GENETIC VARIABILITY OF FLIGHT METABOLISM IN *DROSOPHILA MELANOGASTER*. III. EFFECTS OF *GPDH* ALLOZYMES AND ENVIRONMENTAL TEMPERATURE ON POWER OUTPUT

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ABSTRACT

The effect of allozyme variation at the *sn*-glycerol-3-phosphate dehydrogenase (*Gpdh*) locus on variation in the mechanical power output of the flight muscles of *Drosophila melanogaster* was investigated. The influence of different rearing and flight temperatures and of their interactions with the *Gpdh* allozymic genotypes (allotypes) on flight ability also were analyzed. Populations from three continents were used, and *Gpdh* allotypes were generated from crosses between randomly paired isofemale lines made autozygous for each of the two alleles by inbreeding. Measurements made during tethered flight, together with wing morphology, were used to estimate power output using both WEIS-FOGH's and ELLINGTON's formulas.—Analyses of variance (ANOVA) indicated significant main effects for both environmental components (rearing and flight temperatures) but for only one of the three genetic components (genetic backgrounds within continent); *Gpdh* allotypes and populations (continent of origin) were not significant. The interaction between rearing and flight temperature was highly significant, indicating some physiological adaptation. The effect of *Gpdh* allozymes depended on both rearing and flight temperature and was either significant or marginally so, depending on which set of formulas was used. In either case, the *S/S* allotype showed a 2–4% greater power output than the *F/F* allotype at low temperature for both interactions. In addition, the *S/S* allotype showed significantly greater power output than the *F/F* allotype among flies raised at 15° and flown at 15°, whereas the reverse was true for flies raised at 30° and flown at 30°. Significant differences among the three allotypes for GPDH activity level were found in general, with *S/S* having the highest, *F/S* intermediate and *F/F* the lowest activity, and an inverse relationship existed between rearing temperature and activity.—The temperature effects on power output are consistent with the geographical and seasonal variation observed at the *Gpdh* locus in nature. In general, the results show that *Gpdh* can be considered a minor polygene affecting quantitative variation in the power output during flight and that genotype-by-environment interaction is an important component of that effect.

THE evolutionary significance of enzyme variability has been a long-standing and controversial problem in population genetics (LEWONTIN 1974;

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KIMURA 1983). For variation involving alleles at a structural locus, a direct approach would be to estimate fitness differences among genotypes under natural conditions. However, most organisms do not have life histories that are amenable to fitness estimation in the field or even in the laboratory and, furthermore, incredibly large sample sizes are required to detect small fitness component differences (LEWONTIN 1974). Even if accurate fitness estimates are obtained, it may not be clear whether they apply to the locus under observation or if they are influenced by linkage disequilibrium with other loci. In addition, it is very difficult to determine if appropriate environmental factors or other selective agents are operational during the fitness estimation.

In the study reported here, the scope of the problem is narrowed to consider the contribution of enzyme variability to variation in the type of complex physiological or quantitative morphological traits that affect adaptation, at least under some circumstances. Specifically, the effect of allozyme variation at the *Gpdh* locus on variation in the mechanical power output of the flight muscles of *Drosophila melanogaster* was investigated. The potential problem of spurious effects due to linkage disequilibrium was alleviated in two ways. First, large samples of chromosomes of each allozymic type from populations on three different continents were analyzed. Second, the enzyme *sn*-glycerol-3-phosphate dehydrogenase (GPDH, EC 1.1.1.8) has a specific and very important role in flight metabolism and, consequently, in the functional variable, power output. The interaction of *Gpdh* genotype with different thermal environments to which *D. melanogaster* might naturally be exposed also was investigated. The choice of temperature as a relevant factor was suggested by geographic clines and seasonal variation in *Gpdh* allele frequencies as discussed below. The results show that, in natural populations, *Gpdh* can be considered a minor polygene affecting power output during flight.

