Environment-dependent fitness differences in *Drosophila melanogaster*: temperature, domestication and the alcohol dehydrogenase locus

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(a) Phenotypic fitnesses were estimated over the temperature range 15°C to 29°C for genotypes at the alcohol dehydrogenase (*Adh*) locus. The traits measured were egg and larval survival, development time and mating ability in single generation experiments, and total fitness by gene frequency changes in discrete generation populations.

(b) Significant temperature-dependent fitness associations involving the *Adh* locus were obtained for isofemale strains which had been in the laboratory for eighteen months. Heterozygote advantage was general in the single generation experiments, and in the populations the *AdhF* allele increased in frequency.

(c) In contrast, populations obtained directly from the field did not show these fitness associations. It was not therefore possible to attribute the genotypic fitness differences detected for the laboratory strains directly to the *Adh* locus. Indeed, the contrast between the laboratory-adapted and field collected flies may provide a model of likely change under domestication whereby the higher variability of the laboratory strains may be a manifestation of the "stress" involved in the adaptation of populations to laboratory conditions.

(d) The results emphasise the need to commence at the phenotypic level in attempting to understand fitness relationships in natural populations and it is suggested that direct assessments of the effects of environmental stresses of ecological significance may provide an initial strategy.

INTRODUCTION

This paper attempts to associate genotypic changes at the alcohol dehydrogenase locus (*Adh*) with phenotypic fitness differences in *Drosophila melanogaster*. Fitness was measured in single generations by egg and larval survival, development time and mating ability; and total fitness was estimated in discrete generation populations by gene frequency changes (Prout, 1971*a, b*). We commenced with the premise that the gene is the unit of selection (Clarke, 1975; Dawkins, 1981) so that in certain environments it should be possible to demonstrate direct selection upon alleles at single electrophoretic loci. Since expressed genetic variation for fitness tends to be greater when environmental stresses are extreme (Parsons, 1983), single locus selection should be most apparent in relatively extreme environments. The experiments presented here assess the relations between the common *Adh* genotypes and phenotypic fitness in the temperature range 15°C to 29°C. Therefore, we commence with a single locus and search, *a posteriori*, for correlations of adaptive significance. This

approach will be contrasted with an *a priori* approach which begins with the organism, assayed phenotypically in a variety of ways according to circumstances.

The *Adh* locus is polymorphic in natural populations (van Delden, 1982). The broad and complementary clines detected on several continents (Johnson and Schaffer, 1973; Malpica and Vassallo, 1980; Oakeshott *et al.*, 1982; David, 1982; Oakeshott, McKechnie and Chambers, 1984) directly or indirectly implicate temperature as a selective agent and hence its choice as a major environmental variable here. The clinal data also possibly indicate that a variable associated with resources, or that a combination of resources and temperature (Malpica and Vassallo, 1980) are involved in the maintenance of the polymorphism. Considering resources, the presumed physiological role of *Drosophila* ADH in ethanol tolerance and utilisation suggests that ethanol could be an important selective agent. However, evidence from field and laboratory studies is equivocal with respect to this hypothesis (McKenzie and Parsons, 1974; McKenzie and McKechnie, 1978; Hickey and

McClean, 1980; Oakeshott *et al.*, 1980; Gibson *et al.*, 1981a; van Delden, 1982; McKechnie and Geer, 1984). These results are important since ethanol tolerance can be regarded as an ecological phenotype of clear adaptive significance (Holmes *et al.*, 1980; Parsons, 1983) and also as a metabolic phenotype (Johnson, 1979), presumably controlled by many genes including *Adh* (McKenzie and Parsons, 1974; Middleton and Kascier, 1983).

An extensive study of the *Adh* polymorphism in the wine cellar at Chateau Tahbilk, Seymour, Victoria has shown that allele frequency varied with temperature (McKechnie and McKenzie, 1983) and appeared to be independent of ethanol concentration. In fitness studies, McKechnie and McKenzie (1983) found significant variation in female progeny production whereby female heterozygotes produced more progeny. However, there was no indication of relative changes in progeny production among genotypes with changing temperature. McKenzie and McKechnie (1981) did not find differences between the fertilities of the homozygous females and the abilities of the male genotypes to sire progeny. Finally, larval survival and adult longevity at different temperatures and in the presence of ethanol could not be interpreted in terms of fitness differences at the *Adh* locus for strains derived from Chateau Tahbilk (Ziolo and Parsons, 1982; McKechnie and Morgan, 1982).

Mating appears to be non-random for *Adh* genotype in the Chateau Tahbilk population—the heterozygote exhibits a higher mating propensity than either homozygote (McKenzie and McKechnie, 1981). A related laboratory study indicated that male heterozygotes mated more frequently with virgin females than did either homozygote (McKenzie and Fegent, 1980). Differences in mating abilities of flies of different *Adh* genotype have also been detected by Pot *et al.* (1980) and Knopien *et al.* (1980). Finally, heterozygote advantage is a common observation for the *Adh* locus in a variety of laboratory and field studies (van Delden *et al.*, 1978; Franklin, 1981; van Delden, 1982; Oakeshott, Gibson and Wilson, 1984). There appears to be an association between individual heterozygosity levels and fitness measured in a number of ways, especially when conditions are stressful (reviewed in Parsons, 1983), suggesting that the heterozygote advantage observed for *Adh* could be due to stress in contrast to single locus overdominance.

In summary, *Adh* allele frequencies can be considered to vary with temperature as a "first order" effect by comparison with the other vari-

ables cited. In the experiments to be discussed, fitness will be assessed in the range 15°C to 29°C using (a) 20 isofemale strains derived from Chateau Tahbilk and which had been in laboratory culture for 18 months prior to the experiments, and (b) flies and larvae derived directly from the field from Chateau Tahbilk and Fergusons Vineyard, Yarra Glen, Victoria. The possibility that the extremes of this temperature range are not sufficiently stressful in regard to levels of expressed genetic variation and phenotypic fitness differences is also considered. Further, following Oakeshott, Gibson and Wilson (1984) who have shown that a significant change in *Adh* allele frequency in response to selection on ethanol media is associated with laboratory populations and not with base populations closely related to nature, the possibility that significant results for the *Adh* locus are principally detected for laboratory adapted strains is considered. Finally, the contrast between (a) and (b) above permits the possibility of discussing the results in the context of domestication.

