Frequency and temperature dependent selection at the alcohol dehydrogenase-1 locus of *Drosophila buzzatii*

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Genotype frequencies at the alcohol dehydrogenase-1 (*Adh*-1) locus of *D. buzzatii* were analysed for deviations from Hardy–Weinberg equilibria in the progeny of laboratory populations established at five initial *Adh*-1^b allele frequencies and kept at either 18°C, 25°C or 30°C. At 25°C, no observed genotype frequencies were significantly different from Hardy–Weinberg expectations. Observed frequencies of heterozygotes were generally less than expected for populations at 18°C and 30°C. Fitness differences among genotypes were greater in males than in females, with *Adh*-1^b homozygotes having highest fitness at 18°C and *Adh*-1^c homozygotes having highest fitness at 30°C. The results are discussed in relation to previous field and laboratory studies on *D. buzzatii* and to the *Adh* polymorphism of *D. melanogaster*.

INTRODUCTION

Of the two *Adh* loci in *Drosophila buzzatii*, *Adh*-2 is essentially monomorphic, while *Adh*-1 is highly polymorphic, with mean frequencies for 50 field populations averaging 55 per cent *Adh*-1^b and 45 per cent *Adh*-1^c (Barker and Mulley, 1976). Evidence for natural selection affecting allele frequencies at the *Adh*-1 locus was found by Barker and East (1980); following artificial perturbation of a natural population, a return to pre-perturbation *Adh*-1 allele frequencies occurred within a year.

The question of selection on the *Adh* polymorphism in *D. melanogaster* due to environmental factors such as alcohol or temperature has not been resolved (Oakeshott *et al.*, 1985; Oakeshott *et al.*, 1984; Oakeshott *et al.*, 1982b; van Delden, 1982). The exploration of *Adh*-1 variation in *D. buzzatii* may provide new insight into mechanisms of selection on an *Adh* polymorphism since this species has a better defined ecology and differs in the organisation of the *Adh* gene. *D. melanogaster* is cosmopolitan and possesses one *Adh* locus. On the other hand, *D. buzzatii* is niche

specific, feeding and breeding exclusively in rots of cactus species (Barker and Mulley, 1976), and has two closely linked *Adh* loci, thought to result from a gene duplication (Oakeshott *et al.*, 1982a). If, despite these differences, allele frequency changes are observed as a result of exposure of *D. buzzatii* to environmental stresses in the laboratory, which are similar to those found for *D. melanogaster*, then the argument for differential selection acting on *Adh* alleles is, in general, strengthened.

Several field and laboratory studies have suggested that temperature may be a selective agent on *Adh*-1 allele frequencies in *D. buzzatii* (Mulley *et al.*, 1979; Watt, 1981; Barker *et al.*, 1986). This study was designed to determine the effects of rearing temperature on genotype frequencies in laboratory populations of *D. buzzatii* over one generation. In addition, these populations were initiated at different *Adh*-1^b allele frequencies to test for possible frequency dependent selection (Kojima and Tobari, 1969; Yoshimaru and Mukai, 1979).

MATERIALS AND METHODS

Stocks were reared on a medium comprising 19 per cent blended *Opuntia stricta* cactus, 6 per cent yeast, 4 per cent sucrose, 1 per cent nipagin,

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1×10^{-4} per cent propionic acid, 2×10^{-5} per cent streptomycin and 3×10^{-4} per cent terramycin (w/v). Forty ml of medium were used to culture flies in bottles of 140 ml total volume.

Female *D. buzzatii* were captured at Hemmant, Queensland (locality 31 of Barker and Mulley, 1976) and their progeny cultured as single pair full sib matings for five generations. *Adh-1* genotype was monitored by electrophoresis at each generation and 61 independently derived homozygous *Adh-1^b* and 19 independently derived homozygous *Adh-1^c* isofemale lines were founded. Two homozygous stock populations, one from the 61 *Adh-1^b* lines and the other from the 19 *Adh-1^c* lines, were set up and maintained at 200 pairs of parents (10 bottles) for a further seven generations prior to the experiment.

A factorial experimental design was used to determine the effects on *Adh-1* genotype frequency after a single generation of two treatments, viz. initial *Adh-1* allele frequency (5 levels), and temperature (3 levels). To achieve the five frequency levels, six-day old virgin flies from the homozygous stock populations were mass mated for 24 hours at 25°C, in one of four crosses, viz. $\text{♀}^{bb} \times \text{♂}^{bb}$, $\text{♀}^{bb} \times \text{♂}^{cc}$, $\text{♀}^{cc} \times \text{♂}^{bb}$ and $\text{♀}^{cc} \times \text{♂}^{cc}$. Females from the four cross types were then combined in five classes of Hardy-Weinberg equilibrium proportions to give expected allele frequencies in the progeny for the *Adh-1^b* allele of 0.9, 0.7, 0.5, 0.3 and 0.1. The total number of females in any one bottle was 100.

Twelve bottles were prepared for each of the five *Adh-1^b* frequency classes. Four bottles per frequency class were then set at one of three temperatures, 18°C, 25°C or 30°C. The experiment was repeated one week later to give an additional four replicates for each treatment.

