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Fitness of wild-caught *Drosophila melanogaster* females: allozyme variants of GPDH, ADH, PGM, and EST

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Abstract

We have collected several hundred *Drosophila melanogaster* flies (near Davis, California), isolated them individually, without anesthesia, at the collecting site, and estimated the fitness components of the wild-caught females under different environmental conditions. The fitness parameters measured are fecundity, oviposition rate, and productivity (egg-to-adult viability, development rate, and number of progeny). The environmental variables are two temperatures (22°C and 28°C) and two densities ('scant' and 'crowded'). After the fitness measurements are completed for each individual female, its genotype is determined at four loci encoding enzymes: GPDH and ADH, located on chromosome II; and PGM and EST-C, located on chromosome III. Density has a large significant effect on productivity; temperature has significant effects on fecundity, oviposition rate, and development rate. The experiments show that allozyme polymorphisms are associated with selection effects. Fitness differences between allozyme genotypes occur for all fitness components, except oviposition rate. But which genotype is superior depends on the environmental conditions; heterozygotes exhibit higher fitness than homozygotes in a number of cases, but inferior in others. A unique feature of the present experiments is that the experimental flies are wild-caught females rather than laboratory-bred individuals.

Introduction

Studies of natural populations in a great variety of organisms have shown that allozyme polymorphisms are ubiquitous, with two or more alleles found at nontrivial frequencies in a substantial fraction of all gene loci (e.g. Wright, 1978; Nei & Graur, 1984; Nevo, Beiles & Ben-Shlomo, 1984; Avise, 1994). The evolutionary significance of much of this variation remains, however, unclear. A main issue is whether most enzyme polymorphisms are the result of mutation and genetic drift of selectively equivalent alleles (the neutralist position) or whether most enzyme polymorphisms are maintained by some form of balancing selection (the selectionist position).

A number of laboratory investigations have attempted to elucidate the issue by ascertaining whether individuals with different allozyme genotypes at a specific locus exhibit different fitnesses under one or other set of environmental conditions. Studies with *Drosophila* species have manifested cases of overdominance (Marinkovic & Ayala, 1975a,b; Peng, Moya & Ayala, 1991), frequency-dependence (Kojima & Yarbrough, 1967; Kojima & Tobari, 1969; Snyder & Ayala, 1979a), overcompensation (Tosic & Ayala, 1980, 1981; Peng, Moya & Ayala, 1991), and a host of other selection modes (e.g. Serradilla & Ayala, 1983a,b; Oudman et al., 1994; Oakeshott et al., 1994). An interesting observation is that when different fitness components (such as viability, fertility, and rate of development) are assayed, it is often the case that no particular genotype is best for all components; rather the relative fitnesses of genotypes are reversed when different parameters are considered or when they are measured in different environmental conditions (Marinkovic & Ayala, 1975a,b; Tosic & Ayala, 1980; Peng, Moya & Ayala, 1991).

The laboratory investigations of *Drosophila* are typically carried out with the progenies of individual caught females that have been inbred, in one way or

Table 1. Genetic polymorphism at four enzyme loci in a natural population of *Drosophila melanogaster* near Davis, California

Locus	Allele frequency									H–W	Heterozygote frequency		
	N	1	2	3	4	5	6	7	8		Observed	Expected	*P*
Gpdh	289	0.822	0.159	0.019						< 0.05	0.225	0.299	< 0.05
Adh	419	0.667	0.333							< 0.05	0.499	0.444	< 0.05
Pgm	417	0.835	0.106	0.040	0.010	0.006	0.002	0.001	0.001	NS	0.276	0.291	NS
Est-C	402	0.687	0.300	0.007	0.006					NS	0.411	0.439	NS

N is number of individuals assayed. Alleles are listed in order of decreasing frequency in the Davis population. H–W gives the χ^2 probability that the observed genotype frequencies are in Hardy–Weinberg equilibrium. *P* is the χ^2 probability for the agreement between the observed and expected (calculated according to Hardy–Weinberg equilibrium) frequency of heterozygotes. NS, not significant. Alleles 1 and 2 in this table correspond in the other tables to *F* and *S* for *Gpdh* and *Adh* and to 100 and 102 for *Pgm* and *Est-C*.

another, for the purpose of obtaining true-breeding cultures with respect to the allozymes of interest. Each particular allozyme genotype is often represented in the experiments by several lines, each isolated from a different wild-caught female. The averages among lines with a given genotype are used to represent the fitness effects of that genotype over the different genetic backgrounds (linkage disequilibrium) with which it happens to be associated in the laboratory lines. In addition, the fitness parameters are often measured on the progenies of crosses between two lines with identical allozyme genotype, so as to neutralize the inbreeding effects created during the isolation of the lines. Although these practices seek to alleviate the effects associated with the laboratory process of extraction of desired genotypes, it remains the case that inbreeding, linkage disequilibrium, and laboratory-dependent selection impact to unknown extent fitness studies with laboratory-bred individuals. It would seem desirable to study the fitness of the wild-caught flies rather than in their inbred progenies, in order to diminish these biases.