MATERIALS AND METHODS

Laboratory strains

Ten isofemale strains homozygous for each of *Adh*^{FF} and *Adh*^{SS} were obtained from the Genetics Department, Monash University. These strains, were maintained as individual discrete generation populations (half-pint milk bottles) at 25°C for eighteen months prior to the experiments. All experiments were conducted at 15°C, 20°C, 25°C and 29°C. The composition of the food was as follows: 2000 ml water, 36 g agar, 108 g raw sugar, 72 g dry yeast, 10.0 ml propionic acid, 24.0 ml nipagen. The vials contained 10.0 ml of food unless otherwise specified, and the population bottles, food to a height of 2.5 cm.

Eggs and larvae of the three common *Adh* genotypes were obtained as follows: The homozygous strains were pooled by transferring 30 virgin adults from each population to a fresh bottle and subculturing this population a number of times. Virgin homozygotes collected from these populations were randomly transferred to empty half-pint milk bottles. Food covered watch-glasses were taped to the mouths of these bottles which were inverted and cultured at 25°C for 6 hours for egg laying. At the end of this period the adults were transferred to fresh bottles and watch-glasses and cultured at 25°C for a further 6 hours. The adults were then discarded and the watch-glasses were cultured at 25°C for 18 hours after which time

larvae were available. Heterozygous eggs and larvae were obtained from reciprocal crosses of virgin homozygotes as described above. When possible, experiments were conducted with progeny of $Adh^{FF}\text{♀} \times Adh^{SS}\text{♂}(FS)$ and $Adh^{SS}\text{♀} \times Adh^{FF}\text{♂}(SF)$. When this was not possible, the progeny were pooled. Virgin adults of the three common *Adh* genotypes were derived as follows: Virgin homozygotes were collected as described above and 5 populations were established for each homozygote at 25°C by randomly transferring 200 adults to fresh bottles. Experimental virgin homozygotes were collected from these populations every 4 hours. Simultaneously, 5 populations were established at 25°C for each reciprocal cross— $Adh^{FF}\text{♀} \times Adh^{SS}\text{♂}$ and $Adh^{SS}\text{♀} \times Adh^{FF}\text{♂}$ by introducing 150 virgins of each homozygote genotype into a bottle. Experimental virgin heterozygotes were collected from these populations every 4 hours and these were pooled. Virgin adults of each *Adh* genotype and sex were cultured in vials with no more than 15 flies in a vial, at 25°C for 3 days prior to the experiments.

(a) Egg and larval to adult survival and development time

To estimate egg to adult survival and development time 25 eggs of each genotype were transferred to a vial. There were 3 replicates for Adh^{FS} and Adh^{SF} at each temperature. For Adh^{FF} and Adh^{SS} , there were 4 replicates at 25°C and 5 replicates at the other temperatures. Surviving adults were collected daily.

Larval to adult survival and development time were estimated in independent cultures and competition experiments. In the former, 20 larvae of each genotype were transferred to the same vial and there were 6 replicates at each temperature. Surviving adults were collected daily. In the latter, 20 larvae of each genotype were transferred to the same vial and there were 6 replicates at each temperature. Heterozygotes were obtained from pooled Adh^{FS} and Adh^{SF} larvae. Surviving adults were collected daily and their *Adh* genotypes were determined by Cellogel electrophoresis.

(b) Mating abilities

The mating abilities of the three common *Adh* genotypes were estimated for 3 unmated genotypic constitutions as follows: Three-day old virgin adult flies were acclimatised at each temperature for 2 days and were then transferred to half-pint milk bottles containing 0.5 cm of food which had been acclimatised at each temperature for 6 hours. The unmated genotypic constitutions were denoted 0.25*F*, 0.5*F* and 0.75*F*, representing the *Adh*^{*F*}

allele frequency among the unmated adults which were in the proportions 10*Adh*^{*FF*}, 30*Adh*^{*FS*}, 60*Adh*^{*SS*}; 30*Adh*^{*FF*}, 60*Adh*^{*FS*}, 30*Adh*^{*SS*} and 60*Adh*^{*FF*}, 30*Adh*^{*FS*}, 10*Adh*^{*SS*} respectively. There were 2 replicates at 15°C and 3 replicates at the other temperatures. The adults remained together for 150 minutes at 15°C, 60 minutes at 20°C, 45 minutes at 25°C and 40 minutes at 29°C. These periods were chosen on the basis of McKenzie (1978). At the end of the mating periods the females were transferred to single vials and the males were discarded following determination of their *Adh* genotype (Cellogel electrophoresis). The *Adh* genotypes of the females were determined and for those which mated, 25 of their progeny were scored and the genotype of their mate inferred.

(c) Discrete generation population experiments: Populations were established at each temperature for the above *Adh*^{*F*} allele frequencies, denoted 0.25*F*, 0.5*F* and 0.75*F*, by transferring virgin adults in the above genotypic proportions to half-pint milk bottles. The adults were acclimatised at each temperature for 2 days prior to transfer to the bottles which had been acclimatised at each temperature for 6 hours. The adults remained in the bottles for 240 hours at 15°C, 65 hours at 20°C, 35 hours at 25°C and 30 hours at 29°C after which time they were discarded. These periods were chosen on the basis of McKenzie (1978) and ensured that similar egg, larval and adult densities obtained at each temperature. After establishment, the populations were maintained in discrete generations as follows: The subsequent generation was monitored daily and when approximately 350 to 450 adults had emerged, these were transferred to a fresh bottle. These flies remained together for the mating and egg laying periods given above after which time they were removed and the *Adh* genotypes of 50 adults were determined by Cellogel electrophoresis with the remaining flies being discarded. The populations established at 20°C for an initial allele frequency of 0.75*F* were subcultured at generation 13 to 25°C and 29°C. These subcultured populations were maintained for a further 20 generations at 25°C and 29°C.

Field derived flies and larvae

Approximately 2000 *D. melanogaster* adults were collected at two vineyards, Chateau Tahbilk and Fergusons, by sweeping with a net over a pile of grape refuse during March, 1981 and were divided into two lots of 1000 flies. One lot was used to generate experimental larvae and adults as follows: Adults from each site were randomly assigned to

three lots of approximately 330 flies and transferred to empty half-pint milk bottles. Food covered watch-glasses were taped to the mouths of the bottles which were inverted and cultured at 25°C for 6 hours. The adults were then transferred to half-pint milk bottles containing 2.5 cm of food at 25°C for 5 days after which time they were discarded. Larvae were collected from the watch-glasses following a further 18 hours culture at 25°C and virgin adults for the mating experiment were collected at 4 hourly intervals from the above population bottles. The virgin adults were held in vials (15 adults per vial) at 25°C for 3 days prior to the experiment. The second lot of 1000 flies were used to initiate populations by equally distributing them among 3 population bottles denoted A, B and C for Chateau Tahbilk and Fergusons respectively. Following a 5-day egg laying period at 25°C the adults were transferred to fresh bottles and this procedure was repeated until populations derived from each founding population were available for each temperature. These populations were randomly assigned to each temperature as they became available.