The value of using flight metabolism in *D. melanogaster* for studying the physiological effects of enzyme variation has been described in detail in the preceding two papers of this series (CURTSINGER and LAURIE-AHLBERG 1981; LAURIE-AHLBERG *et al.* 1985). Briefly, the biochemical pathways by which carbohydrates, the apparent sole energy reserves for flight (CHADWICK 1947; WIGGLESWORTH 1949; SACKTOR 1965), are metabolized to produce ATP are well characterized (SACKTOR 1975; CRABTREE and NEWSHOLME 1975). Of particular interest, the α-glycerophosphate cycle provides a shuttle system in which reducing equivalents from the cytosolic pool of NADH pass the mitochondrial barrier and NAD^+ is regenerated for the glyceraldehyde-3-phosphate dehydrogenase reaction. Coupled with the extremely efficient delivery of oxygen to the flight muscles via the tracheolar system, the α-glycerophosphate cycle allows flight metabolism to proceed aerobically for extended periods (SACKTOR 1975; KAMMER and HEINRICH 1978). Thus, Drosophila does not acquire an oxygen debt even after prolonged flight (CHADWICK 1947, 1953), and the metabolic rates of insect flight muscle are the highest known for any animal tissue (SACKTOR 1965). As a result, one might expect the rate of ATP production in flight muscles to be quite sensitive to *in vivo* functional differences among variants or to different activity levels of enzymes in the cycle.

Insect flight has also been investigated extensively from the biomechanical point of view (ALEXANDER 1984), and these studies provide a basis for estimating the mechanical power imparted to the wings during hovering or slow forward flight. The precise determination of this power output is a difficult problem because of the unsteady, rotational effects of the wings that are believed to be important in insect flight (MAXWORTHY 1981; ELLINGTON 1984d). In addition, extensive kinematic analyses of the wing and body motions would be required. Nevertheless, by using a quasi-steady-state assumption, by assuming simple harmonic motions of the wings and by using incomplete kinematic data, WEIS-FOGH (1972, 1973) derived formulas for lift and power than adequately satisfied the lift requirements of many insects, including *Drosophila virilis*. He pointed out, however, that unsteady effects were not ruled out. ELLINGTON (1984a–f) included some unsteady effects in a more recent analysis and collected more extensive kinematic data. This approach has lead to significant improvements in the estimation of lift, but only to a slight change in the power requirements. Both WEIS-FOGH's and ELLINGTON's power formulas are used in this study and give comparable results. These power estimates are highly correlated with the rate of oxygen consumption during flight and, thus, provide a sensitive measure of the rate of flight metabolism (LAURIE-AHLBERG *et al.* 1985).

GPDH is one of two enzymes that make up the α-glycerophosphate cycle. That this enzyme plays a central role in flight metabolism is indicated by the inability of *D. melanogaster*, homozygous for mutant "null" alleles at the *Gpdh* locus, to fly (O'BRIEN and MACINTYRE 1972; O'BRIEN and SHIMADA 1974; KOTARSKI *et al.* 1983). Perhaps because of its importance in flight, structural variation at the *Gpdh* locus, as revealed by sequential gel electrophoresis, isoelectric focusing and heat denaturation, apparently is subjected to strong purifying selection, at least in the family Drosophilidae. Of over 200 drosophilid species examined, only eight have shown within species heterogeneity, and only two of these eight species have variants at frequencies high enough to be considered polymorphic (LAKOVAARA, SAURA and LANKINEN 1977; COLLIER 1977; COYNE *et al.* 1979; LAKOVAARA and KERÄNEN 1980). One of these two species in *D. melanogaster*, which is polymorphic for the $Gpdh^F$ and $Gpdh^S$ alleles in virtually every natural population surveyed (OAKESHOTT *et al.* 1982). However, each allozyme class appears to be very homogeneous structurally, because no hidden variation has been found within the allozymes (BEWLEY 1978; COYNE *et al.* 1979).