Horizontal starch gel electrophoresis using Buffer III of Shaw and Prasad (1970), modified with the addition of 1 mg/ml MgCl_2 to the gel buffer, was used to score individual progeny. Gels were stained with 4.5 ml 2-propanol, 50 mg β -NAD, 30 mg MTT, and 3 mg PMS in 100 ml Tris-HCl pH 8.5, and incubated at 37°C for 30 to 60 minutes (Barker *et al.*, 1986). A sample of 50 one-day old adults of each sex from each bottle (taken proportionately from the daily emergence distribution) was assayed for *Adh-1* genotype, although some bottles produced fewer flies than required, and for the 25°C, 0.1 initial frequency treatment, some additional flies were assayed. At 30°C, bacterial contamination caused the loss of one replicate in each of the 0.1, 0.3 and 0.5 treatments, and two replicates in the 0.7 treatment. One replicate of the 18°C, 0.9 treatment was deleted from the

data set because of extremely aberrant observed numbers (50 *bb*, 50 *bc*), presumably due to an error at setting up this bottle.

RESULTS

Two major effects on *Adh-1* genotype frequencies were found. Firstly, significant deviations from Hardy-Weinberg expected genotype numbers were observed at both 18°C and 30°C, but not at 25°C (table 1). These deviations took the form of heterozygote deficiencies for all five of the initial *Adh-1^b* allele frequency classes at 18°C, and for four at 30°C. The exception at 30°C was the 0.9 initial frequency class where a significant excess was recorded. In a graphic description of the table 1 data (fig. 1) a trend for genotype frequency dependent selection can be seen at 30°C, where the fitness of the *Adh-1^b* homozygotes increases as *Adh-1^b* frequency decreases. The observed heterozygote deficiencies in the pooled data for the 18°C and 30°C treatments (excluding 30°C, 0.9) were not due to a Wahlund effect, since 48 of the 66 replicate bottles in these nine treatments had an observed deficiency of heterozygotes (29 of these significant), 16 an observed excess (5 significant), and two no difference.

Secondly, genotype fitnesses varied with temperature and differences were larger in males than in females (table 2). *Adh-1^b* homozygotes had highest fitness at 18°C, while *Adh-1^c* were highest at 30°C.

DISCUSSION

Temperature had a large effect on *Adh-1* heterozygosity, with heterozygote numbers reduced at both low (18°C) and high (30°C) temperatures, but not at the intermediate temperature (25°C).

As both high and low temperatures gave similar results, it is difficult to relate this study to the field results (Barker *et al.*, 1986) where heterozygote frequency varied seasonally (with respect to Hardy-Weinberg equilibrium), rising in summer and falling in winter. This discrepancy may be because the range of temperatures tested in this study is much narrower than those which *D. buzzatii* experience in the wild, or because of the homogeneity of the laboratory environment in contrast to diurnal temperature variation in the field, or because effects on larvae are compensated by different effects at other life cycle stages.

Table 1 Observed and expected numbers and their χ^2 values for each *Adh-1* genotype for each treatment combination of temperature and initial gene frequency. Expected numbers were calculated using initial frequency values of p and q from the four cross types giving rise to the progeny

Temperature	Input frequency <i>Adh-1</i> ^b	Observed			Total No.	Observed frequency <i>Adh-1</i> ^b	Expected†			$\chi^2_{(2)}$	$\frac{het_{obs}}{het_{exp}}$
		<i>bb</i>	<i>bc</i>	<i>cc</i>			<i>bb</i>	<i>bc</i>	<i>cc</i>		
18°C	0.9	595	103	0	698	0.926	565	126	7	12.8‡	0.82
	0.7	474	227	91	792	0.742	388	333	71	58.4§	0.68
	0.5	225	343	226	794	0.499	199	397	199	14.4§	0.86
	0.3	103	293	398	794	0.314	71	333	389	19.4§	0.88
	0.1	5	109	676	790	0.075	8	142	640	10.8‡	0.77
25°C	0.9	646	132	13	791	0.900	641	142	8	3.9	0.93
	0.7	384	315	85	784	0.691	384	329	71	3.4	0.96
	0.5	188	395	209	792	0.487	198	396	198	1.1	1.00
	0.3	59	329	403	791	0.283	71	332	388	2.6	0.99
	0.1	12	126	678	816	0.092	8	147	661	5.4	0.86
30°C	0.9	611	181	5	797	0.880	646	143	8	13.1‡	1.27
	0.7	315	163	65	543	0.730	266	228	49	32.8§	0.71
	0.5	167	305	178	650	0.492	163	325	163	2.7	0.94
	0.3	82	237	373	692	0.290	62	291	339	19.9§	0.81
	0.1	21	81	584	686	0.090	7	123	556	43.8§	0.66

† Based on input frequency.

‡ $P < 0.01$.

§ $P < 0.001$.