(a) Larval to adult survival and development time: To measure larval to adult survival and development time 75 larvae were transferred to a single vial. There were 6 replicates for each site and temperature. Surviving adults were collected daily and their *Adh* genotypes were determined by Cellogel electrophoresis. To obtain an estimate of the expected genotypic frequencies at each site, fewer larvae (25) were cultured in more food (16.5 ml) at 25°C. There were 8 replicates for each site and these were denoted "controls".

(b) Mating abilities:

The mating abilities of the common *Adh* genotypes were estimated at 15°C, 20°C, 25°C and 29°C for flies derived from Chateau Tahbilk only as follows:

30 five-day old virgin males and 30 five-day old virgin females were transferred to half-pint milk bottles containing 0.25 cm of food. The virgin flies were acclimatised at each experimental temperature for 3 days prior to transfer to the bottles which had been acclimatised at each temperature for 6 hours. The flies remained together for the specific mating periods described for the laboratory strains and there were 3 replicates at each temperature. At the end of the mating periods the females were transferred to single vials and the males were discarded following determination of their *Adh* genotype (Cellogel electrophoresis). The *Adh* genotypes of the females were determined and for those which mated, the genotypes of 25 of

their progeny were determined and the genotype of their mate inferred.

(c) Discrete generation population experiments: Populations established for each founding generation and site were randomly assigned to each temperature. The populations were maintained as discrete generations in the manner of the laboratory populations. At particular generations, including the founding one, approximately 100 flies were set aside and their *Adh* genotypes were determined by Cellogel electrophoresis.

RESULTS

Laboratory adapted strains

(a) Egg and larval survival and development time Table 1 gives the results for egg and larval survival of the *Adh* genotypes in independent culture and competition experiments. Temperature was a significant factor in each experiment—more flies survived at 20°C and 25°C compared with 15°C and 29°C suggesting an extreme environment effect. There also appeared to be a greater temperature effect for larval survival in the competition experiment. The approximate orders for increasing genotypic survival were as follows:

egg to adult survival, independent culture:

$$FF = FS > SF = SS$$

larval to adult survival, independent culture:

$$FS = SF \geq FF \geq SS$$

larval to adult survival, competition experiment:

$$FS > FF > SS.$$

Table 2 gives the results for development time, expressed as $T_{1/2}$, the time taken for half of the surviving adults to emerge. Mean $T_{1/2}$ values at each temperature and ANOVAs are shown. Temperature was a significant factor with the characteristic and expected result of more rapid development as temperature increased. The approximate genotypic orders for more rapid development time were as follows:

egg to adult development time, independent culture:

$$FF = FS < SS = SF$$

larval to adult development time, independent culture:

$$FF = SF < FS = SS$$

Table 1 The Egg and larval to adult survivals of the three common *Adh* genotypes estimated at 15°C, 20°C, 25°C and 29°C for eggs and larvae obtained from isofemale strains which had been in laboratory culture for 18 months prior to the experiments(a) The Egg to adult survival of the common *Adh* genotypes estimated in independent culture, where 25 eggs of each genotype were cultured in a single vial. Heterozygotes were derived from the progeny of reciprocal homozygote crosses—*Adh*^{FS} and *Adh*^{SF}.

(i) Means

(ii) ANOVA

Temp. (°C)	Adh genotype				Df	SS	MS	F	
	FF	FS	SF	SS					
15	10.3±1.9	10.0±2.2	5.3±1.3	5.8±2.8	Temp. (T)	3	20.4	4.8	4.6*
20	16.6±2.1	13.0±2.2	8.0±2.9	6.3±3.3	Genotype (G)	3	100.6	33.5	22.5†
25	13.6±3.4	14.7±0.1	9.0±1.4	9.0±3.4	T×G	9	9.1	1.0	0.7
29	13.8±1.9	12.3±1.7	6.7±1.7	6.6±2.2	Residual	46	68.6	1.5	

(b) The Larval to adult survivals of the common *Adh* genotypes estimated in independent culture, where 20 larvae of each genotype were cultured in a single vial. Heterozygotes were derived from the progeny of reciprocal homozygote crosses—*Adh*^{FS} and *Adh*^{SF}.

(i) Means

(ii) ANOVA

Temp. (°C)	Adh genotype					Df	SS	MS	F
	FF	FS	SF	SS					
15	10.7±2.9	12.0±2.9	12.0±2.8	10.7±2.7	Temp. (T)	3	29.3	9.8	4.0*
20	12.7±4.9	13.3±0.09	10.3±5.3	11.8±3.4	Genotype (G)	3	25.5	8.5	3.5*
25	12.5±2.7	16.7±2.1	13.3±2.1	11.0±3.0	T×G	9	11.1	1.2	0.5
29	9.2±1.9	12.3±0.05	12.0±3.7	6.7±2.5	Residual	56	136.4	2.4	

(c) The Larval to adult survivals of the common *Adh* genotypes estimated in competition experiments, where 25 larvae of each genotype were cultured in the same vial. Heterozygotes were derived from the pooled progeny of reciprocal homozygote crosses—*Adh*^{FS}.

(i) Means

(ii) ANOVA

Temp. (°C)	<i>Adh</i> genotype				<i>Df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>
	<i>FF</i>	<i>FS</i>	<i>SS</i>					
15	12.5 ± 2.8	17.0 ± 3.7	9.8 ± 1.6	Temp. (<i>T</i>)	3	46.0	15.3	6.5*
20	15.5 ± 3.6	19.0 ± 2.9	13.3 ± 2.3	Genotype (<i>G</i>)	2	114.0	57.0	24.3†
25	17.7 ± 7.8	21.5 ± 1.0	13.5 ± 4.6	<i>T</i> × <i>G</i>	6	8.4	1.4	1.7
29	13.2 ± 3.9	16.3 ± 3.4	7.2 ± 2.4	Residual	60	141.1	2.4	

* $p < 0.025$ † $p < 0.005$

larval to adult development time, competition experiment:

$$FF = FS < SS.$$

The significant temperature genotype interaction detected in the competition experiment is indicated by comparisons of the means in table 2. *AdhSS* developed slowest at all temperatures, and whilst *AdhFF* and *AdhFS* were similar at 15°C and 29°C, *AdhFF* developed more rapidly at 20°C and *AdhFS* more rapidly at 25°C.