In the present paper we report our efforts to determine the fitness of wild-caught females by investigating directly their fecundity, oviposition rate, and productivity; this third parameter is measured by the egg-to-adult viability, number of adult progeny, and rate of development of their F_1 progenies. The experiments are performed in different environmental conditions: two temperatures and, for the number of progeny and their development rate, also at two densities. Fitness parameters are measured for genotypes at four different loci, two on the second chromosome (*Gpdh* and *Adh*) and two on the third chromosome (*Pgm* and *Est-C*). An unavoidable handicap of these studies is uneven replication, since the allozyme genotypes can only be determined after the fitnesses have been measured, when the wild-caught can be sacrificed. The positive side of this handicap is that the different genotypes are represented in the experiments at the approximate frequencies at which they exist in nature. Still another handicap is that only the most common genotypes at each locus can be studied. Some genotypes are represented by very few females in the samples, so that statistical ascertainment is impossible for these rare genotypes. Thus, at each locus only two genotypes are analyzed, but these account for 87–98% of all individuals, depending on the locus.

Materials and methods

Collection

Wild *Drosophila melanogaster* were collected in an apple orchard near Davis, California, by net sweeps over fallen apples and over banana baits set 10–20 meters apart from one another. About two thirds of the numerous flies collected were *D. simulans* that were discarded; most others were *D. melanogaster*. Females and males were, without anesthesia, immediately separated to avoid any matings after collection, and the females were individually placed, in separate cultures, several hundred in total. Standard *Drosophila* medium (cornmeal and molasses, with a few drops of a very diluted bakers' yeast solution) was used in these cultures and throughout the experiment; the medium used for egg collecting was blackened with vegetable charcoal to facilitate egg counting.

Experimental design

Five fitness components were estimated individually for each wild-caught female: (1) *fecundity*, number of eggs laid per day over 5 days; (2) *oviposition rate*, mean egg-laying time (estimated by multiplying the number of eggs laid the first day by 1, those laid the second day by 2, etc. and dividing the sum by the total number of

Table 2. Three components of fitness in wild-caught *D. melanogaster* females at two temperatures, as a function of genotype

Component	Temperature	GPDH		ADH		PGM		EST	
		F/F	F/S	F/F	F/S	100/100	Heterozygotes	100/100	100/102
Fecundity	22°C	18.6 ± 3.8 (34)	21.8 ± 5.1 (20)	13.9 ± 3.1 (26)	24.6 ± 4.6 (28)*	21.7 ± 3.7 (40)	14.6 ± 4.5 (17)	19.0 ± 3.5 (30)	20.4 ± 5.4 (23)
(number of eggs)	28°C	26.2 ± 5.2 (29)	36.0 ± 6.5 (19)	31.8 ± 6.2 (23)	29.3 ± 5.2 (29)	28.9 ± 4.4 (41)	33.3 ± 8.9 (12)	30.3 ± 6.2 (25)	29.2 ± 5.7 (21)
Oviposition rate	22°C	3.52 ± 0.15 (31)	3.14 ± 0.19 (17)	3.62 ± 0.18 (22)	3.36 ± 0.13 (27)	3.32 ± 0.14 (39)	3.63 ± 0.18 (12)	3.50 ± 0.17 (28)	3.27 ± 0.17 (19)
(days)	28°C	2.90 ± 0.14 (25)	3.16 ± 0.16 (19)	2.79 ± 0.13 (19)	3.12 ± 0.14 (28)	3.07 ± 0.10 (37)	2.78 ± 0.28 (11)	3.01 ± 0.12 (24)	2.96 ± 0.19 (18)
Viability	22°C	0.73 ± 0.05 (31)	0.76 ± 0.07 (17)	0.72 ± 0.07 (22)	0.78 ± 0.05 (27)	0.74 ± 0.05 (39)	0.73 ± 0.08 (12)	0.72 ± 0.05 (28)	0.81 ± 0.07 (19)
(frequency)	28°C	0.77 ± 0.05 (25)	0.79 ± 0.06 (19)	0.85 ± 0.04 (19)	0.73 ± 0.05 (28)	0.77 ± 0.04 (37)	0.86 ± 0.03 (11)*	0.76 ± 0.05 (24)	0.81 ± 0.04 (18)

Number of replications is given in parentheses.
*The difference between the two genotypes is statistically significant, $P < 0.05$.

eggs, which yields a mean of 3 if an equal number of eggs are laid each of the 5 days); (3) *viability*, frequency of eggs that develop into adults; (4) *number of progeny*, number of F_1 individuals produced per female; (5) *development time*, mean number of days from egg to adult (estimated by counting daily the number of adults emerged during the previous 24 h in each vial).

The wild-caught females were allowed to rest for 24 h after collection, and then treated in two different ways. Some were individually placed in a 30 ml (8.5 cm × 2 cm) glass vial with a plastic spoon covered with blackened medium. The spoon was replaced daily for 5 days: and the eggs counted and transferred to 30 ml vials with standard medium, in groups of up to 20 eggs per vial, all from the same female. All five fitness components were estimated for these flies. With respect to two productivity components (F_1 progeny number and development time), this set of females is considered the 'scant' density group, since no more than 20 larvae develop in each culture. A total of 128 wild-caught females were included in this group.

A second group of females were each placed in a 250 ml culture bottle with standard medium and allowed to lay eggs for 5 days. Only two productivity components (F_1 progeny number and development rate) were estimated for these females. This condition is called the 'crowded' density. This group consisted of 300 wild-caught females.

