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Allozyme polymorphism at the *αGpdh* and *Adh* loci and fitness in *Drosophila melanogaster*

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We have studied in *Drosophila melanogaster* the effects of allozyme variation at the *αGpdh* and *Adh* loci on the following fitness components: female fecundity, egg hatchability, egg-to-adult survival under near-optimal and competitive conditions, rate of development under near-optimal and competitive conditions, and mating capacity of males. Significant effects of the *αGpdh* locus on rate of development under competitive conditions (*SS* > *FS* > *SS*), and of the *Adh* locus on egg hatchability (*FF* = *SS* > *FS*) and egg-to-adult survival under competitive conditions (*FF* > *SS* = *FS*) were revealed. Possible natural selection mechanisms involved in the maintenance of allozyme polymorphisms at the *αGpdh* and *Adh* loci are described. These mechanisms and the fitness of In(2L)t may account for the persistence and clinal distribution of the two allozyme polymorphisms in nature.

INTRODUCTION

The existence of some "*in vitro*" differences between the allozymes of the *αGpdh* (Miller *et al.*, 1975) and *Adh* loci (Rasmuson *et al.*, 1966; Gibson, 1970; Vigue and Johnson, 1973; Day *et al.*, 1974*a*, *b*; Oakeshott, 1976) is well documented.

Nevertheless, to prove the physiological "*in vivo*" repercussions of these differences is not easy, and their reported relationships with environmental variables such as temperature and alcohol substrates is not very clear.

Temperature. The results obtained in experimental populations are not very coherent (Berger, 1971; Van Delden *et al.*, 1978; Alahiotis and Pelecanos, 1980; Vigue *et al.*, 1982; Sánchez and Rubio, 1983*a*, *b*; Oakeshott *et al.*, 1985; Kohane and Parsons, 1986). The latitudinal clines for the *αGpdh* and *Adh* loci are well established (Oakeshott *et al.*, 1982, 1984*b*) but Oakeshott *et al.* (1982) clearly reject the positive correlation between maximum temperatures and genic frequencies at the *αGpdh* and *Adh* loci. Besides, Gionfriddo and Vigue (1978) do not find any correlation between environmental temperature and temporal allelic frequency fluctuation at the *Adh* locus. Thus, it seems that the relative thermostabilities of the αGPDH and ADH allozymes have a small direct effect over the temperature tolerance in *Drosophila melanogaster* (Oakeshott *et al.*, 1982). On the other hand, adult survival (Schenfeld and McKechnie, 1979) and larva-to-adult viability (Charles-Palabost, 1982) of the three *αGpdh* genotypes are not temperature dependent, although there is evidence of heterozygote disadvantage at high temperature (McKechnie *et al.*, 1981).

Environmental alcohol. For the *Adh* locus, adult survival tests do not yield conclusive results (Briscoe *et al.*, 1975; Oakeshott, 1976; Van Delden *et al.*, 1978), although almost always the *F*/*F* genotype is more resistant and the *Adh-F* allele frequency grows in experimental populations. Even so, the key to alcohol detoxification does not depend exclusively upon the ADH catalysis (McKenzie and Parsons, 1974; Oakeshott, 1976; McKenzie and McKechnie, 1978; Gibson *et al.*, 1981; Van Delden and Kamping, 1983; Oakeshott *et al.*, 1984*a*; Kerver and Van Delden, 1985; Gibson and Wilks, 1988).

In a first approximation to the topic we have carried out experiments at constant temperature and in a standard medium lacking alcohol, in order to estimate the allozyme fitnesses without the influence of these factors.

The *αGpdh* and *Adh* loci are in chromosome II and a linkage disequilibrium associated with the cosmopolitan In(2L)t has been detected between them. The In(2L)t includes the *αGpdh* locus but

not the *Adh* locus, although the latter is near one of the two extremes of the In(2L)t. The association between In(2L)t and the *αGpdh-F* and *Adh-S* alleles is common (Watanabe and Watanabe, 1977; Inoue *et al.*, 1984). The meaning of these non-random associations is subject to contradictory interpretations: for some (Prakash and Lewontin, 1968, 1971; Dobzhansky, 1970) they are clear examples of selective coadaptation; for others (Nei, 1975; Nei and Li, 1975, 1980) they can be explained as random genetic drift or as a disequilibrium of the initial linkage when the inversion took place and are, therefore, compatible with allelic neutrality.