(b) Mating abilities

The numbers of males and females of each genotype which mated are given in table 3 for

pooled replicates at each temperature. The respective χ^2 values for comparisons between these observed values and expected values based upon the unmated genotypic proportions are also shown. Such comparisons should indicate genotypic differences in mating abilities since in no circumstance did all flies mate, although there were relatively more matings for the initial frequencies 0.25*F* and 0.75*F* and at 29°C.

Considering the initial *AdhF* allele frequency of 0.25*F* (table 3(a)), there were no significant χ^2 values indicating that the genotypes of both sexes mated in the same proportions as found in the unmated populations. For 0.5*F* (table 3(b)) there were significant χ^2 values for the females at 25°C

Table 2 The egg and larval to adult development times of the three common *Adh* genotypes estimated at 15°C, 20°C, 25°C and 29°C for eggs and larvae obtained from isofemale strains which had been in laboratory culture for 18 months prior to the experiments. Development time is expressed as $T_{1/2}$ in days

(a) The egg to adult development time of the common *Adh* genotypes estimated in independent culture, where 25 eggs of each genotype were cultured in a single vial. Heterozygotes were derived from the progeny of reciprocal homozygote crosses—*Adh*^{FS} and *Adh*^{SF}.

(i) Means

(ii) ANOVA

Temp. (°C)	<i>Adh</i> genotype				<i>Df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>
	<i>FF</i>	<i>FS</i>	<i>SF</i>	<i>SS</i>				
15	40.13 ± 0.61	40.00 ± 0.41	40.79 ± 1.41	40.25 ± 0.30	Temp. (<i>T</i>)	3	9437.0	3145.7
20	16.93 ± 0.18	16.97 ± 0.15	16.85 ± 0.11	17.37 ± 0.39	Genotype (<i>G</i>)	3	21.0	7.0
25	10.79 ± 0.30	10.76 ± 0.06	11.15 ± 0.39	10.79 ± 0.11	<i>T</i> × <i>G</i>	9	3.5	0.4
29	7.75 ± 0.06	7.78 ± 0.03	7.96 ± 0.30	7.79 ± 0.12	Residual	45	17.0	0.4

(b) The larval to adult development times of the common *Adh* genotypes estimated in independent culture, where 20 larvae of each genotype were cultured in a single vial. Heterozygotes were derived from the progeny of reciprocal homozygote crosses—*Adh*^{FS} and *Adh*^{SF}.

(i) Means

(ii) ANOVA

Temp. (°C)	<i>Adh</i> genotype				<i>Df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>
	<i>FF</i>	<i>FS</i>	<i>SF</i>	<i>SS</i>				
15	27.44 ± 0.31	27.22 ± 0.37	27.37 ± 0.37	27.55 ± 0.06	Temp. (<i>T</i>)	3	4384.4	1461.5
20	13.79 ± 0.31	14.29 ± 0.40	13.35 ± 0.14	13.89 ± 0.23	Genotype (<i>G</i>)	3	4.0	1.3
25	9.25 ± 0.26	9.53 ± 0.17	9.22 ± 0.08	9.45 ± 0.25	<i>T</i> × <i>G</i>	9	1.1	0.1
29	7.14 ± 0.12	7.36 ± 0.19	7.08 ± 0.10	7.42 ± 0.23	Residual	55	6.4	0.1

(c) The larval to adult development times of the common *Adh* genotypes estimated in competition experiments, where 25 larvae of each genotype were cultured in the same vial. Heterozygotes were derived from the pooled progeny of reciprocal crosses—*Adh*^{FS}.

(i) Means

(ii) ANOVA

Temp. (°C)	<i>Adh</i> genotype			<i>Df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>
	<i>FF</i>	<i>FS</i>	<i>SS</i>				
15	27.38 ± 0.23	27.35 ± 0.28	28.62 ± 0.27	Temp. (<i>T</i>)	3	4608.0	1536.0
20	13.40 ± 0.35	13.54 ± 0.36	14.34 ± 0.44	Genotype (<i>G</i>)	2	9.0	4.5
25	9.19 ± 0.24	8.98 ± 0.21	9.35 ± 0.20	<i>T</i> × <i>G</i>	6	2.5	0.4
29	7.22 ± 0.25	7.22 ± 0.31	7.70 ± 0.24	Residual	60	6.0	0.1

* $P < 0.025$.

† $P < 0.005$.

and 29°C where there was a relative excess of heterozygotes. Considering the homozygotes, they were similar at 25°C whilst at 29°C there was a relative deficiency of *Adh*^{SS}. For males there was a relative excess of heterozygotes at all temperatures especially at 29°C, and this was significant at 20°C, 25°C and 29°C, but not at 15°C. The homozygotes performed similarly at all temperatures. For 0.75*F* (table 3(c)) there were no significant χ^2_2 values for the females, and for the males there was a significant excess of heterozygotes and a relative deficiency of *Adh*^{SS} at 29°C. In summary, whenever there was a significant departure from the expected values, this was associated with an excess of heterozygotes which

appeared greatest at 29°C, and was especially evident for males. The relative performances of the homozygotes were less consistent.

The effect of temperature variation in determining the number of genotypes of each sex which mated was assessed by χ^2 tests of independence for genotype and temperature for pooled replicates at each initial *Adh*^F allele frequency. For the males, no value was significant (0.25*F*, $\chi^2_6 = 1.26$; 0.5*F*, $\chi^2_6 = 8.93$; 0.75*F*, $\chi^2_6 = 3.61$). For the females there was a significant value for 0.5*F* (0.25*F*, $\chi^2_6 = 3.84$; 0.5*F*, $\chi^2_6 = 14.30$, $p < 0.05$; 0.75*F*, $\chi^2_6 = 2.24$) where a relative excess of heterozygotes was observed at 25°C and 29°C. These results thus reflect those given in Table 3 and discussed above.

Table 3 The numbers of males and females of each *Adh* genotype which mated in the experiments which assessed the mating abilities of flies differing in *Adh* genotypes. Observed values are shown for pooled replicates at each temperature (15°C, 20°C, 25°C and 29°C) and for each initial *AdhF* allele frequency (0.25*F*, 0.5*F*, 0.75*F*). χ^2 values for comparisons between observed values and expected values, based upon the unmated genotypic proportions are given