All fitness components were measured at two temperatures, 22°C and 28°C, with half the cultures from each group placed at each temperature. All handling of the experimental flies, until the time when they were frozen for electrophoresis, was done without anesthesia.

Electrophoresis

Four gene loci were assayed, two located on the second chromosome and two on the third chromosome, coding for the following enzymes: glycerol-3-phosphate dehydrogenase (GPDH at II, 17.8), alcohol dehydrogenase (ADH at II, 50.1), phophogluconate mutase (PGM at III, 43.4) and esterase C (EST at III, 47.7). The wild-caught females were frozen at −80°C immediately after completing their 5 egg-laying days, and were later assayed following standard procedures of horizontal starch electrophoresis (Ayala et al., 1972).

The two most common genotypes observed (one homozygous and the other heterozygous) accounted for 93% of the total for GPDH, 92% for ADH, and 87% for EST. Only the two most common genotypes are included in the analyses of these three loci; the few females exhibiting the rarer genotypes were insufficient for statistical evaluation. In the case of PGM, eight different alleles and 14 genotypes appeared in our sample, but the homozygotes for the most common allele accounted for 70% of the sample, whereas the heterozygotes for all alleles accounted for 28% of the sample. Two 'genotypes' are considered for PGM, the most common homozygote and a group consisting of all heterozygotes.

Statistical analysis

All statistical tests are made using the BMDP statistical software package (copyright Regents of the University of California), programs 3D, 7D and 2V.

One-way analyses of variance and Student's *t*-tests are used to compare the fitness of the two genotypes at each locus for each fitness component and each set of environmental conditions. In the case of the two productivity components, male and female progenies are analyzed separately as well as combined. We used Levene's *F* (Levene, 1960) to ascertain whether the variances are equal. When they are equal we used paired ('pooled') *t*-tests; when they are not, we used unpaired ('separated') *t*-tests. For the analyses of variance we used the tests of Welch (1938) and Brown and Forsythe (1974). These two tests as well as the *t*-tests all yield similar results, with only very trivial differences.

The *t*-tests were made without transformation of the data. For the analyses of variance, the square root was used for female fecundity and number of progeny produced, the natural logarithm for oviposition rate, the arcsine for viability, and the reciprocal for development rate. Each gene locus is separately analyzed.

We performed two-way analyses of variance, carried out for all fitness components. Three-way analyses were possible only for two productivity components, number of progeny and development rate (these components include density as a third variable, in addition to temperature and genotype). We report the three-way analyses only for these two components, but note cases where the two-way analyses indicate statistical significance not uncovered by the three-way ANOVA. For fecundity, oviposition rate, and viability, we provide the results of the two-way analyses of variance.

Results

Table 1 gives the allele frequencies at the four allozyme loci. There are two common alleles at each locus, accounting for 94–100% of the total. Similarly, only two genotypes appear in the samples in nontrivial numbers: the homozygotes for the most common

Table 3. Mean parameter values and standard error for the fitness components

Fitness component	Scant		Crowded	
	22°C	28°C	22°C	28°C
Fecundity	19.3 ± 1.3 (57)	30.6 ± 1.1 (53)	–	–
Oviposition rate	3.42 ± 0.06 (51)	2.97 ± 0.05 (48)	–	–
Viability	0.75 ± 0.01 (51)	0.79 ± 0.02 (48)	–	–
Number of progeny				
Females	8.2 ± 0.5 (57)	14.5 ± 0.5 (53)	38.3 ± 1.7 (120)	34.3 ± 1.9 (115)
Males	8.3 ± 0.7 (57)	12.3 ± 0.4 (53)	37.5 ± 1.2 (120)	33.6 ± 1.7 (115)
All	16.5 ± 1.2 (57)	26.8 ± 0.9 (53)	75.8 ± 2.9 (120)	68.9 ± 3.6 (115)
Development time				
Females	12.38 ± 0.06 (47)	9.11 ± 0.02 (46)	14.47 ± 0.05 (110)	11.70 ± 0.07 (94)
Males	12.83 ± 0.08 (47)	9.40 ± 0.03 (46)	14.68 ± 0.05 (110)	11.86 ± 0.07 (94)
All	12.55 ± 0.07 (47)	9.24 ± 0.02 (46)	14.57 ± 0.05 (110)	11.77 ± 0.07 (94)

The standard errors are calculated from the variance among the eight genotype means. Sample size (in parentheses) is the number of wild-caught females assayed for each parameter.

allele and the heterozygotes for the two common alleles. Fitness components are hereafter reported only for these two common genotypes, except for *Pgm* where all heterozygotes have been combined. The genotype frequencies at the two second-chromosome loci significantly depart from the Hardy–Weinberg expectations, owing to a deficiency of heterozygotes at *Gpdh*, but an excess at *Adh*.

Table 2 gives, for the two common genotypes at each locus, the results for three fitness components. The measurements were made for each wild-caught female. The genotype of the female was then determined by electrophoresis and the data for all females with a given genotype combined. The electrophoretic genotype was also determined for at least 10 F_1 progeny from each female for the purpose of ascertaining the genotype of the father, with the original intention of using 'mating type' and 'male mating capacity' as additional fitness components in our analysis. It turns out, however, that about 50% or more of the progenies are fathered by more than one male. This high incidence of 'concurrent multiple paternity' is of great interest in itself (Ochando, Reyes & Ayala, 1996), but makes impossible the intended analysis of the paternal contribution to fitness.