In this work we try to infer possible natural selection mechanisms upon allozyme polymorphisms at the *αGpdh* and the *Adh* loci, estimating several fitness components. We also try to account for the persistence and clinal distribution of the two allozyme polymorphisms in nature as functions of the obtained fitness data.

MATERIALS AND METHODS

Drosophila melanogaster flies were collected at Los Areneros near Oviedo (Asturias, North of Spain) using banana baited traps. The collected males were immediately assayed by electrophoresis whereas each female was placed in a vial with standard *Drosophila* medium. When larvae or pupae were present in the vials, females were also assayed by electrophoresis.

The electrophoretical analysis for αGPDH and ADH enzymes were carried out using standard starch gel electrophoresis (Grell *et al.*, 1965; Grell, 1967; O'Brien and MacIntyre, 1969). Two allozymes for each locus were detected: F (fast) and S (slow).

Following the Marinkovic and Ayala (1975*a*, *b*) method, single-pair crosses were made within each isofemale strain. When both parents were identical homozygote, *FF* or *SS* at each locus, their descendants (*FF* or *SS* homozygotes at the two loci) were maintained by mass culture to constitute an original double-homozygote isofemale strain. We produced 12 isofemale strains of the *FFFF* genotype (group A), 12 of the *FFSS* genotype (B), 12 of the *SSFF* genotype (C), and six of the *SSSS* genotype (D) (from now on the first locus is always *αGpdh* and the second, *Adh* locus). Each of these 42 isofemale strains came from a different inseminated wild female and, therefore, each retain the variability from the chromosomes of one male and one female.

To test each genotype in a genetic background representative of the genetic composition of the original population (Marinkovic and Ayala, 1975*a*, *b*), series of crosses were made between the original isofemale strains, as follows. If the strains of group A are represented as A_1, A_2, A_3, etc., we first crossed $A_1 \times A_2$, $A_2 \times A_3$, etc.; then we crossed $A_{1,2} \times A_{3,4}$, $A_{2,3} \times A_{4,5}$, etc. The same for the groups B and C. Within group D, we crossed $D_{1-4} \times D_{5,6}$, $D_{2-5} \times D_{6,1}$, etc. Thus, within each group we have obtained six combined strains, each originated from four isofemale strains in the groups A, B and C, and from the six isofemale strains in group D. To obtain the homozygous or heterozygous final synthetic strains, we crossed the combined strains of same ($A_{1-4} \times A_{6-9}$, or $B_{2-5} \times B_{9-12}$, or $C_{5-8} \times C_{10-1}$, or $D_{1-6} \times D_{2-5}$, etc.) or different genotype ($A_{1-4} \times B_{1-4}$, or $B_{5-8} \times C_{5-8}$, or $C_{9-12} \times D_{3-2}$, etc.), respectively (table 1). Thus, we obtained 30 final synthetic strains, three of each of the ten possible genotypic combinations. Each of these final synthetic strains is derived from six, eight or ten original isofemale strains and the three final synthetic strains of same genotype join genetic background come from 6, 12, 18 or 24 original isofemale strains (table 1).

Table 1 Crosses to originate the 30 final synthetic strains, three strains of each genotypic combination