(a) 0.25*F* initial *AdhF* allele frequency

(b) 0.5*F* initial *AdhF* allele frequency

(c) 0.75*F* initial *AdhF* allele frequency

Initial <i>AdhF</i> allele frequency	Temp. (°C)	Female genotype				Male genotype			
		<i>FF</i>	<i>FS</i>	<i>SS</i>	Total	<i>FF</i>	<i>FS</i>	<i>SS</i>	Total
(a) 0.25 <i>F</i>	15	6	13	31	50	5	18	27	50
					$\chi^2 = 0.50$				$\chi^2 = 0.90$
	20	6	18	20	44	3	17	24	44
					$\chi^2 = 3.88$				$\chi^2 = 1.76$
	25	7	25	39	71	9	26	36	71
					$\chi^2 = 0.91$				$\chi^2 = 2.57$
	29	9	25	50	84	10	29	45	84
					$\chi^2 = 0.04$				$\chi^2 = 1.36$
(b) 0.5 <i>F</i>	15	33	53	30	116	26	62	28	116
					$\chi^2 = 1.01$				$\chi^2 = 0.62$
	20	29	56	32	117	22	75	20	117
					$\chi^2 = 0.37$				$\chi^2 = 9.38^\dagger$
	25	23	74	21	118	24	75	19	118
					$\chi^2 = 7.69^*$				$\chi^2 = 9.11^*$
	29	26	69	15	110	15	79	16	110
					$\chi^2 = 9.32^\dagger$				$\chi^2 = 20.62^\ddagger$
(c) 0.75 <i>F</i>	15	72	24	8	104	55	41	8	104
					$\chi^2 = 3.69$				$\chi^2 = 4.51$
	20	83	38	12	133	76	48	9	133
					$\chi^2 = 0.35$				$\chi^2 = 3.21$
	25	84	32	14	130	74	43	13	130
					$\chi^2 = 2.80$				$\chi^2 = 0.62$
	29	80	30	14	124	69	49	6	124
					$\chi^2 = 1.94$				$\chi^2 = 7.43^*$

* $P < 0.025$.

† $P < 0.01$.

‡ $P < 0.005$.

(c) Discrete generation population experiments
The results for the discrete generation population experiments are given in figs. 1 to 5 expressed as *AdhF* allele frequencies. For these data, *t*-tests were carried out between the initial and final generations sampled, which was considered a satisfactory procedure since the experiments measured change in *AdhF* allele frequency in response to population culture at a constant temperature. Further, except for all initial allele frequencies at 15°C and for 0.25*F* and 0.75*F* at 29°C, the number of generations monitored exceeded 29. Thus it is reasonable to assume that an equilibrium was being approached in the latter populations. Considering the exceptions, the discussion below indicates that significant changes were observed for all initial

allele frequencies at 15°C and for 0.75*F* at 29°C. Thus, the only temperature-initial allele frequency combination for which such an assessment may require qualification is 29°C, 0.25*F*. An overall summary is given in table 4.

Considering temperature, at 15°C, significant increases in *AdhF* allele frequency were observed for all initial allele frequencies. However, it must be noted that for 0.75*F*, *AdhF* allele frequency initially decreased for the first four generations and subsequently increased. At 20°C, *AdhF* allele frequency significantly increased for 0.5*F* and did not change for all other initial frequencies. At 25°C, *AdhF* allele frequency significantly increased for 0.75*F* and did not significantly change for all other initial frequencies including the 20°C, 0.75*F*

Figure 1 Discrete generation population cage experiments conducted with flies derived from isofemale strains which had been in laboratory culture for 18 months prior to the experiments. The change in *Adh^F* allele frequency observed at 15°C for the initial allele frequencies 0.25F (a), 0.5F (b) and 0.75F (c). Values are given for each generation sampled and each replicate. Mean values at each generation sampled are also shown.

Figure 2 Laboratory strains, discrete generation population experiments: The change in *Adh^F* allele frequency observed at 20°C for the initial *Adh^F* allele frequencies of 0.25F (a), 0.5F (b) and 0.75F (c). Details are given in fig. 1.

Figure 3 Laboratory strains, discrete generation population experiments: The change in *Adh^F* allele frequency at 25°C for the initial *Adh^F* allele frequencies of 0.25F (a), 0.5F (b) and 0.75F (c). Details are given in fig. 1.

Figure 4 Laboratory strains, discrete generation population experiments: The change in *Adh^F* allele frequency at 29°C for the initial *Adh^F* allele frequencies of 0.25F (a), 0.5F (b) and 0.75F (c). Details are given in fig. 1.

Figure 5 Laboratory strains, discrete generation population experiments: The change in *AdhF* allele frequency in the populations which were established at 20°C for an initial *AdhF* allele frequency of 0.75F and then subcultured to 25°C (a) and 29°C (b).

Table 4 A summary of the discrete generation population cage experiments conducted with flies derived from isofemale strains which had been in laboratory culture for 18 months prior to the experiments (figs. 1 to 5). Comparisons have been made between the initial and final generation sampled for *AdhF* allele frequency. Means and standard errors have been assessed in both two-sided and one-sided *t*-tests. + represents a significant increase in *AdhF* allele frequency and nc, no change in allele frequency.

Initial <i>AdhF</i> allele frequency	Temp. (°C)			
	15	20	25	29
0.25F	+*	nc	nc	nc
0.5F	+†	+*	nc	nc
0.75F	+*	nc	+†	+*
0.75F subcultured populations			nc	nc

(i) two-sided *t*-test * $p < 0.05$
† $p < 0.005$.

(ii) one-sided *t*-test; *AdhF* allele frequency significantly increased at 25°C for 0.25F and at 29°C for 0.5F ($p < 0.05$).

populations which were subcultured to 25°C. As noted in table 4, for a one-sided test there was a significant increase for 0.25F. At 29°C, *AdhF* allele frequency significantly increased for 0.75F and did not change for 0.25F, 0.5F and the subcultured populations. It must be noted that for 0.25F, the allele frequency initially increased and subsequently decreased to a mean value similar to the initial frequency. Again, for a one-side test *AdhF* allele frequency significantly increased for 0.5F.

Finally, χ^2_1 comparisons between the observed numbers of *Adh* genotypes and expected numbers assuming Hardy-Weinberg proportions for each replicate indicated that only two values were significant (0.25F, 15°C, replicate 2, $\chi^2_1 = 3.99$, $P < 0.05$; 0.25F, 29°C, replicate 1, $\chi^2_1 = 4.41$, $p < 0.05$). A relative excess of heterozygotes was detected in both populations possibly indicating an extreme environment effect.

Field derived flies and larvae

(a) Larval to adult survival and development time

It was not intended nor was it possible to manipulate the input genotypic proportions in this experiment. Thus, survival differences among genotypes can only be detected by comparisons between the results for the controls and the results at each temperature if significantly more flies survive in the former. The controls represent the situation where larvae were cultured in optimum conditions, defined as 25 larvae in 16.5 ml of food at 25°C. For Chateau Tahbilk, significantly more flies survived in the controls compared with each temperature ($p < 0.005$). However, for Fergusons, significantly more flies survived in the controls compared with 15°C and 20°C ($p < 0.005$) and 29°C ($p < 0.05$) but not for 25°C ($p > 0.10$). Thus, since survival was relatively similar in the controls compared with each temperature, the possibility of detecting genotypic differences for larval survival and development time may be less in the Fergusons data.

