

# *Est-6* in *Drosophila melanogaster*: effects of genetic composition, temperature and oviposition period on fitness in laboratory populations polymorphic for rare alleles

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The Esterase-6 locus in *Drosophila melanogaster* is known to be involved both in the metabolism of esters and in sperm use and remating of females. Several studies have also produced evidence for selective differences between the allozymic variants at this locus. In this paper we have studied the selection differences when two rare alleles (*Est-6* VS and *Est-6* VF) are competing on the one hand, and when a common allele (*Est-6* F) is in competition against the rare allele *Est-6* VF on the other. Isogenic lines with homogeneous background were used.

In general in the first experiment we found a heterozygous advantage and in the latter strong selection against the rare allele. There was no evidence for any frequency dependent selection. The selective differences were also studied with respect to three different input gene frequencies, three temperatures and in two different periods of oviposition. All the factors were found to influence both the total number of flies emerging and their genotypic composition. There was also interaction between the selective effects of these factors.

## INTRODUCTION

In the last few years the *Est-6* locus in *Drosophila melanogaster* has become particularly interesting in the light of new findings relating to one of the physiological functions of the enzyme Esterase-6 as well as to the geographical pattern of variation of the polymorphism at this locus.

Esterase-6 (E.C. 1.1.1., Wright, 1963) seems to be involved in the mechanism of reproduction affecting both storage and use of sperm in females (Richmond *et al.*, 1980; Richmond and Senior, 1981; Gilbert, 1981; Gilbert *et al.*, 1981). The involvement of Esterase-6 in sperm use and daily productivity in terms of progeny is known to be affected by temperature (Gilbert and Richmond, 1982). Moreover Esterase-6 together with a male specific lipid (cis-vaccenylacetate), a component of the seminal fluid, seems to constitute a substrate-enzyme system that is involved in the pheromonal

regulation of the timing of remating in females (Mane *et al.*, 1983).

Esterase-6 also appears to be involved in the metabolism of esters naturally occurring in food, as suggested by a number of results (Danford and Beardmore, 1979; 1980; Zera *et al.*, 1983).

A large scale geographical survey on the distribution of *Est-6* allele frequencies (Oakeshott *et al.*, 1981; Anderson and Oakeshott, 1984) shows the existence of a recurrent latitudinal cline in three continents both in *Drosophila melanogaster* and in the homologous gene-enzyme system in the sibling species *Drosophila simulans*. Such a pattern seems difficult to explain as being due solely to random processes.

Two common and four rare electrophoretic variants have been described in natural populations. Isoelectrophoretic alleles relative to heat stability, substrate specificity and activity (see references in Costa *et al.*, 1982) have also been found.

Moreover functional differences among allozymes have been reported (Danford and Beardmore, 1979; Costa *et al.*, 1983; Mane *et al.*, 1983). Selection in egg-to-adult viability has been observed in laboratory populations, apparently in relation to the polymorphism at the *Est-6* locus, and selective intensity is significantly modified by temperature, density and composition of the medium (Wright, 1963; Kojima and Yarbrough, 1967; Morgan, 1976; Birley and Beardmore, 1977; Costa and Beardmore, 1980). In a few cases however, experiments cast doubt on the hypothesis of adaptive significance of the *Est-6* polymorphism (Dolan and Robertson, 1975; Clegg *et al.*, 1976; 1978).

Except in one case (Costa and Beardmore, 1980), the laboratory studies on the dynamics of this polymorphism known to us have examined the *Est-6* alleles common in the wild (*Est-6* Slow and *Est-6* Fast).

Here we present the results in egg-to-adult viability obtained with laboratory populations polymorphic at the *Est-6* locus in which a rare allele (*Est-6* Very Fast) was tested at different frequencies against a common allele (*Est-6* Fast) or against another rare one (*Est-6* Very Slow). The definition of "rare" is based on the frequency normally found in the wild (Danieli and Costa, 1977).

In experiments of this kind, there is always the possibility that any effect found might be caused by heterogeneity in the genetic background or by linkage disequilibrium. Our lines were prepared, as described in the next section, so as to strongly reduce the intensity of such interactions.