Genotypes	Crosses		
$\frac{FF}{FF}$	$A_{1-4} \times A_{6-9}$	$A_{2-5} \times A_{9-12}$	$A_{5-8} \times A_{10-1}$
$\frac{FF}{FS}$	$A_{1-4} \times B_{1-4}$	$A_{5-8} \times B_{5-8}$	$A_{9-12} \times B_{9-12}$
$\frac{FS}{FS}$	$B_{1-4} \times B_{6-9}$	$B_{2-5} \times B_{9-12}$	$B_{5-8} \times B_{10-1}$
$\frac{FF}{SF}$	$A_{2-5} \times C_{2-5}$	$A_{6-9} \times C_{6-9}$	$A_{10-1} \times C_{10-1}$
$\frac{FF}{SS}$	$A_{1-4} \times D_{1-4/5-6}$	$A_{5-8} \times D_{2-5/6-1}$	$A_{9-12} \times D_{3-6/1-2}$
$\frac{FS}{SF}$	$B_{1-4} \times C_{1-4}$	$B_{5-8} \times C_{5-8}$	$B_{9-12} \times C_{9-12}$
$\frac{FS}{SS}$	$B_{2-5} \times D_{1-6/3-4}$	$B_{6-9} \times D_{2-1/4-5}$	$B_{10-1} \times D_{4-1/2-3}$
$\frac{SF}{SF}$	$C_{1-4} \times C_{6-9}$	$C_{2-5} \times C_{9-12}$	$C_{5-8} \times C_{10-1}$
$\frac{SF}{SS}$	$C_{1-4} \times D_{1-4/5-6}$	$C_{5-8} \times D_{2-5/6-1}$	$C_{9-12} \times D_{3-6/1-2}$
$\frac{SS}{SS}$	$D_{1-4/5-6} \times D_{2-1/4-5}$	$D_{1-6/3-4} \times D_{3-6/1-2}$	$D_{2-5/6-1} \times D_{4-1/2-3}$

To study the In(2L)t, the polytene chromosomes of third-instar larvae were analysed according to Levine and Schwartz (1970). Five larvae of each of 24 double-homozygote combined strains were analysed.

We estimated selection for fecundity, zygotic selection and sexual selection. Experiments did not coincide in time to avoid correlated estimations. Only viability and rate of development for each density were estimated in the same experiment.

The following fitness components were studied:

Female fecundity, estimated in two replicates with three females and four males each. The number of eggs laid by each group was counted daily during seven days (from third to ninth day of life).

Egg hatchability or egg-to-larvae viability, estimated in six replicates of 50 eggs each.

Egg-to-adult viability under near-optimal density, estimated from 20 eggs per vial in each one of ten replicates; and under competitive density, four replicates of 100 eggs per vial.

Rate of development under near-optimal density, estimated from 20 eggs per vial in each one of ten replicates; and under competitive density, four replicates of 100 eggs per vial.

Mating capacity of males, estimated in five replicates. Each replicate consisted of one male in presence of 12 virgin females during 24 hours. Afterwards, each female was placed in an individual vial to observe if descendants appeared or not. The females came from all the synthetic strains.

All the experiments were carried out in an incubator at 24±1°C and a 12L:12D light-darkness cycle.

Factorial analyses of variance were used. These analyses require symmetry of the data and, therefore, only one datum for double heterozygotes. In all cases we used the data corresponding to coupling heterozygotes because no significant differences were found between the two double heterozygotes for any of the components. When the data were proportions (egg hatchability, viabilities under the two densities and mating capacity of males) we applied the arcsine $\sqrt{\text{proportion}}$ transformation.

In factorial analyses of variance data of all replicates in each of the final synthetic strains were utilised. Thus, the analyses reveal if there are differences between synthetic lines of the same genotype and, therefore, if the genetic background contributes to fitness differences.

RESULTS

The original population

The 68 males and 229 females collected from the wild were assayed by electrophoresis to reveal the αGPDH and ADH allozymes. Wild males and females showed no significant differences for genotypic nor allelic frequencies at the *αGpdh* locus. The same occurring at the *Adh* locus (Izquierdo, 1987).

Tables 2, 3 and 4 give only female data. The male combined genotype data were not available as they were used for the confirmation of other data. Therefore, from now on we will only use the female data for the Los Areneros natural population.

At the *αGpdh* locus, genic frequencies are near 0·5 the *αGpdh-S* being somewhat higher (table 2).