Table 5 gives the results for genotypic larval survival for the controls and the two sites at each temperature. Pooled replicates are shown. χ^2_2 values for comparisons between the controls and the experimental values and the *AdhF* allele frequency in each case are also given. No χ^2_2 value was significant indicating that the genotypes survived in similar proportions as observed in the controls at each temperature and for each site.

The results for larval development time, expressed as $T_{1/2}$, are given in table 6, and indicate that the only significant factor was temperature. Therefore, no significant differences between *Adh* genotypes could be detected at any temperature for larval survival and development time when these fitness components were estimated in competition experiments using field derived larvae. The *AdhF* allele frequency in these experiments was approximately 0.67. However, temperature variation did affect these fitness components—more flies survived at 20°C and 25°C compared with 15°C and 29°C.

Table 5 The larval to adult survival of the common *Adh* genotypes estimated for larvae obtained as progeny of flies collected at two field sites, the Chateau Tahbilk and Fergusons vineyards. The control represents the case where larvae were cultured in optimum conditions, defined as 25 larvae in 16.5 ml of food at 25°C. Pooled values are given, and χ^2 comparisons between the respective control values and each experimental temperature are shown. The *Adh^F* allele frequencies in each case are also given for pooled replicates

Temp. (°C)	Chateau Tahbilk						Fergusons					
	<i>Adh^F</i>	<i>FF</i>	<i>FS</i>	<i>SS</i>	Total	χ^2	<i>Adh^F</i>	<i>FF</i>	<i>FS</i>	<i>SS</i>	Total	χ^2
control												
25	0.68	79	83	14	176		0.65	75	74	23	172	
15	0.67	150	148	34	332	2.22	0.63	113	137	40	290	3.19
20	0.66	150	167	36	353	2.82	0.69	162	147	30	339	5.63
25	0.67	143	179	20	342	4.63	0.67	164	171	39	374	2.32
29	0.70	155	132	30	317	3.61	0.67	156	145	40	341	0.63

Table 6 The Larval to adult development times of the common *Adh* genotypes estimated for larvae obtained as progeny of flies collected at two field sites, the Chateau Tahbilk and Fergusons vineyards. Development time is expressed as *T*_{1/2} in days

(a) Means

Chateau Tahbilk			Fergusons			
	Adh genotype					
Temp. (°C)	FF	FS	SS	FF	FS	SS
15	29.64 ± 0.10	29.38 ± 0.34	29.51 ± 0.46	36.68 ± 0.55	36.35 ± 0.39	36.57 ± 0.50
20	14.42 ± 0.17	14.23 ± 0.17	14.36 ± 0.25	13.71 ± 0.10	13.75 ± 0.06	13.88 ± 0.24
25	9.77 ± 0.13	9.75 ± 0.10	9.81 ± 0.10	9.51 ± 0.18	9.47 ± 0.18	9.48 ± 0.21
29	7.88 ± 0.11	7.83 ± 0.06	7.89 ± 0.10	7.73 ± 0.07	7.74 ± 0.04	7.81 ± 0.17

(b) ANOVA

Temp. (°C)	Chateau Tahbilk				Fergusons			
	<i>Df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>
Temp. (<i>T</i>)	3	5067.0	1689.0	23458.3*	Temp. (<i>T</i>)	3	9607.0	3202.3
Gen. (<i>G</i>)	2	0.1	0.05	0.7	Gen. (<i>G</i>)	2	0.2	0.07
<i>T</i> × <i>G</i>	6	0.3	0.05	0.7	<i>T</i> × <i>G</i>	6	0.3	0.05
Residual	57	4.1	0.07		Residual	60	5.5	0.09

* $p < 0.005$.

(b) Mating abilities

In this experiment the unmated genotypic proportions were not manipulated. Further, mating ability differences between *Adh* genotypes can only be detected by comparisons between observed values and expected values based upon the unmated genotypic proportions if all flies do not mate. Table 7 gives for each sex, replicate and temperature, the number of flies of each *Adh* genotype in the unmated populations, together with the genotypes of the flies which mated. The *Adh^F* allele frequencies among the unmated flies of each sex, based upon pooled replicates, are also given. It is clear that fewer flies mated at 15°C compared with the other temperatures and that all the flies did not mate. However there was one replicate (replicate

2 at 20°C and 25°C respectively) where almost all the flies mated.

Comparisons between the observed numbers of flies of each *Adh* genotype which mated and the expected numbers based upon the unmated genotypic proportions for pooled replicates indicated no significant differences for the females at each temperature (15°C, $\chi^2 = 0.33$; 20°C, $\chi^2 = 0.66$; 25°C, $\chi^2 = 0.22$; 29°C, $\chi^2 = 0.12$). Exclusion of replicate 2 at 20°C and 25°C did not change this result (20°C, $\chi^2 = 0.13$; 25°C, $\chi^2 = 0.32$). For the males there was a significant deficiency of heterozygotes at 15°C (15°C, $\chi^2 = 6.28$, $p < 0.05$; 20°C, $\chi^2 = 0.66$; 25°C, $\chi^2 = 0.10$; 29°C, $\chi^2 = 2.62$). This result was inconsistent with the laboratory strains and the Chateau Tahbilk studies. Again,

Table 7 (a) Field derived flies—the mating abilities of *Adh* genotypes. The number of flies of each *Adh* genotype which mated at each temperature are shown for all replicates and each sex. Also given are the numbers of flies at each *Adh* genotype which were present in the unmated populations

O—observed number which mated

U—number in the unmated populations

Temp. (°C)	Replicate	Female genotype					Male genotype			
			<i>FF</i>	<i>FS</i>	<i>SS</i>	Total	<i>FF</i>	<i>FS</i>	<i>SS</i>	Total
15	1	<i>O</i>	7	5	1	13	11	1	1	13
		<i>U</i>	19	10	1	30	18	11	1	30
	2	<i>O</i>	9	5	1	15	10	2	3	15
		<i>U</i>	18	10	1	29	16	11	3	30
	3	<i>O</i>	11	5	1	17	8	8	1	17
		<i>U</i>	14	13	3	30	17	10	1	28
20	1	<i>O</i>	15	5	1	21	8	8	5	21
		<i>U</i>	20	7	3	30	12	11	7	30
	2	<i>O</i>	15	13	1	29	15	10	4	29
		<i>U</i>	15	14	1	30	15	11	4	30
	3	<i>O</i>	13	10	2	25	13	10	2	25
		<i>U</i>	14	12	3	29	15	12	3	30
25	1	<i>O</i>	14	11	0	25	4	19	2	25
		<i>U</i>	16	14	0	30	9	19	2	30
	2	<i>O</i>	12	5	1	18	10	7	1	18
		<i>U</i>	18	9	1	28	11	16	1	28
	3	<i>O</i>	16	10	3	29	15	12	2	29
		<i>U</i>	17	10	3	30	15	12	3	30
29	1	<i>O</i>	12	9	2	23	14	8	1	23
		<i>U</i>	16	12	2	30	16	9	5	30
	2	<i>O</i>	13	9	3	25	14	11	0	25
		<i>U</i>	16	11	3	30	19	11	0	30
	3	<i>O</i>	10	10	0	20	11	8	1	20
		<i>U</i>	14	15	1	30	17	11	2	30

