GENETIC EFFECTS OF FLUCTUATING TEMPERATURE IN POPULATIONS OF *DROSOPHILA MELANOGASTER*¹

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BEARDMORE and LEVINE (1963) compared populations of *Drosophila pseudo-obscura* kept under a constant temperature of 22°C with populations kept under a 24 hour sinusoidal temperature oscillation of 17° to 27°C. The offspring of the variable temperature populations had higher larval viability than the offspring of the constant temperature populations, whether measured at 17°, 22°, 27°, or in the 17°/27°C fluctuating temperature. The individuals tested had not been exposed to different environments, so the differences in viability are likely to have been a consequence of genetic differences resulting from the effects of natural selection in the two environments.

I have tried to find if this difference in fitness is a general result of temperature fluctuation by comparing the fitness of populations of *Drosophila melanogaster* living at a constant temperature of 25°C with those living under a similar sinusoidal variation of 20° to 30°C with oscillations over one day, 32 days, and 96 days. Since the generation time at 25° or under 20°/30°C daily oscillation is about 16 days, in the 32-day cycle the greater part of an individual's reproduction would be in the opposite part of the cycle from that in which it had developed, and there would be a negative correlation between temperature conditions from one generation to the next. In the 96-day cycle, a gradual selection for genotypes adapted to high temperature followed by selection for genotypes adapted to low temperature could be expected, and there would be a positive correlation between temperature conditions from one generation to the next. In the second year of the experiment, two more populations were established at constant temperatures of 20° and 29°C.

It is difficult to make an adequate measure of the relative fitness, or potential success, of populations, because the population more fit in one environment may be less fit in another. For this reason measurements were made on two components of fitness which are important in any environment—productivity and competitive ability—as well as adaptability to stress environments created by adding two kinds of salt to the normal food of the larvae. All measurements of relative fitness were made at a constant 25°C temperature so that if higher fitness

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were observed in the variable populations, their superiority would be more convincing.

A possible basis for differences in fitness would be differences in the level of genetic variability maintained by the selective pressures in the various environments. To find whether there is a relationship between genetic variability and fitness, the genetic variability in all populations was measured in three systems: genetic variance for sternopleural bristle number, diversity at the Esterase-6 locus, and lethal gene frequency.

MATERIAL AND METHODS

The populations: The progeny of nine inseminated female *Drosophila melanogaster* which were captured in a fruit warehouse in Groningen (The Netherlands) were maintained separately in bottles for seven months prior to the initiation of these experiments. Two thousand flies from these nine strains were introduced into a population cage in May 1966. From this artificial population, twelve food cups were taken each day for nine days, with one cup placed into each of twelve population cages each day. These twelve populations received a random sample of about 3000 eggs and larvae from the mixture of strains in the initiating population.

Three population cages were placed in each of four incubators. One incubator (populations C1, C2, C3) maintained a constant 25°C temperature. The second incubator (populations D1, D2, D3) underwent a regular sinusoidal temperature oscillation from 20° in the early morning to 30°C in the afternoon and back with a period of 24 hours (BEARDMORE 1960). The third incubator had regular sinusoidal oscillation of 20°–30°C over a period of 32 days (populations M1, M2, M3). The fourth incubator had a regular sinusoidal oscillation of 20°–30°C over a period of 96 days (populations T1, T2, T3). In May 1967 two additional populations were initiated with food cups from C and D populations. These populations were kept at uniform temperatures of 20° and 29°C. None of the incubators had humidity control or light. Fans kept conditions similar in all parts of each incubator.

The populations maintained a size of about 3000–6000 individuals from their initiation in May 1966 until the fall of 1967, except for a period in January 1967 when population samples were taken from Groningen to Swansea. About a thousand adult flies per population and another thousand larvae in food cups were transferred into new population cages in Swansea. For two weeks the populations were kept in the same room, then placed in a new set of four incubators on the previous temperature schedules. The monthly incubator had a malfunction shortly thereafter which killed all members of two of the three populations. M1 survived with a very low population size of several hundreds. M2 and M3 were reconstituted with egg samples taken from D1, D2, D3 and T1, T2, and T3, respectively. These substitutions appear to have had little effect on the relative performance of the M populations in fitness tests. From the fall of 1967, when papers were no longer present in the food cups, less space was available for pupae and the population size remained between 1500 and 4000 individuals.

In the long-term fluctuating environments, population size increases at low temperature and decreases at high temperature. The flies which have developed at low temperature are larger than those from high temperature. There is, of course, effectively more food available at low temperatures because the flies are metabolizing more slowly during these periods.