Table 2 Locus *αGpdh*. Genotypic and genic frequencies in the wild females

Genotypes	Observed number	Expected number	Frequency
FF	73	55·75	0·3188
FS	80	114·48	0·3493
SS	76	58·77	0·3319
	229	229·00	1·0000
Genic frequencies	*αGpdh-F*	$p_\alpha = 0{\cdot}4934$	
	αGpdh-S	$q_\alpha = 0{\cdot}5066$	

Table 3 Locus *Adh*. Genotypic and genic frequencies in the wild females

Genotypes	Observed number	Correction for continuity	Expected number	Frequency
FF	176	175·5	171·18	0·7686
FS	44	44·5	53·62	0·1921
SS	9	8·5	4·20	0·0393
	229	229·0	229·00	1·0000
Genic frequencies	*Adh-F*	$p_A = 0{\cdot}8646$		
	Adh-S	$q_A = 0{\cdot}1354$		

Table 4 Observed and expected frequencies of two-loci combined genotypes in the wild females

αGpdh Genotypes		*Adh* Genotypes						
		FF		*FS*		*SS*		
		Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Total
FF	Obs.	39		26		8		73
	Exp.		56·10		14·03		2·87	
FS	Obs.	65		15		0		80
	Exp.		61·48		15·37		3·14	
SS	Obs.	72		3		1		76
	Exp.		58·41		14·60		2·99	
Total		176		44		9		229

Genotypic frequencies differ significantly ($P < 0{\cdot}001$) from the Hardy-Weinberg equilibrium due to an excess of both homozygotes. The *Adh-F* allele frequency is much higher than that of *Adh-S* (table 3) and genotypic frequencies significantly ($P < 0{\cdot}05$) differ from the Hardy-Weinberg equilibrium due to an excess of both homozygotes. In this area (Asturias) it is not rare to find *D. melanogaster* populations with a deficit of heterozygotes (Izquierdo, 1987 and references therein).

On the other hand, the effective size of the Los Areneros natural population was estimated (Corrales, 1982) at about 10,000 individuals. Samples taken there over several years revealed the maintenance of allozyme frequencies (Corrales, 1982) at similar levels to those reported in this work, and the inversion frequencies (Sanchez Refusta, unpublished data) at the levels reported by Roca *et al.* (1982).

The *FFFS*, *FFSS* and *SSFF* genotypes showed higher frequencies than expected in the independent combination hypothesis, while the *FFFF*, *FSSS* and *SSSS* showed lower frequencies than expected (table 4). The difference between observed and expected values was significant ($P < 0{\cdot}001$). Following the Weir and Cockerham (1979) procedure, a negative ($D = -0{\cdot}053$) and highly significant ($P < 0{\cdot}001$) linkage disequilibrium was observed.

We have found the In(2L)t in two of the six combined strains of group A (genotype *FFFF*) and in all the six combined strains of group B (genotype *FFSS*). Given the constitution of these combined strains we may deduce that when the In(2L)t appears in two combined strains, probably only one original isofemale strain would carry it. When the In(2L)t appears in all the six combined strains of the same group, from three to 12 original isofemale strains would carry it. Given the habitual association between In(2L)t and *αGpdh-F* and *Adh-S* alleles we can assume the existence of In(2L)t in all the 12 original isofemale strains of *FFSS* genotype.

Fitness components in the synthetic lines

Female fecundity. The mean numbers of eggs laid per female and day range from 86·5 (genotype *FFFF*) to 96·6 (genotype *SSFF*). The factorial analysis of variance showed no significant differential influence of the *αGpdh* or *Adh* loci or their interaction on this component.

Egg hatchability. Table 5 shows the mean numbers of emerged larvae. The factorial analysis of variance (table 6) showed significant ($P < 0{\cdot}05$) differential influence of the *Adh* locus on egg hatchability. Both homozygotes exhibited higher

Table 5 Egg hatchability: Mean numbers of emerged larvae

αGpdh Genotypes	*Adh* Genotypes			*αGpdh* Genotypes
	FF	*FS*	*SS*	
FF	46·2	44·8	46·8	45·9
FS	46·1	45·8	46·7	46·2
SS	47·2	45·3	46·6	46·4
	46·5	45·3	46·7	

Mean value of the *FSSF* genotype: $Y = 44{\cdot}6$.