(b) *AdhF* allele frequency among unmated flies, based upon pooled replicates.

	males	females
15°C	0.76	0.76
20°C	0.74	0.66
25°C	0.77	0.66
29°C	0.72	0.75
mean	0.75	0.71

exclusion of replicate 2 at 20°C and 25°C did not change this result (20°C, $\chi^2_2 = 0.73$; 25°C, $\chi^2_2 = 0.20$).

(c) Field derived discrete generation populations
The results for the discrete generation population experiment using flies derived directly from the field are shown in fig. 6. The *AdhF* allele frequencies are given for all populations and generations sampled at each temperature with means. These data have been assessed by *t*-tests between the initial and final generations sampled. The initial mean *AdhF* allele frequencies for the Chateau Tahbilk and Fergusons populations were 0.59 ± 0.05 and 0.69 ± 0.06 respectively. There was no

evidence for significant *AdhF* allele frequency change at all temperatures for both the Chateau Tahbilk and Fergusons populations. Comparisons between the observed numbers of *Adh* genotypes and expected numbers assuming Hardy-Weinberg proportions indicated that only one value was significant—the founding generation, population B, derived from Chateau Tahbilk ($\chi^2_1 = 11.99$, $p < 0.05$) where there was a deficiency of heterozygotes. Thus, the above results were not consistent with the laboratory derived populations, the Chateau Tahbilk data, the clinal data discussed in the Introduction, and the experiments of van Delden *et al.* (1978) and Wilson *et al.* (1982).

Figure 6 Discrete generation population cage experiments conducted with field derived flies: The change in *Adh^F* allele frequency observed in populations derived from Chateau Tahbilk (a) and Fergusons (b) and maintained at 15°C, 20°C, 25°C, and 29°C. Values are given for each population and generation sampled.

DISCUSSION

Fitness and temperature

The results show significant fitness differences between *Adh* genotypes for laboratory adapted but not field derived flies and larvae, so that the *Adh* allele frequency changes in the laboratory populations cannot be interpreted as being directly attributable to the *Adh* locus. It should be noted that hidden genetic variation exists at the *Adh* locus (Gibson *et al.* 1980, 1981*b*; Wilks *et al.*, 1980; Chambers *et al.*, 1981; van Delden, 1982) and that this may have contributed to the results. Whilst acknowledging this, the population cage results indicate that the genotypic fitness estimates obtained with the laboratory adapted strains may be difficult to relate to natural populations. Thus, whilst significant differences for fitness at single loci may be demonstrable for laboratory adapted strains, their relation to the field may be obscure.

It is important to briefly discuss the significant results for the laboratory adapted strains in the context of the literature. van Delden and Kamping (1979) found that the development time of *Adh^{FF}* was slightly more rapid than *Adh^{FS}* and both were faster than *Adh^{SS}* in standard food at 25°C. Their results are in general agreement with those presented here. The fitness data presented also indicate that high genotypic survival was associated with rapid genotypic development time (tables 1 and 2). Giesel and Zettler (1980) found a positive genetic correlation between survival and development time in *D. melanogaster*. These authors used highly inbred lines and hybrids where fitness depends upon the number and severity of recessive deleterious alleles fixed by inbreeding. Positive correlations among fitness components are expected for such alleles (Rose and Charlesworth 1981*a, b*). The positive genetic correlations among fitness components found here for *Adh* could result from the use of isofemale strains which had been in laboratory culture for 18 months. Finally, the egg to adult survival and development time data are not consistent with other studies (van Delden, 1982) since the *Adh* allele and presumably alleles of other loci obtained from the female parent appear to determine the result (tables 1 and 2). This observation suggests that, in certain circumstances, the earlier functional activity of the female haploid genome (Wright and Shaw, 1969; Korochkin, 1981) can be reflected in fitness.

Considering mating ability, the majority of significant differences were associated with males (table 3) in accord with the Chateau Tahbilk data (see Introduction) and the general conclusion that

male mating speed is a most important fitness component in *Drosophila* (Parsons, 1974). Further, in accord with the Chateau Tahbilk data, each significant departure from random mating involved heterozygote excess which was especially evident at 29°C, suggesting an extreme environment effect.

The population cage results (figs. 1 to 6) are not generally consistent with the literature (see Introduction) which indicates that *Adh^S* allele frequency decreases with distance from the equator and with increasing altitude. An increase in *Adh^S* allele frequency would therefore be expected in populations cultured at the relatively higher temperatures of 25°C and 29°C, but was not found at any temperature (table 4). The Chateau Tahbilk studies of McKechnie and McKenzie (1983), indicate that the *Adh^S* allele frequency decreases with temperature increase in a range from 8°C to 22°C. The comparable temperatures in this study were 15°C and 20°C and *Adh^S* allele frequency significantly decreased for all initial allele frequencies at 15°C and for 0.5 *F* at 20°C. Thus, there is a degree of consistency between the laboratory populations and the field populations from which the former were derived. However, the obvious absence in the laboratory populations of a return to the *Adh^F* equilibrium usually observed at Chateau Tahbilk (approximately 0.65) is not expected since this equilibrium is generally conserved there (McKechnie and McKenzie, 1983; McKenzie *et al.*, in preparation).

Further van Delden *et al.* (1978) found that their laboratory populations, initiated at different allele frequencies, showed a convergence of frequencies on regular food at 25°C. The equilibrium values were similar to those in the base populations. The authors interpreted their results as some form of balancing selection. In their populations cultured at 29°C a lower equilibrium frequency of *Adh^F* was observed and this is in accord with the clinal data. Wilson *et al.* (1982) detected weak selective differences between the *Adh^F* and *Adh^S* alleles in laboratory populations cultured at 22°C on standard laboratory food. The fitness estimates obtained indicated that the *Adh^F* allele would eventually become fixed and this was not in agreement with the populations from which the experimental lines were extracted. In the population experiments presented here there was only one case of *Adh^F* allele fixation—in the populations cultured at 25°C for an initial frequency of 0.75 *F*. This result was not confirmed when the 20°C 0.75 *F* populations were subcultured to 25°C at generation 13 and maintained for a further 20 generations.

