.0015. The average frequency of *Adh* nulls that we observed in the 1983 population samples gave a figure of 0.0003. Voelker, Schaffer and Mukai, (1980) did not detect any spontaneous null mutations at the *Adh* locus, but observed a rate of 16.49×10^{-6} at the *Gpdh* locus.

The frequencies of *Adh* null alleles which we have found in five Australian populations of *D. melanogaster* contrast with those obtained in previous surveys in the northern hemisphere, and show that in at least some populations *Adh* null activity alleles can be maintained at, or close to, polymorphic frequencies.

Acknowledgements We are grateful to Ann Wilks and Anh Cao for excellent research assistance. A.L.F. was supported by a Commonwealth Scholarship and Fellowship Plan award.

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