The relative humidity (and with this the dampness of the food) varies inversely with the temperature. Microorganisms are also affected by temperature differences, so that molds grow in the food cups more frequently at high temperature than at low. Since the variables correlated with temperature change may be more important than temperature itself, it is impossible to say that the results obtained are consequences of temperature differences alone.

Maintenance: Populations were kept in the perspex population cages described by BEARDMORE, VAN DELDEN and ALKEMA (1963). These cages are 275 × 170 × 95 mm internal dimensions, with four ventilation holes (300 cm² surface area) covered with wire mesh, and fifteen holes in the floor for food cups.

The food medium used was that of MITTLER and BENNETT (1962) with slight modifications. Two polyethylene food cups with about 11 cm² food area were placed in the population cages every third day. Fourteen of the holes were used so that by changing the cups in rotation, each cup remained in the cage for 21 days. In the event of infection by mold or bacteria, the infected cups were removed and replaced by empty cups. This schedule was followed for every population except that at a constant 29°C temperature, in which fifteen holes were used, three cups were changed every third day, and the cups remained in the cage for only 15 days.

Measures of fitness: i) *Productivity.* Estimates of productivity were made by allowing a sample of 100 females (obtained by shaking flies out of a hole in the bottom of the cage) to lay eggs in a bottle for 24 hr. About 20 males were included in the sample so that virgin females would be inseminated. Each sample of flies was kept in a fresh bottle for 24 hr prior to introduction into the laying bottle, so that the effect of the ether should wear off and so that food, crowding, and egg-laying opportunities should be similar in the period before the test. The number of emerging adults was the measure of productivity. There were nine replicates for each population in the first test period and twelve replicates in the second.

ii) *Competitive ability.* Competition against a stock of *Drosophila melanogaster* carrying the dominant markers Curly, Lobe⁴, and Plum in repulsion was carried out as described by KNIGHT and ROBERTSON (1957) with some modifications. 50–60 fertilized *Cy L⁴/Pm* females were introduced into a bottle for 24 hr. These flies were removed, and 50–60 females sampled from a cage population were put into the same bottle for 24 hr. Larval competitive ability is largely determined by differences in development time, and a head start of one day is sufficient to allow similar numbers of *Cy L⁴/Pm* adults to emerge at the same time as the wild adults. These adults were collected as virgins (in roughly equal numbers for each collection). Three days after the first collection and one day after the last, mixtures were made of 40 females of each kind and 40 males of each kind. After a period of an hour or more, to allow the effects of etherization to wear off, the males were introduced into the bottle containing the females. Mating and egg laying took place for two days before the flies were discarded. The measure of competitive ability used was the proportion of wild chromosomes (wild plus one half of the *Cy L⁴/+* and *+/+/Pm* classes divided by total adjusted emergence) in the next generation. The actual number of emerging *Cy L⁴/Pm* flies represented only half the gametes formed, since homozygous *Cy* or *Pm* zygotes are not viable, so this figure was doubled to obtain the adjusted emergence. This measure is the biological product of the mating success of the males, fertility, fecundity of females over a two-day period, and egg-to-adult survival. There were eight replicates for each population in the first test period and twelve replicates in the second.

iii) *Egg-to-adult survival in various food media:* Egg samples were collected in the population cages, on normal food to which adsorbent charcoal had been added to give the surface higher contrast with the eggs. A hundred eggs (50 in the first experiment) were picked up with a stylet probe and placed on the food surface of a bottle. The number of eggs which completed larval and pupal development on this food and emerged as adults was taken as the measure of survival. There were twelve replicates for each population.

Three kinds of food were used: the normal food, normal food plus sodium chloride (0.6M), and normal food plus calcium chloride (0.25M). About fifty percent of the larvae survived in the NaCl food, with a development period about 30% longer than on normal food. In the CaCl₂ food, survival was about thirty percent with a 50% increase in development period.