Table 6 Egg hatchability: Factorial analysis of variance

Source of variation	Degrees of freedom	Sums of squares	Mean squares	*F*	*P*<
Locus *αGpdh*	2	12·39	6·19	0·15	ns
Locus *Adh*	2	251·49	125·75	3·08	0·05
Interaction	4	39·63	9·91	0·24	ns
Within genotypes	153	6245·91	40·82		
Synthetic strains	18	1029·79	57·21	1·48	ns
Replicates	135	5216·12	38·64		

egg hatchability than heterozygotes. No significant differential influence could be detected either for the *αGpdh* locus nor for the *αGpdh* and *Adh* loci interaction.

Egg-to-adult viability under near-optimal density. The mean numbers of adults arisen range from 17·5 (*FSSS*) to 18·8 (*FSFF*). Neither the *αGpdh* nor the *Adh* loci show significant differential influence on this component. The same applies for the interaction between the loci.

Egg-to-adult viability under competitive density. Table 7 shows the mean numbers of adults arisen. The factorial analysis of variance (table 8) revealed a significant ($P < 0{\cdot}05$) differential influence of the *Adh* locus on this component. Under competitive density, *Adh-F/Adh-F* homozygotes showed a higher viability than *FS* or *SS* genotypes. No significant differential influence could be detected either for the *αGpdh* locus nor for the interaction between the loci. The variation among synthetic strains of the same genotype was highly significant ($P < 0{\cdot}001$), therefore indicating an important influence of the background; even so, allelic differences in the *Adh* locus produced significant viability differences other than those due to genetic background.

Application of Scheffé's method (Sokal and Rohlf, 1981) to the mean values of combined two-loci genotypes revealed than the means corresponding to *FFFF*, *FSFF* and *SSFF* genotypes are significantly ($P < 0{\cdot}05$) higher than the other. That is, the *Adh-F/Adh-F* homozygotes exhibit the highest viability in all genotypic combinations with the *αGpdh* locus.

Table 7 Egg-to-adult viability under competitive density: Mean numbers of adults emerged

αGpdh Genotypes	*Adh* Genotypes			*αGpdh* Genotypes
	FF	*FS*	*SS*	
FF	92·7	84·7	87·8	88·4
FS	91·8	86·2	85·3	87·8
SS	91·9	88·0	88·3	89·4
	92·1	86·3	87·2	

Mean value of the *FSSF* genotype: $Y = 88{\cdot}1$.

Rate of development under near-optimal density. The mean numbers of days for egg-to-adult development range from 10·83 (*SSFS*) to 10·99 (*FFFF*). Neither the *αGpdh* nor the *Adh* loci exert significant differential influence on this component. The same for the interaction between the loci.

Rate of development under competitive density. Table 9 shows the mean numbers of days for egg-to-adult development. The factorial analysis of variance (table 10) revealed a significant ($P < 0{\cdot}01$) differential influence of the *αGpdh* locus on this component. Under competitive density, *αGpdh-S/αGpdh-S* homozygotes have a greater rate of development than *FS* or *FF* genotypes. No significant differential influence could be detected

Table 8 Egg-to-adult viability under competitive density: Factorial analysis of variance

Source of variation	Degrees of freedom	Sums of squares	Mean squares	*F*	*P*<
Locus *αGpdh*	2	35·13	17·56	0·26	ns
Locus *Adh*	2	583·65	291·82	4·37	0·05
Interaction	4	49·26	12·32	0·18	ns
Within genotypes	99	2697·15	27·24		
Synthetic strains	18	1201·35	66·74	3·61	0·001
Replicates	81	1495·79	18·47		

Table 9 Rate of development under competitive density: Mean numbers (in days) for egg-to-adult development

αGpdh Genotypes	*Adh* Genotypes			*αGpdh* Genotypes
	FF	*FS*	*SS*	
FF	11·95	12·08	11·99	12·01
FS	11·77	11·88	11·84	11·83
SS	11·55	11·72	11·90	11·72
	11·76	11·89	11·91	

Mean value of the *FSSF* genotype: $Y = 11{\cdot}75$.

either for the *Adh* locus nor for the interaction between the loci. The variation among synthetic strains of the same genotype is significant ($P < 0{\cdot}05$), indicating an influence of the background on this component; even so, the *αGpdh* locus influence produces significant differences other than those due to genetic background.