Fecundity is measured as the average number of eggs laid by one female over 5 consecutive days (days 2–6 after capture). The females lay consistently and substantially more eggs (59%, on average) at 28°C than at 22°C (Table 3), although 22°C is considered within the optimal temperature range for *D. melanogaster*, and 28°C marginal. The differences between the two temperatures are very heterogeneous between loci and between the two genotypes of each locus. The two largest differences and the two smallest ones occur at ADH and PGM, but the larger difference is between the two homozygotes at ADH (an increase by 129%), and between the two heterozygotes at PGM (increase by 128%); the differences in fertility between the two temperatures are only 19% between the two ADH heterozygotes, and only 33% between the two PGM homozygotes.

Oviposition rate is calculated so that the mean is 3 days if the same number of eggs are laid in each day. At 28°C, the females lay on the average more eggs during the early days than later, whereas the opposite is true at 22°C; the average difference between the two temperatures is 0.55 days (Table 3). The greatest differences occur between the ADH homozygotes as well as between the PGM heterozygotes, 0.83 days in each case. These are the same two genotypes that exhibit the largest between-temperature differences for fecundity; in both loci, these genotypes perform much better at the higher than at the lower temperature.

Viability is measured as the percent of eggs that develop into adults. All but two genotypes exhibit the same viability at both temperatures (i.e. the differences are small and not significant). The two exceptions are the same genotypes noted above; namely, the ADH homozygotes and the PGM heterozygotes. In both cases again, the genotypes perform better at the higher temperature; the viability difference is in each case 0.13, which represents an 18% increase at 28°C over the viability at 22°C.

The results for the number of F_1 individuals produced and for development time are given in Tables 4 and 5. These parameters are measured at two densities,

Table 4. Productivity (F_1 progeny number and development rate) of wild-caught *D. melanogaster* females at two larval densities and two temperatures for two second-chromosome enzyme loci

Component and conditions		GPDH		ADH	
		F/F	*F/S*	*F/F*	*F/S*
Number of progeny					
22°C Scant	Females	7.9 ± 1.8 (34)	9.1 ± 2.2 (20)	5.9 ± 1.5 (26)	10.5 ± 2.1 (28)*
	Males	7.6 ± 1.9 (34)	9.7 ± 2.6 (20)	5.5 ± 1.5 (26)	10.6 ± 2.2 (28)*
	All	15.5 ± 3.5 (34)	18.8 ± 4.6 (20)	11.4 ± 2.7 (26)	21.1 ± 4.2 (28)*
22°C Crowded	Females	42.0 ± 1.9 (94)	29.5 ± 5.3 (17)**	41.1 ± 3.1 (41)	39.6 ± 2.3 (69)
	Males	40.0 ± 1.9 (94)	31.5 ± 5.3 (17)	38.9 ± 3.1 (41)	39.4 ± 2.4 (69)
	All	81.9 ± 3.7 (94)	60.9 ± 10.4 (17)*	80.0 ± 6.0 (41)	79.0 ± 4.5 (69)
28°C Scant	Females	12.7 ± 2.6 (29)	16.8 ± 3.1 (19)	14.8 ± 3.0 (23)	14.1 ± 2.5 (29)
	Males	10.6 ± 2.3 (29)	14.3 ± 3.2 (19)	13.4 ± 2.9 (23)	11.5 ± 2.3 (29)
	All	23.3 ± 4.9 (29)	31.1 ± 6.0 (19)	28.2 ± 5.7 (23)	25.6 ± 4.8 (29)
28°C Crowded	Females	32.8 ± 2.9 (80)	34.5 ± 6.1 (27)	40.4 ± 4.1 (49)	29.9 ± 3.6 (60)*
	Males	32.2 ± 2.9 (80)	36.9 ± 6.0 (27)	38.8 ± 4.2 (49)	28.9 ± 3.4 (60)*
	All	65.0 ± 5.8 (80)	76.4 ± 11.8 (27)	79.2 ± 8.1 (49)	58.8 ± 6.9 (60)*
Development time (days)					
22°C Scant	Females	12.24 ± 0.14 (25)	12.59 ± 0.22 (14)	12.52 ± 0.18 (16)	12.18 ± 0.15 (24)
	Males	12.74 ± 0.20 (24)	13.01 ± 0.25 (15)	12.67 ± 0.25 (18)	12.87 ± 0.19 (23)
	All	12.47 ± 0.16 (28)	12.72 ± 0.21 (16)	12.49 ± 0.18 (20)	12.51 ± 0.17 (26)
22°C Crowded	Females	14.58 ± 0.06 (88)	14.15 ± 0.23 (13)*	14.43 ± 0.12 (37)	14.55 ± 0.08 (64)
	Males	14.75 ± 0.07 (87)	14.40 ± 0.27 (13)	14.65 ± 0.13 (37)	14.75 ± 0.09 (63)
	All	14.66 ± 0.06 (88)	14.28 ± 0.25 (13)*	14.55 ± 0.12 (37)	14.65 ± 0.08 (64)
28°C Scant	Females	9.09 ± 0.05 (22)	9.09 ± 0.09 (18)	9.16 ± 0.08 (17)	9.10 ± 0.07 (25)
	Males	9.39 ± 0.10 (23)	9.35 ± 0.08 (16)	9.40 ± 0.12 (18)	9.42 ± 0.07 (24)
	All	9.22 ± 0.06 (24)	9.20 ± 0.07 (18)	9.27 ± 0.07 (19)	9.23 ± 0.07 (26)
28°C Crowded	Females	11.68 ± 0.17 (66)	11.35 ± 0.25 (21)	12.01 ± 0.20 (43)	11.50 ± 0.19 (46)
	Males	11.91 ± 0.15 (65)	11.56 ± 0.24 (21)	12.22 ± 0.20 (43)	11.65 ± 0.16 (44)*
	All	11.75 ± 0.16 (66)	11.44 ± 0.24 (21)	12.10 ± 0.20 (43)	11.55 ± 0.18 (46)*