## MATERIALS AND METHODS

### (i) Lines used

The lines derive from a large natural population sampled in the Venetia region (N. Italy). Five isogenic homozygous lines per genotype were first extracted. Then, in order to randomise the genome and increase the homogeneity of the genetic background, the homozygous lines (*Est-6* VS/*Est-6* VS, *Est-6* F/*Est-6* F, *Est-6* VF/*Est-6* VF) were intercrossed (*Est-6* VS/*Est-6* VS vs *Est-6* VF/*Est-6* VF and *Est-6* F/*Est-6* F vs *Est-6* VF/*Est-6* VF) for a period of 4 years of continuous culture and lines homozygous for the three alleles were then extracted from these populations. The population sizes during this process were maintained at a level (2000–3000 individuals) so as to considerably reduce any linkage disequilibrium which might have been present initially.

Eight *Est-6* VS and 10 *Est-6* VF homozygous lines (derived from the first intercross) were used in experiment I, and 10 *Est-6* F and 3 *Est-6* VF homozygous lines (from the second intercross) in experiment II.

### (ii) Design of experiments

In each experiment three laboratory populations were made up with three different genetic compositions ( $p(\text{VF}) = 0.2; 0.5; 0.8$ ). Each population was started with 600 three day old females fertilised by males of the same or of a different genotype so that the expected zygotic composition of the F1 *Est-6* genotypes would agree with H.-W. expectations. The heterozygotes were the progeny of the reciprocal crosses between the two homozygous genotypes in equal proportions.

The population cages were set up with polyethylene cups containing 25 ml of medium inserted in the base, as described by Beardmore *et al.* (1963). The food medium was prepared with 13 g of agar, 110 g of sucrose, 80 g of maize flour and 145 g of yeast per litre of water in the Experiment I; 13 g of agar, 80 g of sucrose, 80 g of maize flour and 40 g of yeast in Experiment II. The mixture was cooked and boiled for 40 minutes, then cooled to 42°C and 15.6 ml/litre of a solution of nipagin (60 g in 340 ml of 96 per cent ethanol) added to prevent bacterial and fungal growth. The population cages were kept at 24°C in order to allow the females to lay eggs in the cups. The cups were removed after 48 hours of oviposition and substituted with a second series of cups containing fresh medium, in order to analyse any possible difference in fecundity or viability with respect to the period of oviposition. To analyse the possible effect of temperature on the genetic composition of the F1, cups from each population cage were put at two different temperatures (17°C and 23°C) in experiment I and at three different temperatures (17°C, 23°C and 30°C) in experiment II. Taking all possible combinations of the variables (genetic composition, temperature and period of oviposition) we had in all 12 blocks in experiment I and 18 blocks in experiment II. In both the experiments five replicates were set up for each block. All the emerging F1 individuals were collected daily at the same time of day, sexed and stored at -30°C until the electrophoretic assays were performed.

Since in Experiment II one more temperature (30°C) was used than in Experiment I, in order to render the daily technical work manageable, we reduced the productivity by reducing the quantities of yeast and sucrose in the medium.

*(iii) Electrophoretic analysis*

In order to estimate genotypic frequencies about 50 flies were assayed from each replicate, except at 30°C in experiment II where the viability was significantly reduced so that 50 flies were sometimes not available. To avoid any possible bias due to differences in the time of development of different genotypes the flies were chosen so that each day of emergence was proportionally represented, and at random within each day of emergence. Electrophoresis and staining procedures were as previously reported (Costa and Beardmore, 1980).

*(iv) Statistical analysis*

For the analysis of the experimental data, we adopted discrete multivariate analyses using log linear models including main effects of each factor and all interactions (see Gokhale and Kullback, 1978). The test statistic used is  $G^2$ .

To test for the presence of selection in general and for frequency dependent selection in particular we used statistical analyses based on the plots suggested by Bundgaard and Christiansen (unpublished manuscript). Let  $Z_1, Z_2, Z_3$  be the frequencies of the genotypes AA, Aa, aa at a diallelic locus, and let the fitnesses of AA and aa relative to Aa be  $W_1$  and  $W_3$ .