The Scheffé's procedure revealed that the *SSFF* genotype has a significantly ($P < 0{\cdot}05$) higher rate of development than the other genotypes. On the other hand, the *FFFF*, *FFFS* and *FFSS* genotypes have a significantly ($P < 0{\cdot}05$) lower rate of development than the other; that is, homozygosity for *F* allele in locus *αGpdh* decreases the rate of development in all the genotypic combinations with the *Adh* locus.

Mating capacity of males. The mean numbers of inseminated females during 24 hours range from 7·2 (by males from *FSFF* genotype) to 8·3 (*FFFS*). Neither the *αGpdh* nor the *Adh* loci exert significant differential influence on this component. The same applies for the interaction between loci.

DISCUSSION

Fitness differences among single-locus genotypes

The number of isofemale strains involved in each final synthetic strain permit us to examine the genotypic fitnesses of studied loci with a genetical background representative of the natural population. Nevertheless, the small number of effective generations in the crosses to obtain the final synthetic strains cannot confirm whether the observed fitness differences are due to the *αGpdh* and *Adh* loci or to others associated in linkage disequilibrium with them. But, in this case, preserved non-random associations would come from nature and, therefore, their effects would be due to factors also operating there.

αGpdh locus. We have found significant differential influence of this locus on the rate of development in competitive conditions at high density. The *SS* homozygotes show a rate of development in these conditions higher than heterozygotes and *FF* homozygotes ($SS > FS > FF$). This is essentially in agreement with Cavener (1983). Moreover, Charles-Palabost (1982) finds that in quasi-optimal density, at 18°C as at 25°C, the viabilities of the three genotypes are statistically different ($FS \geqq FF > SS$), while the relation becomes ($SS \geqq FS > FF$) under larval competition. These results demonstrate that the temperature variation alone cannot explain the maintenance of *αGpdh* polymorphism, while the variation in density is able to reverse the relative fitness of the two homozygous genotypes. Thus, the larval density is a factor which may account for the persistence and clinal distribution of *αGpdh* polymorphism.

Adh locus. We have found significant influence of this locus on egg hatchability with a selective disadvantage of heterozygotes.

We have also found significant influence on egg-to-adult viability under competitive density. Our results agree with those of the other authors that differences can only be observed under special conditions (Morgan, 1975; Watanabe and Watanabe, 1977; Van Delden *et al.*, 1978; Serradilla and Ayala, 1983) and that *FF* homozygotes are more viable than *SS* ones (Morgan, 1975; Watanabe and Watanabe, 1977; Van Delden *et al.*, 1978) although Serradilla and Ayala (1983) did not find a significant difference between them.

Table 10 Rate of development under competitive density: Factorial analysis of variance

Source of variation	Degrees of freedom	Sums of squares	Mean squares	*F*	*P* <
Locus *αGpdh*	2	1·4718	0·7359	6·04	0·01
Locus *Adh*	2	0·5123	0·2561	2·10	ns
Interaction	4	0·4166	0·1041	0·85	ns
Within genotypes	99	7·1953	0·0727		
Synthetic strains	18	2·1932	0·1218	1·97	0·05
Replicates	81	5·0021	0·0618		

Table 11 Viabilities (percentage) of the three genotypes at *Adh* locus

Genotypes	Egg-to-larva (1)	Egg-to-adult[a] (1)	Egg-to-adult[b] (1)	Larva-to-adult[a] (2)	Larva-to-adult[b] (2)
FF	93·0	92·5	92·1	99·46	99·03
FS	90·6	90·5	86·3	99·89	95·25
SS	93·4	90·0	87·2	96·36	93·36

[a] Under near-optimal density.
[b] Under competitive density.
(1) Estimated in the experiment.
(2) Deduced from estimated.

Given the diversity of conditions, these coincidences are noteworthy. The relation between *FS* and homozygotes is more variable according to the authors.