Number of replications is given in parentheses.
The number of progeny is per female.
*,**The difference between the two genotypes is statistically significant, $P < 0.05, 0.001$.

in addition to the two temperatures. The 'crowded' density includes all eggs laid by a female over 5 days in a culture bottle. The 'scant' density measures the number of F_1 individuals produced per female, at densities never higher than 20 eggs per culture vial. At the scant density, all genotypes consistently produce more progeny at 28°C than at 22°C, about 10.3 more individuals on the average, an increment of 63% (Table 3). The two largest differences occur, as with previous fitness components, between the two ADH homozygotes (11.4 ± 2.7 versus 28.2 ± 5.7 individuals, an increase of 147%) and between the two PGM heterozygotes (12.5 ± 4.2 versus 29.5 ± 8.1 individuals, an increase of 136%). This result reflects, of course, results observed above, since the number of progeny produced at the scant density results from the number of eggs laid and their viability, measured in the same cultures. The differences between temperatures are approximately the same for both sexes, although a slight excess of females over males at the higher temperature occurs for all genotypes.

Because of the experimental design, no measurements of fertilization, oviposition rate, or viability are available for the crowded density. At this density, fewer F_1 individuals are produced at 28°C than at 22°C, the opposite of what we observe at the scant density: 68.9 ± 3.6 individuals on the average at the higher temperature versus 75.8 ± 2.9 at 22°C, an increment of 11% (Table 3). The between-temperature differences are not consistent from one locus to another. For example, at the lower temperature the GPDH homozygotes are more productive (81.9 versus 65.0 individuals), whereas the heterozygotes are less productive (60.9 versus 76.4 individuals). The two genotypes that con-

Table 5. Productivity (F_1 progeny number and development rate) of wild-caught *D. melanogaster* females at two larval densities and two temperatures for two third-chromosome loci

Component and conditions		PGM		EST	
		100/100	Heterozygotes	100/100	100/102
Number of progeny					
22°C Scant	Females	9.1 ± 1.6 (40)	6.2 ± 2.1 (17)	8.1 ± 1.6 (30)	8.7 ± 2.3 (23)
	Males	9.3 ± 1.8 (40)	6.2 ± 2.3 (17)	7.1 ± 1.6 (30)	10.4 ± 2.8 (23)
	All	18.4 ± 3.3 (40)	12.5 ± 4.2 (17)	15.1 ± 3.0 (30)	19.1 ± 5.1 (23)
22°C Crowded	Females	41.0 ± 2.1 (82)	37.2 ± 3.2(38)	43.2 ± 2.5 (60)	32.8 ± 2.9 (46)**
	Males	38.6 ± 2.0 (82)	37.7 ± 3.2 (38)	41.0 ± 2.4 (60)	33.3 ± 3.0 (46)*
	All	79.6 ± 4.0 (82)	74.9 ± 6.3 (38)	84.2 ± 4.7 (60)	66.0 ± 5.7 (46)**
28°C Scant	Females	13.3 ± 2.1 (41)	16.8 ± 4.3 (12)	14.0 ± 3.0 (25)	13.6 ± 2.6 (21)
	Males	12.0 ± 2.0 (41)	12.8 ± 3.9 (12)	12.5 ± 2.8 (25)	11.7 ± 2.8 (21)
	All	25.3 ± 4.1 (41)	29.5 ± 8.1 (12)	26.5 ± 5.7 (25)	25.3 ± 5.2 (21)
28°C Crowded	Females	40.1 ± 3.1 (82)	24.5 ± 4.6 (33)**	37.3 ± 4.2 (46)	35.2 ± 4.2 (42)
	Males	37.9 ± 3.0 (82)	25.0 ± 4.9 (33)*	35.6 ± 4.0 (46)	33.4 ± 4.4 (42)
	All	77.9 ± 6.0 (82)	49.5 ± 9.5 (33)**	72.9 ± 8.1 (46)	68.6 ± 8.4 (42)
Development time (days)					
22°C Scant	Females	12.37 ± 0.16 (30)	12.29 ± 0.16 (11)	12.26 ± 0.14 (23)	12.61 ± 0.22 (15)
	Males	12.82 ± 0.18 (34)	12.70 ± 0.26 (8)	12.54 ± 0.20 (22)	13.29 ± 0.26 (16)*
	All	12.56 ± 0.15 (36)	12.41 ± 0.19 (11)	12.32 ± 0.15 (26)	12.95 ± 0.22 (17)*
22°C Crowded	Females	14.50 ± 0.07 (77)	14.53 ± 0.15 (33)	14.58 ± 0.08 (58)	14.41 ± 0.12 (37)
	Males	14.66 ± 0.08 (76)	14.78 ± 0.16 (33)	14.80 ± 0.09 (57)	14.62 ± 0.13 (37)
	All	14.58 ± 0.07 (77)	14.66 ± 0.15 (33)	14.68 ± 0.09 (58)	14.53 ± 0.12 (37)
28°C Scant	Females	9.05 ± 0.06 (32)	9.23 ± 0.12 (11)	9.04 ± 0.09 (21)	9.15 ± 0.09 (18)
	Males	9.33 ± 0.06 (33)	9.50 ± 0.10 (10)	9.31 ± 0.07 (22)	9.52 ± 0.12 (17)
	All	9.19 ± 0.05 (35)	9.35 ± 0.11 (11)	9.16 ± 0.07 (23)	9.32 ± 0.07 (18)
28°C Crowded	Females	11.64 ± 0.14 (69)	11.85 ± 0.31 (25)	11.73 ± 0.19 (41)	11.85 ± 0.25 (34)
	Males	11.83 ± 0.14 (69)	11.88 ± 0.30 (24)	11.86 ± 0.18 (40)	11.98 ± 0.25 (34)
	All	11.72 ± 0.14 (69)	11.88 ± 0.30 (25)	11.79 ± 0.18 (41)	11.90 ± 0.25 (34)