Further let  $R_1 = Z_1/Z_2$  and  $R_2 = Z_3/Z_2$ . Then  $R_1 \times R_2 = W_1 \times W_3/4$ , and consequently  $\log(R_2) = \log(W_1 \times W_3/4) - \log(R_1)$ . Thus in the absence of frequency dependent selection a plot

of  $\log(R_2)$  against  $\log(R_1)$  should be a straight line with slope  $b = -1$  and intercept  $a = \log(W_1 \times W_3/4)$ . If there is no selection then the intercept should be  $\log(0.25)$ . Thus significant deviations of the data points from the straight line  $Y = a - X$  should be evidence for the presence of frequency dependent selection. If there is no significant deviation from this line but the deviations from the line  $Y = \log(0.25) - X$  are significant this should demonstrate that frequency independent selection is operating.

## RESULTS

In each experimental combination, five replicates were carried out. Three of the 30 combinations gave a value of  $G^2$  for heterogeneity between replicates for sex ratio which was significant at the 5 per cent level. The probability of this event or a more extreme one is 0.127. For the genotype ratios also three of the 30 combinations showed significant heterogeneity. In both cases therefore we considered it legitimate to pool the replicates and all the results described in this paper consider the pooled data.

Table 1 gives the results of the number of flies enclosed in the two experiments for the various combinations of experimental conditions. Table 2 gives the genotypic output observed in the two experiments.

The results of the analysis will be presented in two parts, the first dealing with the yields (*i.e.* the

**Table 1** Number of flies enclosed in the two experiments for each combination of experimental conditions

Period of oviposition	Temp (°C)	Input ratio $p(VF)$	Yield Expt. I			Yield Expt. II		
			Male	Female	Total	Male	Female	Total
0-48 hours	17°C	0.2	535	556	1091	148	215	363
		0.5	633	647	1280	170	222	392
		0.8	459	501	960	135	226	361
	23°C	0.2	475	540	1015	236	315	551
		0.5	597	724	1321	203	278	481
		0.8	673	784	1457	232	329	561
	30°C	0.2	—	—	—	62	118	180
		0.5	—	—	—	54	112	166
		0.8	—	—	—	35	97	132
48-96 hours	17°C	0.2	553	556	1109	152	189	341
		0.5	436	485	921	158	211	369
		0.8	458	499	957	134	165	299
	23°C	0.2	580	679	1259	266	341	607
		0.5	574	612	1186	129	120	249
		0.8	490	526	1016	168	190	358
	30°C	0.2	—	—	—	44	84	128
		0.5	—	—	—	18	46	64
		0.8	—	—	—	22	43	65

**Table 2** Genotypic output observed in the two experiments for each combination of experimental conditions

Period of oviposition	Temp. (°C)	Input $p(VF)$	Genotypic output Experiment I			Genotypic output Experiment II		
			VS/VF	VS/VF	VF/VF	F/F	F/VF	VF/VF
0-48 hours	17°C	0.2	164	77	11	155	129	10
		0.5	66	128	57	54	145	27
		0.8	10	112	130	9	161	48
	23°C	0.2	145	98	7	106	167	1
		0.5	58	128	66	45	168	19
		0.8	5	88	159	13	146	89
	30°C	0.2	—	—	—	129	48	0
		0.5	—	—	—	60	101	5
		0.8	—	—	—	20	82	28
48-96 hours	17°C	0.2	132	108	12	171	52	1
		0.5	65	127	65	95	114	18
		0.8	8	110	133	12	95	71
	23°C	0.2	137	109	9	151	94	2
		0.5	50	136	67	68	105	26
		0.8	13	82	154	16	93	119
	30°C	0.2	—	—	—	81	41	1
		0.5	—	—	—	33	28	1
		0.8	—	—	—	12	47	6

total number of flies emerging) for the various combinations of conditions, and the second dealing with the genotypic distributions of the samples. The analysis carried out was a factorial  $G^2$  analysis (see Gokhale and Kullback, 1978). The factors were the timing of oviposition, the temperature, and the sex (genotypes in the second part). In the first part, the input gene frequency was included as one of the factors. This was not possible however in the analysis of genotype frequencies; in the latter case the results of the three input gene frequencies had to be analysed separately.