The developmental time of *FF* genotype is shorter than *SS* (Van Delden and Kamping, 1979) and *Adh-F* frequency increases under selection for fast developmental *FF* being the fittest genotype (Knibb *et al.*, 1987).

Egg-to-adult viability can be considered as having two components: egg-to-larva and larva-to-adult viabilities. Egg-to-larva (egg hatchability) and egg-to-adult viabilities were independently measured. To deduce the larva-to-adult ratios among the three genotypes we assume no influence of density on egg hatchability, at least, for densities utilised here.

Since *FS* genotype presents the lowest egg-to-larva viability, the absence of significant differences among egg-to-adult viabilities of the three genotypes under near-optimal conditions is only explainable if the *FS* have, under these conditions, higher larva-to-adult viability than both homozygotes (table 11). It seems that egg-to-larva viability would be better adjusted in homozygotes although the heterozygotes would have higher larva-to-adult viability under near-optimal conditions.

Under competitive conditions, at high density, the *FF* homozygotes are more viable than the two other genotypes. The *FF* superiority with respect to heterozygotes could be due to the higher egg hatchability but we can see (table 11) that *FF* also has greater larva-to-adult viability.

The effects of *αGpdh* and *Adh* loci have been assayed in each experimental test of fitness components and we have found a significant influence of *αGpdh* only on the rate of development and of *Adh* only on viability. Effects of *αGpdh* locus on viability (Charles-Palabost, 1982) and *Adh* on rate of development (Van Delden and Kamping, 1979; Knibb *et al.*, 1987) have been found by other authors examining only a single locus. Given the frequent association between the alternative alleles from *αGpdh* and *Adh* loci (table 4), if the samples come from populations with similar frequencies to Los Areneros population and if the combinations with the other locus are not ramdomised because only a single locus is taken into consideration, our results suggest that the effects on viability attributed to *αGpdh* locus might be explained by the effect of *Adh*, since 95 per cent of the *αGpdh-S/αGpdh-S* homozygotes are *SSFF*. In the same way, the effects of *Adh* locus on rate of development might be due to the effect of *αGpdh* since 89 per cent of the *Adh-S/Adh-S* homozygotes are *FFSS*.

Two-loci combined genotypes

For egg-hatchability one can observe (table 5) that *Adh* heterozygosity gives the lowest three values in all genotype combinations with the other locus.

For the egg-to-adult viability at competitive density, the genotypes with homozygosity *FF* at the *Adh* locus (*SSFF*, *FSFF* and *FFFF*) exhibit values significantly higher than the other. The greatest viability of the *Adh-F* allele in homozygosity is shown in all combinations with the other locus alleles. On the other hand (and in the hypothesis that the Los Areneros population is under somewhat competitive conditions, at least when the sample was taken), it seems that viability would be the most decisive component in maintaining frequencies in the population, because all three combined genotypes with the greatest viability are the most frequent in the population (tables 4 and 7).

Contrary to what happens with viability, the influence of the rate of development on gene frequencies in the population seems to be rather scarce or, at least, indirect. Although the most frequent genotype in the population (*SSFF*) is the one with the significantly fastest rate of development, two of the genotypes showing a low rate of development (*FFFF* and *FFFS*) have a rather high

frequency and only one (*FFSS*) is rare (tables 4 and 9). It is not easy to attribute relative selective value to fast or slow development: either fast or slow development can be advantageous depending on how conditions vary in time and space.

On the other hand, the rather high frequency of the *FFFS* genotype in the natural population is not in accordance with its viability and rate of development, as both are the lowest ones (tables 7 and 9). The association between *FS* gametes and In(2L)t in the Los Areneros natural population is almost complete (Corrales, 1982) and therefore the greater part of the *FFFS* individuals are heterozygous for In(2L)t. Watanabe and Watanabe (1977) have demonstrated that In(2L)t heterokaryotypes have low viability but high productivity, which can increase their frequency in populations.