As previously noted, the presence of environmental levels of ethanol together with temperature variation may be more important for *Adh* than is assumed here. Several laboratory experiments indicate that *AdhF* reaches higher equilibrium frequencies in ethanol supplemented food compared with unsupplemented controls (van Delden, 1982). However, these results were generally obtained with populations which had been in laboratory culture for at least a year (Oakeshott, Gibson and Wilson, 1984). There is a similarity between the results obtained here—an increase in *AdhF* allele frequency for all populations where allele frequency changes—and the above. This suggests that whilst ethanol presence appears important, laboratory culture may be more so. Finally, the population data do not provide consistent evidence for heterozygote advantage despite this general result for the estimated fitness components.

The initial assumption that the relatively extreme temperatures of 15°C and 29°C are sufficiently stressful in regard to increased levels of expressed genetic variation for fitness, appears to be satisfied for the *Adh* locus in the laboratory adapted strains. Significant allele frequency changes in the populations and genotypic differences for fitness components were frequently associated with these temperatures. As discussed below, laboratory culture itself can be regarded as an imposed environmental stress. The lack of significant results for the field derived flies and larvae suggests that in this circumstance these temperatures were not sufficiently stressful. For populations closely related to nature, the use of relatively greater stresses of the type likely to be experienced in nature is probably required.

Domestication

The contrast between the laboratory adapted strains and the field derived flies and larvae could be due to changes in the genetic architectures of particular fitness traits which have evolved under a particular selection regime in nature. The switch into the laboratory presumably alters the selection regime, although it is difficult to specify the nature of the changes. Apparently the effect of genetic background, in the form of fitness differences, was enhanced for the laboratory adapted flies and larvae. In addition, laboratory culture can be regarded as an imposed environmental stress, being an artificial situation and so a form of domestication. Thus, the contrast could be due to increased genetic variation for fitness as expressed in extreme environments (Parsons, 1983) where the extreme

environment arises because of transfer from field to laboratory.

The contrast between the laboratory adapted strains and the field derived flies and larvae may provide a model for what may occur during domestication generally. The results presented indicate that phenotypic selection as a consequence of selection and/or environmentally induced effects upon phenotypic development precedes genetic change in *D. melanogaster* populations transferred from the field to the laboratory. The fitness component studies for the field derived flies and larvae indicated that whilst phenotypic fitness varied, there was no associated variation at the genotypic level for the *Adh* locus since there were no significant *Adh* allele frequency changes in the field derived populations. Thus, based upon the results for the *Adh* locus it appeared that primary phenotypic selection was operating in these populations.

This situation contrasts with the laboratory adapted strains where phenotypic and genotypic variability in fitness were detected for the *Adh* locus in most populations. Thus, based upon the *Adh* locus, after 18 months in laboratory culture, phenotypic variability has become associated with genotypic variability. However, it must be noted that the functional relationships between a change in *Adh* genotype and phenotypic fitness differences remain unclear. Since no common *AdhF* allele frequency was approached in the populations derived from the laboratory adapted strains (figs. 1 to 5), considerable phenotypic and genotypic differentiation may be occurring in these populations. However, the possibility of genetic drift in the form of founder effects cannot be excluded in this case. The above discussion therefore accords with Price (1984) who suggests that the process of domestication involves some combination of genetic changes occurring over generations and environmentally induced events re-occurring during each generation. Finally, an increase in genetic and phenotypic variability characterizes domestic populations (Price, 1984) and this is supported by the results presented.

Survival and development time appear to be more affected than mating ability in the domestication of *D. melanogaster* since the former traits varied more between the laboratory and field populations. This also suggests that there is more additive genetic variance for survival and development time compared with mating ability, with mating ability being apparently subject to intense directional selection irrespective of habitat (Parsons, 1974). Thus, given the importance of mating ability

as a fitness component for *D. melanogaster* it follows that its potential for change in the domestication of *D. melanogaster* should be limited.

An organismic approach to gene frequency changes

Finally, we commenced with the assumption that the gene is the determiner of phenotypic fitness variation. Experiments based upon this approach cannot provide a complete understanding of phenotypic fitness in natural populations because of the effects of multiple loci, interactions between loci, linkage relationships and interactions between genotype and environment. Further, this approach which frequently begins with an electrophoretic locus and searches, *a posteriori*, for correlations of adaptive significance may confound causation with correlation (Hoffmann *et al.*, 1984). In this regard, a variable which is correlated with an allozyme genotype is likely to be a component of the overall phenotype. Hence, an *a priori* approach via electrophoretic loci could not provide a complete analysis of this phenotype (Ziolo and Parsons, 1982). The above limitations are demonstrated in the experiments presented here since genotypic variation in fitness at the *Adh* locus was not relatable to temperature related phenotypic fitness variation.

The above approach contrasts with an *a priori* one commencing at the phenotypic level in the assessment of the genetic basis of fitness variation. In the context of this paper where temperature was used as a variable to demonstrate selection upon a single locus, assessment of the genetic basis of ecological phenotypes such as resistance to temperature extremes and desiccation resistance is relevant. These phenotypes are demonstratively adaptive and relatable to evolutionary change at the population level (Parsons, 1983). Such phenotypes tend to be quantitative at the observational level and their genetic analysis may commence with simpler aspects of the biometrical and polygenic approaches to quantitative inheritance (Thompson and Thoday, 1979). For example, in genetically well known organisms such as *D. melanogaster*, genetic activity can be localised to chromosomes or regions of a chromosome, based upon the assessment of extreme isofemale strains, derived from a population (Parsons, 1980) or by selection procedures. Therefore, clarification of the role of the *Adh* locus in temperature related fitness variation in *D. melanogaster* could begin with assessment of the genetic bases of the above phenotypes in a number of *Drosophila* species, in particular contrasting colonising and non-

colonising species. Because of the ecological extremes to which colonising species are subjected, genotypic and phenotypic differences are magnified and are therefore simpler to elucidate compared with more optimal conditions (Parsons, 1984). This approach could be extended to *D. melanogaster* strains derived from a variety of geographical regions, contrasting under stress those derived from relatively extreme and relatively optimal climates. Such an approach may more precisely resolve the relationship of the *Adh* locus with fitness in natural populations of *Drosophila* than is the situation as presently deduced from many recent discussions in the literature.

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