Measures of genetic variability: i) *Genetic variance for sternopleural chaeta number.* The offspring of a random sample of about 200 flies from each population were collected as virgins. Fifty males and fifty females were scored for sternopleural chaeta number and mated assortatively. Five male offspring and five female offspring (three of each in early experiments) were scored from each of the matings. The covariance of the mean number of bristles in the offspring with the midparent values provides an estimate of genetic variance for this character, since nearly all the genetic variance for sternopleural bristle number in the base population had been shown to be additive.

ii) *Gene frequency of Esterase-6 isoalleles:* Isoalleles at the Esterase-6 locus were separated by disc electrophoresis in polyacrylamide gel. Whole flies were grown in sugar solution and the solution absorbed onto bits of filter paper. The filter paper was added to a 3% stacking gel

buffered at pH 6.7–6.9. An 8–9% separating gel was buffered at pH 9.2 and the sample protein drawn through by a current of 2.5 mA. The gels were stained with Fast Blue RR and α -naphthyl-acetate for the presence of an esterase. Heterozygotes were immediately identifiable as double bands; homozygotes could be distinguished by the fraction of the distance which the band had moved between the origin and a band of tracking dye.

iii) *Lethal frequency*: The frequency of lethal genes on chromosomes 2 and 3 was measured by the standard *Cy L/Pm* technique, but with the use of a special stock, A_1B_{18} , supplied by Dr. BRUCE WALLACE. A_1B_{18} has chromosomes 2 and 3 joined by a translocation, with the dominant markers Curly, Lobe, and Ultrabithorax on one compound chromosome and Plum and Stubble on the other. Inversions prevent recombination along most of the length of 2 and 3.

Test environment: All tests were carried out in a 25°C culture room. An ideal situation would require tests at several temperatures, since differences between populations would be likely to be smallest at 25°, and the constant temperature was “home” for one set of populations. But it was necessary to choose between several tests at one temperature or one test at several temperatures, and the former strategy appeared more attractive.

Sampling from the monthly and three-monthly populations: If fitness were a function of temperature in the long-term variable environments, the results of fitness measurements would depend on the time of sampling. Ideally, samples for each test would be taken over three months. The logistics of testing made this impractical, and though samples for fitness tests were taken over an extended period, usually about 24 days, no test period was longer than six weeks.

Though population samples must be biased in containing relatively more flies or recent development, each sample will contain flies which have developed under a range of temperatures. Since the effective temperature for any one sample is diffuse, multiple samples taken over several weeks should represent average values over a considerable portion of even the three-monthly cycle.

The effects of sampling from different parts of the three-monthly cycle can be deduced from the performance of the three-monthly populations as a function of sampling temperature. Of the five characters measured, the only one for which performance might be related to sampling temperature is egg-to-adult survival on normal food, since the pattern of results in the three sampling periods matches the pattern of the mean temperatures at the time of sampling. This kind of match would be expected by chance in one measure out of three, so there is no evidence that the measured level of fitness is affected by the time of sampling. The correlation between sampling period and population performance in egg-to-adult survival on normal food is probably the result of a deterioration in the environment due to infection or overextension of food resources which happened to correspond with the one sample period at low temperature.

ANALYSIS

The data were analyzed under a random effects model, or Model II, analysis of variance. I chose to regard the three replicate populations in each environment as representing a sample of possible populations which could have been produced by the original distribution of eggs. I therefore judge the significance of the observed differences among environments by comparison with the variance among population means within environments. An alternative procedure would be to have used a fixed effects model, or Model I, analysis of variance. The significance of differences among environments would then be judged by comparison with the variance among replicates within populations, but would, strictly speaking, hold only for the three populations actually tested, and would therefore not have the generality of the random effects test. To make generalizations from three populations per environment must always entail a lower confidence level than if the information sought concerns three particular populations, like strains or varieties. This fact is often overlooked by geneticists familiar only with fixed effects models.

Since the variance among populations within environments has only two degrees of freedom per environment, the analysis is based on a pooled estimate with eight degrees of freedom. The possibility of inequality of variance among environments raises a problem, because the analysis of variance is based on a model of equal variances within classes. The analysis can be shown to

be robust against unequal variances when all classes have equal numbers of observations (SCHEFFÉ 1959), and Bartlett's test failed to show a significant inequality of variance among environments except in one of the thirteen tests, the egg-to-adult survival on normal food in 1968. Pooling the variances over environments therefore seems justified.

A subjective judgment had to be made concerning the appropriate number of standard deviations to use in calculating the least significant difference between environments. If comparisons were to be made between just two of the environments by the standard t-test, a difference of 2.31 standard deviations would be significant at the 5% level. If the three comparisons between variable environments and the constant temperature control were to be made by the method of Dunnett, a difference of 2.88 standard deviations would be required. If all 25 possible comparisons among environments and groups of environments were to be made by the method of SCHEFFÉ, a difference of 3.43 standard deviations would be required. I decided that five of the possible comparisons were of interest: the three Dunnett comparisons of variable environments with the constant temperature environment, the variable environments as a group with the constant temperature environment, and the two long-term variable environments with the two environments remaining constant from generation to generation. I chose three standard deviations as the level at which I would attribute significance to observed differences.