### (i) Yield in Experiment I

The simplest model which explains the data takes into consideration the difference in yield between sexes (sex ratio different from 1:1) and a three-way interaction between the other factors (goodness of fit  $G^2[11] = 11.15$ ). The sex ratio is biased in favour of females (see table 3). As for the other three

**Table 3** Sex ratio in the two experiments. The results of Experiment II are subdivided so that section IIa concerns total yields at 17+23°C, section IIb yields at 30°C. \* $P < 0.001$ . The same notation is used in the following tables

Experiment	Males	Females	Total	Male/female	$G^2[1]$
I	6463	7109	13572	0.91	30.8*
IIa	2131	2801	4932	0.76	91.3*
IIb	235	500	735	0.47	97.7*

factors, more flies emerge from the eggs laid in the first 48 hours than in the subsequent 48 hours (table 4) and the yield is higher at 23°C than at 17°C (table 5). Also the yield is highest at  $p(VF) = 0.5$  (table 6).

**Table 4** Number of flies emerged in the two experiments in the two periods of oviposition. 01 refers to individuals emerged from eggs laid in the first 48 hours; 02 to those emerged from eggs of the subsequent 48 hours. The same notation is used in the following tables

Experiment	Period of oviposition		Total	01/02	$G^2[1]$
	01	02			
I	7124	6448	13572	1.10	33.7*
IIa	2709	2223	4932	1.22	48.0*
IIb	478	257	735	1.86	67.5*

\* =  $P < 0.001$

There is a very intense interaction between the input frequency and the pattern of oviposition (table 7). There is also a strong interaction between the temperature and the input gene frequencies (table 8): there is a progressive decrease of the yield at 17°C relatively to that at 23°C (T1/T2) with increasing frequencies of *Est-6 VF*.

### (ii) Yield in Experiment II

Besides the fact that the genotypes in competition are different, this experiment has also an additional temperature (30°C). At this temperature there are

**Table 5** Yields at the temperatures used in the two experiments. T1 = 17°C; T2 = 23°C; T3 = 30°C. The same notation is used in the following tables.

Expt.	Temperature			Total	T1/T2	T3/T2	G <sup>2</sup>	d.f.
	T1	T2	T3					
I	6318	7254	—	13572	0.87	—	64.6*	1
II	2125	2807	735	5667	0.76	0.26	1336.3*	2

**Table 6** Yields for the input frequencies of the *Est-6* VF allele in the two experiments

Expt.	<i>p</i> (VF) = input frequency			Total	0.2/0.5	0.8/0.5	G <sup>2</sup> [2]
	0.2	0.5	0.8				
I	4474	4708	4390	13572	0.95	0.93	12.0*
IIa	1862	1491	1579	4932	1.25	1.06	45.0*
IIb	308	230	197	735	1.34	0.86	26.0*

**Table 7** Interaction between the influence of input frequency and the period of oviposition on the yield

Expt.	Input <i>p</i> (VF)	Period of oviposition		Total	01/02	G <sup>2</sup> [2]
		01	02			
I	0.2	2106	2368	4474	0.89	78.7*
	0.5	2601	2107	4708	1.23	
	0.8	2417	1973	4390	1.23	
IIa	0.2	914	948	1862	0.96	41.2*
	0.5	873	618	1491	1.41	
	0.8	922	657	1579	1.40	
IIb	0.2	180	128	308	1.41	11.4*
	0.5	166	64	230	2.59	
	0.8	132	65	197	2.03	

**Table 8** Interaction between the influences of temperature and input gene frequency on the yield in the two experiments

Expt.	Input <i>p</i> (VF)	Temperature			Total	T1/T2	T2/T3	G <sup>2</sup> [2]
		T1	T2	T3				
I	0.2	2200	2274	—	4474	0.97	—	27.0*
	0.5	2201	2507	—	4708	0.88	—	
	0.8	1917	2473	—	4390	0.78	—	
II	0.2	704	1158	308	2170	0.61	0.27	60.5*
	0.5	761	730	230	1721	1.04	0.32	
	0.8	660	919	197	1776	0.72	0.21	