Conventions as in Table 4.

sistently perform better at the higher temperature for all previous parameters, namely the ADH homozygotes and the PGM heterozygotes, do not exhibit such difference in the crowded cultures; in fact, no between-temperature differences exist for the ADH homozygotes, whereas the PGM heterozygotes perform much better at the lower temperature (49.5 versus 74.9 individuals, a 51% increment; Table 3).

The egg-to-adult development time is, as expected, significantly less at the higher temperature, both in the scant (9.24 ± 0.02 versus 12.55 ± 0.07 days) and in the crowded cultures (11.77 ± 0.07 versus 14.57 ± 0.05 days). Development time is also significantly shorter in the scant than in the crowded cultures, a difference of 2.01 days at 22°C and of 2.53 days at 28°C (Table 3).

Tables 2, 4 and 5 indicate the cases in which the two genotypes at a given locus are significantly different from one another. Differences exist for all fitness components except oviposition rate. However, whether the heterozygotes or the homozygotes are the superior genotypes varies from locus to locus and from one fitness component to another. At GPDH the homozygotes are superior with respect to number of progeny (crowded cultures at 22°C), but inferior with respect to development rate (egg-to-adult is longer in the crowded cultures at 22°C). At ADH, the heterozygotes have much greater fecundity (22°C) and faster development rate (crowded at 28°C) than the homozygotes; with respect to the number of progeny produced, the heterozygotes also are superior at 22°C scant, but are inferior at 28°C crowded. At PGM, the heterozygotes exhibit higher viability (28°C), but produce much fewer progeny in crowded cultures (28°C). At EST, significant differences exist in number of progeny produced (22°C crowded) and development rate (22°C scant); the homozygotes exhibit higher fitness in both cases.

Table 6. Two-way analysis of variance for three fitness components of *D. melanogaster* wild-caught females

Component and locus	Parameters		
	Temperature	Genotype	Interaction
Fecundity			
GPDH	4.71*	2.01	0.79
ADH	4.50*	1.66	1.51
PGM	5.73*	0.20	1.77
EST	3.87*	0.07	0.00
Oviposition rate			
GPDH	2.32	0.02	3.02
ADH	11.54***	0.06	2.50
PGM	8.61**	0.04	3.92*
EST	4.27*	0.62	0.01
Viability			
GPDH	0.01	0.35	0.07
ADH	0.07	0.84	2.84
PGM	0.48	0.06	0.58
EST	0.09	1.80	0.43

The F values are displayed. The degrees of freedom are 1 for the numerator and, approximately, 95 (range 85–106) for the denominator.
*,**,***Statistically significant, $P < 0.05$, 0.01, 0.001, respectively.

Tables 6 and 7 summarize the results of the analyses of variance. Temperature has significant effects on fecundity and oviposition rate, but not on viability (Table 6). With respect to number of progeny and development rate (Table 7), density has consistently highly significant effects; temperature is highly significant for development rate, but not for number of progeny. There are extensive and highly significant interactions between density and temperature, and several significant interactions between genotype and density or temperature, consistent with observations made earlier. Neither number of progeny nor development time manifest a significant genotype effect in the three-way analyses of variance, but such effect appears in the two-way analyses as noted in Table 7, and is consistent with the significant effects noted in Tables 4–5. The total instances of significant differences between genotypes are 2 out of 24 comparisons in Table 2, 12 out of 48 in Table 4, and 8 out of 48 in Table 5; more instances in all cases than the 5% that would be expected by chance, using a 5% significance level.