RESULTS

Productivity: Productivity is an important component of fitness for populations which expand to fill a niche each year, and for populations which exploit transient food sources.

The productivity of a sample of females representing the age structure of the adult population was measured in four trials in August 1966 and five more trials in December 1966. The results are combined in Table 1. In March 1968, approximately 40 generations after the establishment of the populations, productivity tests were carried out again. The 20° population had higher productivity, and the 29° population considerably lower productivity, than any of the twelve populations under comparison.

The observed differences could be in part or wholly due to environmental

TABLE 1
Productivity

Period	Population	Constant	Daily	Monthly	Three-monthly	l.s.d.*
Fall, 1966	1	306	375	336	287	
	2	307	404	292	340	
	3	320	284	305	212	
Environment mean		311	354	311	280	114
Percentage of 25°C value		100	114	100	90	37
Spring, 1968	1	374	381	413	366	
	2	398	441	429	345	
	3	353	406	368	316	
Environment mean		375	409	404	342	68
Percentage of 25°C value		100	109	108	91	18
Combined results		100	111	104	91	17

* The least significant difference is calculated from the variance among populations within environments, adjusted for multiple comparisons.

rather than genetic causes, since productivity was measured after only 24 hours in a common environment.

Comparing the estimates of productivity from the beginning and end of the experiment is not possible in absolute terms, for egg laying is strongly influenced by environment.

Competitive ability: Competitive ability is an important component of fitness when a population is limited by food supply. There is intense competition in population cages.

Competitive ability against a laboratory stock with the markers *Cy L⁺/Pm* was first measured in November 1966. The results, analyzed with data transformed from percentages by the angular transformation to make the part of the variance due to sampling error independent of the proportion surviving, are given in Table 2. There are no significant differences between environments.

A second test of competitive ability was carried out in August 1967, about 30 generations after the beginning of the experiment. The outcome was the same, save that the monthly environment average slipped from first to third place relative to the other environments. Again there are no significant differences, though it is clear upon combining the two analyses that the variable temperature populations taken as a whole and the daily fluctuating populations alone have significantly higher competitive ability than the constant temperature populations.

It might be supposed that there would be a positive correlation between total emergence and the competitive ability of wild flies, since differences between strains are likely to be more important under stress conditions. There was no evidence for this, however. The correlations between emergence and competitive ability were -0.04 for the 48 individual bottles in the first test and $+0.13$ for the 72 bottles in the second test, neither correlation being significant.

TABLE 2
Competitive ability analyzed under the angular transformation

Period	Population	Constant	Daily	Monthly	Three-monthly	l.s.d.*
Fall, 1966	1	54.97	56.38	55.55	55.40	
	2	53.90	55.68	55.78	53.73	
	3	53.63	55.08	56.20	56.65	
Environment mean (%)		65.73	68.27	68.47	67.53	3.60
Percentage of 25°C value		100.0	103.9	104.2	102.7	5.5
Fall, 1967	1	57.82	59.47	57.70	60.65	
	2	58.90	59.40	60.68	59.07	
	3	57.72	61.08	59.12	58.32	
Environment mean (%)		72.15	74.97	73.73	74.01	4.20
Percentage of 25°C value		100.0	103.9	102.2	102.6	5.8
Combined results		100.0	103.9	103.1	102.7	3.6

* The least significant difference is calculated from the variance among populations within environments, adjusted for multiple comparisons.

The observed differences are almost certainly genetic, since competitive ability was measured during the second generation in a common environment.

It is not possible to compare the competitive ability at one time with that at another because the standard is a stock culture, which, having much lower population size, probably has a less stable fitness.

Egg-to-adult viability under NaCl stress: It is unlikely that NaCl stress is part of any population's history; any adaptedness to this condition must be thought of as part of a general state of fitness, a pleiotropic effect of a genetic system which has been selected for some other capability, or a similarity between the response needed to deal with NaCl and a response which has been selected for as part of the past environmental situation. Either of the first two reasons reflects adaptability in the sense of THODAY (1953) and LEWONTIN (1957). Estimates of adaptability in two different stress environments were included in this investigation, partly to distinguish between generalized fitness and specific adaptation as explanations of adaptability, and partly to qualify any generalities made on the basis of one new environment.

The first test, in October 1966 (after about 10 generations), gave results similar to those of all the other tests at that time. Percentage survival was transformed by the angular transformation for analysis, and the results are given in Table 3.