\* =  $P < 0.001$ 

drastic selection effects (see table 5), and in the analysis we have tended to keep the results at 30°C separate. Thus while the model which considers sex ratio and the three-way interaction among oviposition pattern, temperature and input gene frequencies fits the data satisfactorily ( $G^2[11] = 17.1$ ), if we include the results for 30°C, this is no longer true; when these data are included, there

is also a strong interaction between the sex ratio and the temperature. In fact males are strongly selected against at the highest temperature (table 3). Interaction between the temperature and the oviposition pattern is such that selection at 30°C is particularly strong in the second oviposition period (table 4). When only 17°C and 23°C are considered, the results for sex ratio, oviposition

**Table 9** Total output genotypes for the input  $p(\text{VF})$  frequencies in Experiment I. Genotypic and allelic fitnesses are relative to the heterozygote and the *Est-VF* allele respectively

Expt. I $p(\text{VF})$	Output genotypes			Fitness		Output alleles		Fitness VS
	VS/VF	VS/VF	VF/VF	VS/VS	VF/VF	VS	VF	
0.2	578	392	39	0.74	0.80	1548	470	0.82
0.5	239	519	255	0.92	0.98	997	1029	0.97
0.8	36	392	576	0.73	0.73	464	1544	1.20

pattern and temperature are qualitatively the same as in Expt. I (tables 3–5). The effect of input gene frequency is however different. The yield (pooling 17°C and 23°C) is minimum at  $p(\text{VF}) = 0.5$  (table 6). While in general the yield is higher at 23°C relative to 17°C the only exception is when  $p(\text{VF}) = 0.5$  where the yields are effectively the same at 17°C and 23°C (table 8). Excluding 30°C, the interaction between oviposition pattern and input gene frequency is as in Expt. I.

Even though most of the results of Expt. II at 17°C and 23°C are qualitatively the same as in Expt. I, there are nevertheless significant quantitative differences in sex ratio, oviposition pattern and temperature effects. All these effects are more pronounced in Expt. II. It must be kept in mind however that the media used in the two experiments are slightly different.

### (iii) Genotypic and allelic output in Expt. I

Since none of the interactions between factors is significant, table 9 shows the totals for each input gene frequency over all the other factors. At all input gene frequencies, there is heterozygote advantage. We also used the statistical test of significance described earlier for the presence of frequency dependent and frequency independent selection. Using the 12 points calculated from the observed genotypic outputs of table 2, we did not find any evidence for frequency dependent selection ( $F[1, 10] = 1.14$ ;  $P > 0.25$ ) while there is evidence that frequency independent selection is operating ( $F[1, 11] = 10.18$ ;  $P < 0.01$ , table 10).

### (iv) Genotypic and allelic output in Expt. II

Once again, from the 17 usable points of table 2, we find that while there is no evidence for frequency dependent selection ( $F[1, 15] = 0.13$ ;  $P > 0.50$ ) there is strong evidence for frequency independent selection ( $F[1, 16] = 77.19$ ;  $P \ll 0.001$ , table 10).

**Table 10** Analysis of variance for testing for frequency-independent and frequency dependent selection in the two experiments

EXPERIMENT I				
Hypothesis	S.S.	d.f.	F	P
$b = -1$ ; $a = \log(0.25)$	4.73	12		
(Difference)	2.27	1	10.18	< 0.01
$b = -1$ ; $a$ estimated	2.46	11		
(Difference)	0.25	1	1.14	n.s.
$a, b$ estimated	2.21	10		
EXPERIMENT II				
Hypothesis	S.S.	d.f.	F	P
$b = -1$ ; $a = \log(0.25)$	43.19	17		
(Difference)	35.77	1	77.19	$\ll 0.001$
$b = -1$ ; $a$ estimated	7.42	16		
(Difference)	0.07	1	0.13	n.s.
$a, b$ estimated	7.35	15		

Tables 11 and 12 show the genotypic and allelic outputs summed respectively over sex and oviposition or sex and temperature in order to study the effects of the factors on the fitnesses. There is almost always strong selection against the *Est-6* VF allele, the only exceptions being for  $p(\text{VF}) = 0.2$  both at 23°C and the first oviposition period (01). There is also a highly significant difference between the selective effects in the two oviposition periods. While in the first 48 hours the heterozygote is the fittest genotype (except at 30°C), among the eggs laid in the second period there is directional selection.