Discussion

The distribution of allozyme polymorphisms in natural populations has been interpreted as favoring, at least in some cases, that the polymorphisms are maintained by natural selection (Nevo, Beiles & Ben-Shlomo, 1984; Gillespie, 1991; Avise, 1994). Relevant evidence for *Drosophila* populations includes the following kinds of observations (see Gillespie, 1991 for additional evidence based on rates of amino acid replacements, indices of dispersion, and other): (1) The distribution patterns of allele frequencies at a given locus are similar in different populations of the same species, including populations in different continents and in remote islands, unlikely to be sharing frequent immigrants (Ayala, Powell & Dobzhansky, 1971; Ayala et al., 1972, 1974; Ayala, Powell & Tracey, 1972; Singh, Hickey & David, 1982; Nevo, Beiles & Ben-Shlomo, 1984); (2) Similar latitudinal clines occur in different localities and even different continents (Cooke & Oakeshott, 1989; Izquierdo & Rubio, 1989; Ayala, Serra & Prevosti, 1989; Berry & Kreitman, 1993; Oudman et al., 1994); (3) Virtually identical frequency distributions not inherited from a common ancestor are shared by different species; among 14 taxa of the *D. willistoni* group, taxa in a subset (say A, B, C) share similar frequencies, while other taxa (say D, E, F) also share frequencies which are disparate to those of the previous subset; but the taxa associated within a subset vary from locus to locus (say, at a second locus A will be identical to D and C identical to F) (Ayala & Gilpin, 1974; Ayala, 1976); (4) The distribution of nucleotide polymorphisms in the proximity of a nonsynonymous substitution indicates that the allozyme polymorphism (*Adh* in *D. melanogaster*) is ancient and balanced (Kreitman & Hudson, 1991); (5) The pattern of nucleotide variation suggests that the allozyme polymorphism has resulted from a selective sweep that occurred in recent evolutionary times (*Sod*, *Est-6* and *Est-P* loci of *D. melanogaster)* (Hudson et al., 1994; Hudson, Sáez & Ayala, 1997).

Numerous laboratory investigations of the fitness effects of allozyme variation have been performed with *Drosophila*. Some have sought to determine overall fitness, while others have focused on one or several components of the life cycle. The diverse selection effects include the following:

1. Overdominance. In the case of two alleles, whenever the fitness of the heterozygotes is superior to that of the two corresponding homozygotes, a globally stable equilibrium is predicted, with the expected frequencies of the two alleles being simply related to the selective coefficients of the homozygotes. Overdominance has been demonstrated in *Drosophila* for whole chromosomes (e.g. Sved & Ayala, 1970; Mourão, Ayala & Anderson,

Table 7. Three-way analysis of variance of the productivity (F_1 progeny number and development rate) of *D. melanogaster* wild-caught females

Component, sex and locus		Parameters			Interactions			
		Density (D)	Temperature (T)	Genotype (G)	DT	DG	TG	DTG
Number of progeny								
GPDH	Females	60***	1.29	0.00	3.84*	3.07†	4.02*	0.87
	Males	64***	0.15	0.01	2.69	1.93	1.49	0.80
	All	62***	0.57	0.00	3.37	2.60†	2.99	0.77
ADH	Females	95***	0.01	0.06	9.64**	3.66	3.04	0.03
	Males	96***	0.14	0.05	7.21**	2.55	4.03*	0.00
	All	96***	0.04	0.08	8.63**	2.94	3.72*	0.02
PGM	Females	68***	0.05	2.74†	12.79***	2.79‡	0.00	3.80*
	Males	76***	0.11	3.41†	9.47**	0.80	0.04	3.30
	All	72***	0.01	3.14†	11.35***	1.95‡	0.01	3.53
EST	Females	89***	0.60	1.54†	4.84*	1.44	0.97	0.52
	Males	87***	0.04	0.84	3.61	1.35	0.04	0.98
	All	89***	0.19	1.37	4.31*	1.40	0.47	0.68
Development time								
GPDH	Females	287***	580***	0.34	32.5***	2.69‡	0.39	0.35
	Males	273***	642***	0.72	40.2***	2.35	0.58	0.12
	All	299***	643***	0.56	35.7***	2.03‡	0.28	0.22
ADH	Females	422***	686***	3.27	43.4***	0.01‡	0.73†	3.02
	Males	432***	803***	0.27	52.5***	2.60	3.03†	1.15
	All	463***	791***	1.61	47.1***	0.93	2.60†	1.59
PGM	Females	371***	604***	0.58	32.6***	0.05	0.88	0.18
	Males	283***	572***	0.26	30.9***	0.08	0.28	0.59
	All	386***	669***	0.34	36.3***	0.00	0.71	036
EST	Females	413***	684***	0.90	51.4***	1.05‡	0.05	0.40
	Males	328***	686***	3.38†‡	46.4***	4.41*	0.08	1.00
	All	431***	783***	2.93†‡	55.0***	3.53‡	0.06	0.94

The F values are displayed. The degrees of freedom for the error are, on average, 262 (range 237–337).
*,**,***Statistically significant, $P < 0.05$, 0.01, 0.001, respectively.
†Statistically significant in two-way ANOVA of genotype versus temperature, in either scant or crowded cultures, or both.
‡Statistically significant in two-way ANOVA of genotype versus density, in either scant or crowded cultures, or both.