The second test, in October 1967 (after about 30 generations), showed a surprising and decisive difference between all of the 25°C constant temperature populations on the one hand and the 40% less adapted, variable temperature populations on the other. A third test, in March 1968 (after about 40 generations),

TABLE 3

Egg-to-adult survival on food containing 0.6 M NaCl, analyzed under the angular transformation

Period	Population	Constant	Daily	Monthly	Three-monthly	l.s.d.*
Fall, 1966	1	48.1	47.3	48.4	50.4	
	2	45.2	50.3	51.2	47.5	
	3	50.1	51.2	46.4	49.6	
Environment mean (%)		54.9	58.0	56.4	57.2	9.1
Percentage of 25°C value		100	106	103	104	17
Fall, 1967	1	46.2	26.4	30.6	36.5	
	2	43.9	36.8	41.4	32.7	
	3	47.3	36.1	31.3	29.7	
Environment mean (%)		51.4	29.8	32.0	29.7	17.2
Percentage of 25°C value		100	58	62	58	33
Spring, 1968	1	37.8	30.3	29.3	25.8	
	2	39.2	32.4	28.7	19.6	
	3	38.7	26.9	25.1	26.7	
Environment mean (%)		38.9	24.8	21.6	16.6	9.3
Percentage of 25°C value		100	64	56	43	24
1967 and 1968 combined		100	60	59	51	19

* The least significant difference is calculated from the variance among populations within environments, adjusted for multiple comparisons.

confirmed the discovery that the variable temperature populations were considerably less adaptable to NaCl stress than the constant temperature populations.

The 20° and 29°C populations were also tested in the second and third test periods. The 20° population performed better than the environment means in any of the four environments under comparison, and the 29° population performed worse than any but one, the mean of the three-monthly variable populations in Spring 1968.

The differences in salt tolerance are likely to be genetic because the individuals analyzed had not experienced the environmental differences. Environmental maternal effects cannot be ruled out.

It appears that NaCl tolerance decreased during the test period, but different methods of food preparation and increasing efficiency in mixing salt into the food may have changed the effective concentration. Survival differed greatly from trial to trial within each test.

Egg-to-adult viability under CaCl₂ stress: CaCl₂ in the environment has no obvious relevance to populations of *Drosophila melanogaster*, but was investigated for the reason set out in the discussion of NaCl stress. It is good that it was taken as a second measure of adaptability, or as being diagnostic of long-term flexibility; the results are quite different from those in NaCl stress. Tests were carried out at the same time as the NaCl investigations, and the data were again analyzed under the angular transformation (Table 4).

There were never significant differences in environmental means. There is no apparent pattern in CaCl₂ adaptability with respect to environment. Again no

TABLE 4

Egg-to-adult survival on food containing 0.25 M CaCl₂, analyzed under the angular transformation

Period	Population	Constant	Daily	Monthly	Three-monthly	l.s.d.*
Fall, 1966	1	41.2	41.1	36.1	40.4	
	2	40.7	39.7	38.3	39.4	
	3	34.8	42.3	36.9	37.3	
Environment mean (%)		39.4	43.2	36.4	39.6	9.0
Percentage of 25°C value		100	110	92	100	23
Fall, 1967	1	36.9	32.0	28.2	37.0	
	2	34.3	31.6	37.2	36.5	
	3	32.9	33.5	33.5	34.6	
Environment mean (%)		32.4	28.6	29.6	34.6	10.3
Percentage of 25°C value		100	89	92	107	32
Spring, 1968	1	33.5	36.0	28.8	33.0	
	2	31.8	34.8	34.6	31.9	
	3	34.0	28.9	27.4	30.2	
Environment mean (%)		29.8	30.1	25.4	27.6	10.9
Percentage of 25°C value		100	101	85	93	37
Combined results		100	100	90	100	15

* The least significant difference is calculated from the variance among populations within environments, adjusted for multiple comparisons.

reliable conclusion can be drawn as to the change of tolerance with time, though it would seem to have decreased.

Egg-to-adult viability in normal food: Larval viability in normal food was included in the experiments on egg-to-adult survival to find whether variability in egg hatchability and under ideal conditions might explain some of the variability on NaCl- and CaCl_2 -stress environments. The results are given in Table 5.

There are no significant differences between environments, and there is no obvious pattern with respect to environmental influence. The extreme variation observed for the three-monthly population might be an effect of the part of the temperature cycle sampled, or more likely due to changed growth conditions. Egg-to-adult viability increased slightly and steadily in the constant temperature populations, but the overall mean shows no trend.