Although decreased heterozygote fitness is an unusual result it is not without precedent in similar experiments. A reduction in heterozygote frequency related to temperature has been observed for the α -*Gpdh* locus in *D. melanogaster*, where genotypes emerged in Hardy-Weinberg equilibrium at 16°C and 20°C, but at 28°C a significant decrease in heterozygote fitness was observed (McKechnie *et al.*, 1981).

Clinal variation for the *Adh* polymorphism in *D. melanogaster* has been well documented (Oakeshott *et al.*, 1982b) and a study of *D. buzzatii* *Adh-1* allele frequencies in eastern Australia showed significant spatial heterogeneity, with *Adh-*

1^b frequency decreasing with increasing distance from the coast and increasing latitude (Mulley *et al.*, 1979). In a laboratory study of the effects of cold shock (0°C for 5 days), a decrease in the frequency of *Adh-1*^b was found among survivors (Watt, 1981). This decrease is consistent with the direction of the latitudinal variation, but appears to conflict with the present study, where a higher fitness was found for *Adh-1*^b homozygotes at the lowest temperature (18°C), rather than the highest (30°C) (table 2). However, the cold shock experiment of Watt (1981) tested adult survival only, *i.e.*, a fitness component not involved in the present study. Here selection on *Adh-1* genotype frequen-

Figure 1 Standardised deviates (obs-exp/SQRTexp) for each temperature, initial *Adh-1*^b frequency and genotype (from data in table 1). ○ *Adh-1*^{bb}, △ *Adh-1*^{bc}, □ *Adh-1*^{cc}.

Table 2 Standardised deviates (obs-exp/SQRTexp) and χ^2 values for genotype by temperature for each sex

	Genotype				Genotype			
	Female		Male		Female		Male	
	<i>bb</i>	<i>bc</i>	<i>cc</i>	$\chi^2_{(2)}$	<i>bb</i>	<i>bc</i>	<i>cc</i>	$\chi^2_{(2)}$
18°C	2.7	-4.2	1.6	27.68§	4.2	-5.7	1.7	52.80§
25°C	1.1	-2.4	1.3	8.45*	-1.7	0.5	1.2	4.37
30°C	0.8	-2.3	1.5	8.03*	1.4	-3.8	2.4	22.25§

* $P < 0.05$.§ $P < 0.001$.

cies could have occurred at one or many of the life stages of the test animals, including effects on fertility and oviposition behaviour of the parent females which oviposited in each treatment under test conditions. One stage at which selection might be expected is the larval stage, since *Adh-1* activity peaks during that stage (Batterham *et al.*, 1984). In any case, genotype differences for adult survival need not be in the same order as for other components of fitness.

Some controversy exists in the literature over genotype frequency dependent selection at the *Adh* locus of *D. melanogaster*. One study (Kojima and Tobari, 1969) showed genotype frequency dependent selection to occur, with the genotype at lowest frequency having enhanced fitness. A later study (Yoshimaru and Mukai, 1979) found no effect. Weak evidence for genotype frequency dependent selection can be seen in *D. buzzatii* at 30°C, where the fitness of the heterozygote decreases, and that of *both* homozygotes increases as *Adh-1^b* frequency decreases (fig. 1). It is noteworthy that the parental generation was made up of homozygotes only. Hence the point made by Prout (1965), that fitnesses estimated from a comparison of progeny with parental genotype frequencies can show spurious frequency dependent selection, is not relevant here.

As in all other experiments of this type, the possibility that another locus closely linked to *Adh-1* is the primary site for selection cannot be discounted. However, the background genome of test stocks was randomised during the breeding program such that in the absence of epistatic selection only closely linked genes would be likely to remain in disequilibrium with *Adh-1*. Studies of *D. mojavensis*, a member of the *mulleri* sub-group to which *D. buzzatii* belongs, have shown *Adh* to be located on Chromosome III (Zouros, 1976) and preliminary experiments indicate that *D. buzzatii Adh-1* is on the same chromosome. Chromosome III of *D. buzzatii* is not known to have any inversions (Knibb, personal communication) so linkage

disequilibria due to inversions will not be occurring.

This experiment has shown that temperature is a component of the environment affecting *Adh-1* genotype frequencies in laboratory populations of *D. buzzatii*, so *Adh-1* frequencies may be influenced by environmental temperature in nature. However, the lack of agreement between this study and field observations in terms of the direction of the effect suggests that any selection on the *Adh-1* locus due to temperature in wild populations is more complex than that indicated here. Such complexity is evidenced by fitness differences between the sexes at 18°C and 30°C (table 2), where effects on genotype frequencies were found to be greater for males than females, suggesting differential selection between the sexes. Differences in *Adh-1* allele frequency have been associated with other variables such as the age of the cactus cladode rot, its pH and the number of flies emerging from it (Barker *et al.*, 1986), indicating that the micro-environment may be just as important in maintaining the polymorphism as the macro-environment. Two things are clear: observed heterozygote excesses occur for *Adh-1* in the wild (Barker *et al.*, 1986) and heterozygosity can be manipulated by factors such as temperature and initial gene frequency in the homogeneous laboratory environment.

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