1972; Tracey & Ayala, 1974; Wilton & Sved, 1979; Seager & Ayala, 1982; Seager, Ayala & Marks, 1982) as well as for a variety of enzyme loci in *D. pseudoobscura* (Marinkovic & Ayala 1975a,b; Snyder & Ayala, 1979a,b; Tosic & Ayala, 1980, 1981; Milosevic, Moya & Ayala, 1991) and *D. melanogaster* (Serradilla & Ayala, 1983a,b; Peng, Moya & Ayala, 1986, 1991; Lee, Hur & Kim, 1989). Often, 'marginal' overdominance (Wallace, 1959) prevails: the heterozygote is not consistently superior to both homozygotes; rather, the heterozygote is as fit as the fitter homozygote, but which of the two homozygotes is best varies from one to another fitness component or environmental variable tested, so that on the average the heterozygote is superior to either homozygote (e.g. Marinkovic and Ayala 1975a,b).

2. Frequency-dependent selection, which was already invoked by Fisher (1930, 1958) as a mechanism yielding balanced polymorphism whenever the fitness of a genotype is inversely related to its frequency. In *Drosophila*, laboratory evidence of frequency dependence was noted by Wright and Dobzhansky (1946) and Levene, Pavlovsky and Dobzhansky (1954). Frequency-dependent selection associated with mate preference was discovered by Petit (1951) in *D. melanogaster* and has been ascertained in other species of *Drosophila* and other animals (e.g. Petit & Ehrman, 1969; Ayala & Campbell, 1974). Frequency-dependent selection

may come about as a consequence of competition for heterogeneous limiting resources, in which case it may be associated with 'overcompensation': the resources are better exploited by a mixture than by a single genotype, because different genotypes exploit better different resources among those that are limiting the population; as one genotype becomes more common, its fitness decreases because it becomes increasingly dependent upon those resources that are better exploited by other genotypes (Levins, 1965; Antonovics, 1978; Tosic & Ayala, 1980). Evidence of frequency dependence and, in some cases, of overdominance has been associated with several *Drosophila* allozyme polymorphisms (e.g. Snyder & Ayala, 1979a,b; Tosic & Ayala, 1980, 1981; Nunney, 1983; Peng, Moya & Ayala, 1991).

3. Counteracting selective advantages of the genotypes with respect to different life-cycle components or environmental variables. Marinkovic and Ayala (1975a,b) noted this effect in *D. pseudoobscura*: at several enzyme loci, genotypes that, for example, yield the highest female fecundity have low viability or are disadvantaged in male mating performance. However, antagonistic pleiotropy is unlikely to maintain by itself allelic variation (Curtsinger, Service & Prout, 1994).

A distinctive feature of the present experiments relative to those just reviewed is that the experimental flies are wild-caught females rather than laboratory-bred individuals. We sought to ascertain whether the selective effects associated with allozyme polymorphisms would be detected in wild-caught flies, in which laboratory-dependent effects of inbreeding, linkage disequilibrium, and selection are minimized. The experiments manifest that environmental variables (i.e. temperature and density) are of great consequence in the fitness performance of the various genotypes. In addition, selective differences between alternate genotypes appear with respect to all fitness components examined, except oviposition rate. There is evidence of heterozygote superiority with respect to some fitness components (GPDH development rate at 22°C in crowded conditions; ADH fecundity at 22°C, number of progeny at 22°C in scant cultures, and development rate at 28°C in crowded cultures; and PGM viability at 28°C). But heterozygote superiority is not consistently observed; indeed, the homozygotes exhibit higher fitness than the heterozygotes in a number of instances (GPDH number of progeny at 22°C in crowded cultures; ADH number of progeny at 28°C in crowded cultures; PGM number of progeny at 28°C in crowded cultures; and EST number of progeny at 22°C in crowded cultures, and development rate at 22°C in scant cultures).

The experiments show that allozyme polymorphisms are associated with selection effects, but do not give much of an inkling as to how the polymorphisms are maintained. We cannot tell from the present experiments whether frequency-dependent selection or marginal heterosis occurs. What we can corroborate is the phenomenon noted above of counteracting selection effects as a function of life-cycle component; for example the GPDH heterozygotes are superior to the homozygotes with respect to rate of development, but inferior with respect to number of progeny. Counteracting effects also occur with respect to environmental variations; for example, the ADH heterozygotes produce more progeny than the homozygotes at 22°C in scant cultures, but fewer at 28°C in crowded cultures. It seems likely, therefore, that the persistence and evolution of allozyme of polymorphisms may be associated with environmental heterogeneity in space and environmental fluctuations that may interact in complex ways with different life stages. Selection may be detectable by analyzing allozyme distributions in natural populations, but it may be difficult, in most cases at least, to identify with laboratory experiments which modes of selection are impacting which allozyme polymorphisms.

We have not examined whether the allozymes are systematically associated with chromosomal inversions. If that were the case, the allozyme effects might be due to distinctive genetic arrays associated with different inversions, but the allozyme polymorphisms would, in any case, be subject to the selective consequences associated with the arrays. Even if specific associations between allozymes and chromosome inversions do not occur, it is still possible that the allozymes are in linkage disequilibrium with allelic variation at other loci. Indeed, the selection effects that we observe may not be at all the effects of the observed allozymes as such, but of other genes with which the allozymes are associated; although, in such a case as well as in the case of association with inversions, the allozyme polymorphisms would be impacted by the selective effects acting on the linked loci.

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