Fitness: In order to have an overall measure of fitness, which would be useful for comparing environmental effects and finding the relationship between fitness and genetic variability, an index was constructed from the individual components. So that the contribution of each component would reflect the amount of information about fitness which was contained in the observed differences between populations, the deviation of each population from the grand mean was divided by the standard deviation of replicate population means within environments. Thus each population was given the value of the number of standard deviations it stood from the grand mean. Exceptions were the 1967 and 1968 NaCl-stress tests, where the difference between environmental means was very large in comparison with other measures of fitness. In this case the population differences were taken about the environmental means so that only the variation between populations within environments was included in the index. Removing differences between environments in NaCl tolerance in the latter two test periods was justified on the grounds that NaCl tolerance *per se* is unlikely to be an important component of fitness, and that other procedures would be even more subjective. In the absence of any information about the relative importance of

TABLE 5

Egg-to-adult survival on normal food, analyzed under the angular transformation

Period	Constant	Daily	Monthly	Three-monthly	l.s.d.*
Fall, 1966					
Environment mean (%)	79.8	83.0	81.1	81.5	7.4
Percentage of 25°C value	100.0	104.0	101.6	102.1	9.3
Fall, 1967					
Environment mean (%)	80.6	82.2	80.0	77.7	6.5
Percentage of 25°C value	100.0	101.9	99.2	96.4	8.0
Spring, 1968					
Environment mean (%)	81.9	80.9	81.6	85.7	9.0
Percentage of 25°C value	100.0	98.7	99.6	104.5	11.0
Combined results	100.0	101.5	100.1	101.2	4.8

* The least significant difference is calculated from the variance among populations within environments, adjusted for multiple comparisons.

these components, it was thought best to take as the index of fitness the simple sum of productivity, competitive ability, and adaptability (measured in turn as the sum of performance in NaCl- and CaCl₂-stress environments), calculated for each population.

The average index values of populations within environments are given in Table 6, and the relative values of the mean fitness for each environment are shown in Figure 1. Analysis of the combined data shows that the daily fluctuating populations have significantly greater overall fitness than the 25° constant temperature populations. The differences in overall fitness may include an environmental component in productivity, and possibly in salt tolerance.

Unfortunately this index does not give any information on whether the fitness of the populations as a whole is increasing or decreasing.

The correlation between measures of fitness: Correlations between population performances in different tests at the same time, or the same test at different times, can be calculated from the scores of each of the twelve experimental populations.

In the measurements at the beginning of the experiment, after four to six months, the average correlation between population performances in pairs of the four tests—productivity, competitive ability, NaCl- and CaCl₂-tolerance—was -0.02. Likewise, there was no appreciable correlation between individual fitness components and the fitness index obtained by summing the other fitness components. These correlations averaged -0.08. The two presumed measures of adaptability, egg-to-adult survival in NaCl and CaCl₂ food, had a correlation of -0.04, and their correlations with egg-to-adult survival on normal food were 0.16 and 0.14, respectively.

In the second round of tests, after 14 to 18 months, the average correlation

TABLE 6

Fitness index, expressed as number of standard deviations from the grand mean

Period	Population	Constant	Daily	Monthly	Three-monthly	l.s.d.*
Fall 1966	1	-.04	.90	.02	.09	
	2	-.70	.98	.18	-.45	
	3	-.79	.17	-.06	-.28	
Environment mean		-.51	.68	.04	-.21	.82
Fall 1967	1	-.37	-.36	-.69	.68	
	2	.07	.91	1.77	-.40	
	3	-.97	1.12	-.40	-1.37	
Environment mean		-.42	.56	.23	-.36	2.37
Spring 1968	1	-.56	.40	-.19	.49	
	2	.16	1.32	1.48	-.92	
	3	-.81	.58	-.76	-1.20	
Environment mean		-.40	.77	.18	-.54	2.00
Combined results		-.45	.67	.14	-.36	1.07

* The least significant difference is calculated from the variance between populations within environments, adjusted for multiple comparisons.

FIGURE 1.—The relative fitnesses of populations from the experimental environments. Each bar represents the environment mean plus and minus the standard error of the estimate, based on the variance between populations within environments, and the vertical line the least significant difference between two environmental means. C = populations from the 25°C constant temperature environment, D = populations from the daily fluctuating environment, M = populations from the monthly fluctuating environment, T = populations from the three-monthly fluctuating environment.

between pairs of the three tests—competitive ability and tolerance to NaCl- and CaCl_2 -stresses—was 0.45. Correlations between components of the fitness index and the sum of the other components averaged 0.48. Performance in the NaCl and CaCl_2 adaptability tests had a correlation of 0.46. Again, survival in normal food was uncorrelated with that in either NaCl ($r = -0.15$) or CaCl_2 ($r = 0.05$).