## DISCUSSION

No comparison is possible between the two experiments as far as total productivity is concerned due to the slight differences in the media (see Materials and Methods). Nevertheless the model which considers sex ratio, oviposition pattern and temperature fits the data qualitatively in the same way in the two experiments when 17°C and 23°C are

considered. This could indicate that the diversity in larval density between the two experiments did not greatly affect the qualitative pattern of interactions between the considered factors.

The variation in total yield with input gene frequencies is different in the two experiments (table 6) and this may be due to intrinsic reproductive and perhaps other features of the homozygous lines used in the experiments. Preliminary estimates of fecundity of the different genotypes, measured for single fertilised females, showed the existence of differences even if we cannot extrapolate them to our experimental conditions which differed for example with respect to crowding.

As for temperature effects, in addition to the fact that the highest yield was always observed at 23°C, the highest temperature considered (30°C) produced drastic effects and in addition also affected males much more than females. There is a strong interaction between input gene frequency and the pattern of oviposition such that the yield in the first period relative to that in the second is considerable lower at  $p(VF) = 0.2$ . In addition this ratio is much higher at 30°C with respect to 17°C and 23°C (table 7). It seems worth considering the hypothesis that this interaction is due to a difference in the pattern of oviposition among genotypes. Previous results (Gilbert and Richmond, 1982) have suggested that male *Est-6* promotes ejaculate transfer, sperm storage and use in females. A quantitative study of sperm use during the first 50 hours after mating by using *Est-6* null and active stocks of *Drosophila melanogaster* (Gilbert *et al.*, 1981) demonstrated that the rate of sperm release is related to the level of Esterase-6 activity suggesting the involvement of this enzyme, present in the seminal fluid, in sperm motility. Moreover 3 or 4 days after mating few sperms are present in the receptacle and at this time also the

fertilisation rate begins to drop together with the number of eggs (David, 1963; Pyle and Gromko, 1978). As for sperm release it has been hypothesized that Esterase-6 plays a primary role in determining its initial rapid rate (Gilbert *et al.*, 1981) which declines 2 or 3 days after mating. Since functional differences have been detected among *Est-6* allozymes, in particular between rare and frequent variants (Costa *et al.*, 1983), we suggest that the differences in yield observed in our experiments in the two periods of oviposition considered and for different input frequencies are mainly due to the differences in the catalytic properties of the allozymes in the physiological process of sperm use.

In Experiment I no interaction was observed between the factors considered. The heterozygote is always the fittest genotype. It is interesting to note that the two alleles VF and VS which are both rare in nature have very similar fitnesses when competing with each other (table 9).

The genotypic and allelic output in Exp. II, where the *Est-6* polymorphism included the *Est-6* F and *Est-6* VF alleles, indicates the presence of strong selection (tables 11 and 12). In this experiment while heterozygote advantage is again evident at 17°C and 23°C there is a clear shift towards directional selection at 30°C. Contrary to Exp. I, in Exp. II the *Est-6* VF allele is in general strongly selected against with only one exception. The strong selection against *Est-6* VF suggested by these results is in agreement with the fact that in nature it is very rare while *Est-6* F is a common allele in the populations from which our lines are derived (Danieli and Costa, 1977).

Particularly interesting in Exp. II seems the interaction between the mode of selection and the oviposition period at 17°C and 23°C, when a shift from heterosis in the first oviposition period to

**Table 11** Total genotypic and allelic output for the input *Est-6* VF frequencies and temperatures in Experiment II. Fitnesses are as in table 9

Expt. II Temp.	Input $p(VF)$	Output genotypes			Fitness		Output alleles		Fitness F
		F/F	F/VF	VF/VF	F/F	VF/VF	F	VF	
17°C	0.2	326	181	11	0.90	0.49	833	203	1.03
	0.5	149	259	45	1.15	0.35	557	349	1.60
	0.8	21	256	119	0.67	0.23	298	494	2.41
23°C	0.2	257	261	3	0.49	0.09	775	267	0.73
	0.5	113	273	45	0.83	0.33	499	363	1.37
	0.8	29	239	208	0.97	0.44	297	655	1.81
30°C	0.2	210	89	2	1.18	0.18	509	93	1.37
	0.5	93	129	6	1.44	0.09	315	141	2.23
	0.8	32	129	34	1.98	0.13	193	197	3.92