Polymorphism at the αGpdh and Adh loci in natural populations

It can be stated that, at least under certain conditions, fitness differences exist between individuals having different genotypes. But it is necessary to explain the persistence in the population of lower fitness alleles and genotypes, that is, to explain how the fitness relations among the genotypes were such as to produce a stable equilibrium of allele frequencies (Lewontin, 1974). Since under near-optimal density no significant differences appear, contrary to what occurs under competitive density, this could be one of the mechanisms in maintaining polymorphisms at the *αGpdh* and *Adh* loci.

On the other hand, the *αGpdh-F/αGpdh-F* homozygote persists in the population due to the great viability of the *FFFF* genotype and to the high productivity of *FFFS*. Moreover, the *αGpdh-F* allele is maintained in the *FSFF* genotype which has a great viability due to its *FF* homozygosity for the *Adh* locus.

The *Adh-S/Adh-S* homozygote persists almost exclusively in the *FFSS* genotype because 89 per cent of the *Adh-S/Adh-S* genotypes are *FFSS* (table 3). The *Adh-S* allele also persists in the *FFFS* genotype. Productivity of In(2L)t determines the frequencies of the *FFSS* and *FFFS* genotypes.

In this way, under competitive conditions, the *Adh-F* allele seems to be the main element determining frequencies at the *Adh* locus through its influence on viability. It determines to a considerable extent the allele frequencies at the *αGpdh* locus through its genotypic combinations with it. These last frequencies are modulated by the *αGpdh-S* allele influence on the rate of development. This is in accordance with the hypothesis (Oakeshott *et al.*, 1982) that for *αGpdh*, the balancing selection is more important and clinal variation in the relative fitness of the homozygotes less important than for *Adh*. This could explain the frequency levels at both loci found in the Los Areneros population and, in general, those of Europe (Oakeshott *et al.*, 1982; Charles-Palabost, 1984; Izquierdo, 1987). That is, intermediate frequencies of the *αGpdh-F* and *αGpdh-S* alleles and very high frequency of the *Adh-F* allele.

In most of the non-European populations the more frequent alleles are *αGpdh-F* and *Adh-S* (Oakeshott *et al.*, 1982; Charles-Palabost, 1984), their frequencies increasing towards the Equator. These alleles are associated with In(2L)t whose frequency also increases towards the Equator (Knibb, 1982 and references therein) although it seems that these latitudinal clines are not maintained exclusively by temperature (Malpica and Vassallo, 1980; Knibb, 1982; Oakeshott *et al.* 1982, 1984*b*; Kohane and Parsons, 1986).

It can be thought that allele frequencies at both loci persist in different populations by balacing equilibrium between fitness of alleles alone and, on the other hand, fitness of In(2L)t, since the alleles having more fitness are *αGpdh-S* and *Adh-F* according to their respective activity levels (Cluster *et al.*, 1987), while In(2L)t is associated with the *αGpdh-F* and *Adh-S* alleles.

So, towards the Equator we can expect the prevalence of near-optimal conditions (high and uniform temperature, abudance of food, abundance and diversity of laying places, etc.). Being so, the fitness of the *αGpdh-S* and *Adh-F* alleles would not appear and, on the contrary, fitness of In(2L)t increases its frequency and therefore those of the *αGpdh-F* and *Adh-S* alleles associated with it. On increasing the latitude, competitive conditions or, in general, restrictive conditions (lower and more variable temperatures, scarcity of food, scarcity of laying places, etc.) would prevail and the *αGpdh-S* and *Adh-F* alleles having greater fitness in these conditions will increase their frequencies decreasing that of In(2L)t and the associated alleles.

This is in accordance with the observation (Izquierdo, 1987) that in the majority of natural populations, high or low relative frequency of alternative alleles of both loci (*αGpdh-F* and *Adh-S* or *αGpdh-S* and *Adh-F*) coincide. That could also explain the close association between In(2L)t and *αGpdh-F* and *Adh-S* alleles at low latitudes (Knibb, 1983; Inoue *et al.*, 1984). However the In(2L)t clines is insufficient to explain the *αGpdh* and *Adh* clines over relatively high latitudes

(Voelker *et al.*, 1978; Oakeshott *et al.*, 1984*b*; Anderson *et al.*, 1987) where In(2L)t is rare.

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