In the spring of 1968, 21 to 24 months after the beginning of the experiment, the average correlation between pairs of tests fell back to 0.26. The average correlation between components of the fitness index and the sum of the other components was 0.31. Performance in NaCl- and CaCl_2 -stress tests had a correlation of 0.36. Viability on normal food was not significantly correlated with viability in NaCl ($r = 0.13$) or in CaCl_2 ($r = 0.53$). The value of this last correlation is high, but it is to be expected by chance one time in ten from groups of uncorrelated variables when there are ten degrees of freedom for comparison.

The conclusions can be drawn from these data that egg-to-adult viabilities on NaCl- and CaCl_2 -stress media are not strongly correlated with viability on normal food, and that there is an increasing correlation between performance in NaCl

and performance in CaCl_2 , which seems to be a reflection of increased correlation among all the fitness components with time. The increase in correlations among population fitness components must be because the populations evolved to become more distinct from one another, as can be shown by the arguments of the next two paragraphs.

The measured correlations represent two components: the effect of differential population fitness within an environment, and the effect of the environment. These components can be distinguished by calculating correlations between tests based on environmental means alone and on deviations from the environmental mean. In the first set of tests in Fall 1966, the populations had not yet diverged enough from one another to have separate identities. The differences between populations were mostly environmental effects, so the correlations between tests due to environment alone averaged 0.42, while correlations due to population differences within environments averaged 0.06. In the Fall 1967 and Spring 1968 tests, environment alone played no role in similarities among populations in different tests, correlations between tests averaging -0.06 , while correlations due to population differences within environments averaged 0.50.

The same effect can be seen in measures of overall fitness. The correlation between population performances in the fitness index rose from 0.67 (significantly greater than zero) between Fall 1966 and Fall 1967 to 0.92 between Fall 1967 and Spring 1968. Environmental effects on overall fitness were close to the same in all three periods of measurement, and the component of the correlation due to environment changed only from 0.95 to 0.98. The greater similarity between the later test periods is the result of divergence among populations, reflected in the component of correlation due to population differences within an environment of only 0.10 between Fall 1966 and Fall 1967, and of 0.83 between Fall 1967 and Spring 1968.

The nature of the experimental environments: It is of interest to compare the experimental environments on the basis of their biological effects. Such a comparison can be made from results of productivity tests carried out at 20° , 25° , 29° , and daily fluctuating $20^\circ/30^\circ\text{C}$. If the twelve experimental populations ranked similarly in productivity in two environments, these environments were taken to be close to one another. A nearly opposite ranking would indicate that phenotypes well adapted to one environment were poorly adapted to the other. The relationship between environments, calculated as the algebraic distance from a perfect product moment correlation, is shown in Figure 2. It appears that success in the daily fluctuating environment is nearly independent of success in the constant temperature environments.

Measures of genetic variability: Estimates of relative genetic variance for sternopleural bristle number were obtained in each of the three test periods (Table 7). The variable environment populations generally scored higher than the 25°C constant temperature populations, though there were no significant differences among environments. The genetic variance of the constant temperature populations decreased slightly during the course of the experiment, while the variance of the variable temperature populations remained at the initial

FIGURE 2.—The relationship between environments, calculated from the correlation between population performances in each pair of environments as 100 ($1-r$). A distance of 0 units is identity, 100 units independence, and 200 units complete negative correlation.

TABLE 7

Square root of the genetic variance for sternopleural bristle number

Period	Population	Constant	Daily	Monthly	Three-monthly	I.s.d.*
Fall 1966	1	1.68	1.84	1.80	1.53	...
	2	1.69	1.93	1.86	1.63	...
	3	1.52	1.54	1.38	1.73	...
Environment mean		1.63	1.77	1.68	1.63	.44
Percentage of 25° value		100	109	103	100	27
Fall 1967	1	1.56	1.90	1.51	1.77	...
	2	1.58	1.33	1.39	1.65	...
	3	1.57	1.59	1.40	1.69	...
Environment mean		1.57	1.61	1.43	1.70	.37
Percentage of 25° value		100	102	91	109	23
Spring 1968	1	1.49	1.65	1.92	1.78	...
	2	1.59	1.96	1.77	1.59	...
	3	1.42	1.50	1.48	1.54	...
Environment mean		1.50	1.70	1.72	1.64	.44
Percentage of 25° value		100	114	115	109	29
Combined results		100	108	103	106	13

* The least significant difference is calculated from the variance between populations within environments, adjusted for multiple comparisons.

level. In the Fall 1966 and Spring 1968 test periods, the relative genetic variance for bristle number was significantly positively correlated with the fitness index of the twelve populations.