**Table 12** Total genotypic and allelic outputs for the input *Est-6* VF frequencies and oviposition periods in Experiment II. Fitnesses are as in Table 9

Expt. II	Oviposition period	Input $p(\text{VF})$	Output genotypes			Fitness		Alleles		Fitness F
			F/F	F/VF	VF/VF	F/F	VF/VF	F	VF	
IIa	01	0.2	261	296	11	0.44	0.30	818	318	0.64
		0.5	99	313	46	0.63	0.29	511	405	1.26
		0.8	22	307	137	0.57	0.21	351	581	2.42
	02	0.2	322	146	3	1.10	0.16	790	152	1.30
		0.5	163	219	44	1.49	0.40	545	307	1.78
		0.8	28	188	190	1.19	0.51	244	568	1.72
IIb	01	0.2	129	48	1	1.34	0.17	306	50	1.53
		0.5	60	101	5	1.19	0.10	221	111	1.99
		0.8	20	82	28	1.95	0.17	122	138	3.54
	02	0.2	81	41	1	0.99	0.20	203	43	1.18
		0.5	33	28	1	2.36	0.07	94	30	3.13
		0.8	12	47	6	2.04	0.06	71	59	4.81

directional selection against *Est-6* VF in the second one was observed (table 12). However this may not be due to selective mechanisms but to the relative differences that might exist in the timing of oviposition among fertilised females depending on the male *Est-6* genotype as mentioned above.

The overall picture apparently suggests that selection patterns are similar for the two experiments in spite of possible differences in the genetic background among the lines used in the two experiments. This could mean that the *Est-6* locus is really involved in determining detectable fitness component(s). Further these results are similar to those obtained by several authors working with lines with possible dissimilarities in their genetic backgrounds (MacIntyre and Wright, 1966; Kojima and Yarbrough, 1967; Kojima and Huang, 1972; Birley and Beardmore, 1977; Minawa and Birley, 1975; Morgan, 1976; Oakeshott, 1979; Abedin *et al.*, 1980; Costa and Beardmore, 1980). While one can see in general selection against the *Est-6* VF allele in the results of tables 11 and 12, there are two exceptions, both for  $p(\text{VF}) = 0.2$ , one at a temperature of 23°C and the other for the first 48 hours of oviposition. From the analysis of the yield these are the optimal growth conditions. In other words the allele *Est-6* VF is at a disadvantage when conditions are suboptimal, and further it is possible that it shows selection when rare enough.

On the other hand apparent selective responses regarding a locus under study may be due to the fact that other linked loci are the true targets of selection. The lines used in this study were isolated by randomising their genomes and reducing the possibility of the persistence of genetic variation at closely linked loci; nevertheless we cannot

exclude the possible effects of other loci on the results obtained. We have performed other one-generation experiments to test the actual role of the *Est-6* locus in determining fitness by using highly inbred lines obtained with a procedure reported elsewhere (Costa *et al.*, 1985). There too we obtained results in agreement with the hypothesis that attributes an adaptive role to the *Est-6* polymorphism. Nevertheless the dynamics of this polymorphism studied over several generations in experimental populations set up with our highly inbred lines suggests that part of the selection associated with the locus under more or less pronounced outbred conditions has been eliminated (Costa *et al.*, in preparation). It seems likely then that there is a good deal of interaction of the *Est-6* locus with other systems possibly in linkage disequilibrium in the wild or in laboratory populations derived from outbred lines even though studies devoted to discover linkage disequilibrium in wild populations in the chromosomal arm carrying the *Est-6* locus in natural populations of *Drosophila melanogaster* have not been successful (see references in Costa *et al.*, 1985).

It has by now become evident that the *Est-6* polymorphism has an adaptive significance based either on its already demonstrated involvement in the reproductive process (Gilbert and Richmond, 1982) or on its role in the metabolism of esters (Zera *et al.*, 1983; Costa *et al.*, 1985). The results reported here together with the other findings for outbred and inbred populations cited above, suggest however that the *Est-6* locus is not only involved in selective processes *per se* but also because of its interactions with other loci. How this occurs remains to be studied, but considering



the overall pattern of results produced by studies on the *Est-6* locus it is suggested that this polymorphism in the wild is maintained by complex and interacting modes of selection.

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