Gene frequency at the Esterase-6 locus was measured after about eight generations and again after about 40 generations. The variable temperature populations generally had lower gene frequencies of the less frequent allele than the constant temperature populations, though the only significant difference when the results were expressed as the diversity of genotypes (by the method of SHANNON, given by PIELOU 1966) was that the monthly populations were significantly less diverse than the constant temperature populations. The diversity at this locus remained the same during the course of the experiment. In spite of consistent differences among the environments, diversity was never correlated with genetic variance for bristle number or with the fitness index of the twelve populations.

The frequency of recessive lethal genes on chromosomes 2 and 3 was measured in the foundation population and during the three test periods. There were never any significant differences among environments, nor were the results correlated with either measure of genetic variability or with the fitness index. The frequency of lethal genes increased during the early generations and decreased later.

DISCUSSION

The results of this investigation show the danger in relying on a single component of fitness to assess the relative fitnesses of populations. The variable populations had higher competitive ability and lower NaCl tolerance, while they included both high and low observations of productivity. There was no pattern in CaCl₂ tolerance. One could argue that competitive ability and productivity are more important measures of fitness than adaptability to circumstances that *Drosophila* populations are never likely to meet; on the other hand, flexibility is an important character, and one which (in the absence of experimental evidence) would be expected to develop under natural selection in a variable environment.

An index formed from several components of fitness provides a more secure basis for identifying the factors involved in adaptation to variable or constant environments (VAN DELDEN 1968.) In this case, overall population fitness is related to the frequency of change in the environment, at least among the variable and 25°C constant temperature populations. The 20° and 29°C populations were added later and are not properly part of the experimental design, but where they have been included for comparison it is clear that a five degree difference in the constant temperature can have larger effects on population fitness than a regular variation of ten degrees. A similar experiment based on variation around 15° or 20°C might have quite different results.

The surprising result of BEARDMORE and LEVINE (1963), that the constant temperature populations have lower fitness than variable populations in their home environment, is found again in these populations. These authors suggested that since their best environment was different from the population cage environ-

ment, it might be new to both kinds of populations. In the present experiment, the culture vessels are different, crowding is less severe than in population cages (there is no crowding at all in the NaCl- and CaCl_2 -stress measures), there is a regular cycle of light and dark, humidity is different, and there probably exist other differences between the selective environment and the test environment. Phenotypic flexibility may be more important in the new environment than strict phenotypic adaptation. These results support the idea that natural selection under a variable environment tends to result in greater population fitness than natural selection in a mean uniform environment.

It has not been possible to explain the greater population fitness under a varying environment on the basis of a greater genetic variability having been maintained by varying selective pressures. Genetic variance for sternopleural bristle number was generally higher in the variable temperature populations than in the 25°C constant temperature populations, while diversity at the Esterase-6 locus was generally lower and lethal gene frequency showed no consistent differences. No two of the measures of genetic variability were correlated, indicating that there may be no biological reality corresponding to the concept of the genetic variability of a population, and only one was correlated with fitness. This may be only because the temperature conditions in the 25°C and variable populations are not sufficiently far apart in comparison with the tolerance of the gene systems involved. It may also be that measures of genetic variance in a gene system are more useful indicators of the genetic variability in a population than diversity at a single locus or lethal gene frequency.

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SUMMARY

Twelve populations of fruit flies derived from a wild population have been maintained for two years in laboratory culture. The populations were divided into four groups, one of which was kept at a constant temperature of 25°C while the other groups were kept under temperature cycles fluctuating between 20° and 30°C with periods of one day, one month, and three months, respectively.—Productivity, competitive ability, and egg-to-adult survival under NaCl- and CaCl_2 -stress were measured two or three times in samples from each population. The pattern of environmental effects was different for each test. In an index combining the results of all tests, overall population fitness was found to be greater under more frequent environmental variation. These results support the idea that natural selection under a variable environment tends to result in greater population fitness than natural selection in a uniform environment.—Population fitness was found to be correlated with one of three measures of genetic variability.

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