In *D. melanogaster* there is a latitudinal cline in allele frequencies that is similar in direction on three continents, where the $Gpdh^F$ allele decreases in frequency as latitude increases (JOHNSON and SCHAFFER 1973; OAKESHOTT *et al.* 1982; OAKESHOTT, MCKECHNIE and CHAMBERS 1984). In addition, BERGER (1971) observed seasonal changes in *Gpdh* allele frequencies in natural populations in the northeastern United States [but see CAVENER and CLEGG (1981) for lack of seasonal variation in a southeastern population]. The seasonal changes involved a decrease in the $Gpdh^F$ allele frequency in the fall. These results would be compatible with the geographic clines if temperature was an

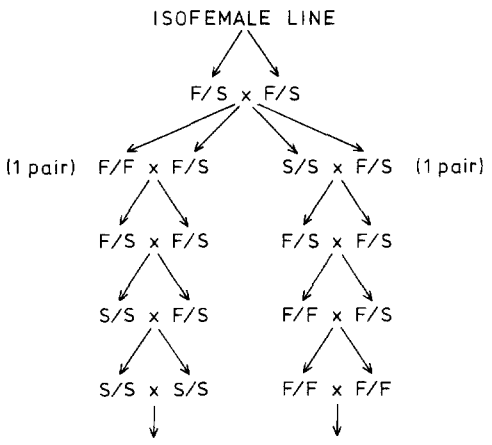

FIGURE 1.—The inbreeding scheme used to obtain the pair of autozygous allozyme sublines from each orginally polymorphic isofemale line.

underlying environmental basis for fitness determination. In fact, MILLER, PEARCY and BERGER (1975) have reported temperature-dependent differences in several kinetic parameters associated with the homo- and heterodimeric forms of GPDH, although BEWLEY, NIESEL and WILKINS (1984) report a lack of temperature-dependent kinetic differences. The kinetic data of MILLER, PEARCY and BERGER (1975) could provide an explanation for the seasonal and clinal variation, if these differences in kinetic parameters actually have some effect on a fitness-related phenotypic character. The study reported in this paper attempts to provide that connection by examining the genotype-by-temperature interaction and its effect on flight performance, as measured by total mechanical power output of the flight muscles. If the flight phenotype is affected by kinetic differences between the allozymes, these observations predict that the $Gpdh^F$ homozygote is superior at high temperature and that the $Gpdh^S$ homozygote is superior at low temperature. The significance of flight ability resides in the fact that flight behavior is known to be an integral part of feeding, mating, dispersal and oviposition. Therefore, it is likely that variation in power output is ultimately related to variation in reproductive success and is subject to the action of natural selection.

MATERIALS AND METHODS

Experimental stocks: Approximately 200 isofemale lines were collected from each of three different continental locations: Sydney, New South Wales, Australia; Groningen, The Netherlands, Europe; and Raleigh, North Carolina, North America. Flies were collected by using fermenting banana baits in traps sealed with funnels. The funnels were made to present as small as opening as possible to allow Drosophila to enter the traps but not to escape. Thus, all flies entering the traps were sampled, reducing the possibility of misrepresentative sampling of flies based on their flight ability, as might occur by sweeping with a net. All isofemale lines were screened electrophoretically for their allelic composition at the *Gpdh* locus. From the lines polymorphic for the $Gpdh^F$ and $Gpdh^S$ alleles, one *F* allele and one *S* allele were made autozygous by the inbreeding scheme shown in Figure 1. The numbers of isofemale

TABLE 1

Flight variables

Variable	SI units	Abbreviations
Wingbeat frequency	Cycles/sec	*WBF*
Wing amplitude	Radians	*WA*
Wing morphology:		
Chord (average)	m	*c*
Length	m	*l*
Mechanical moments:		
$\int_0^l cdr$ (area)	m^2	*AR*
$\int_0^l cr^2dr$ (second)	m^4	*S*
$\int_0^l cr^3dr$ (third)	m^5	*T*
Body mass	kg	*M*

lines from which pairs of autozygous *F* and *S* sublines were derived are Australia, 36; Europe, 28; and North America, 26; representing a total of 180 chromosomes sampled. The *Gpdh* locus is located genetically at 2-20.5 (O'BRIEN and MACINTYRE 1978). Therefore, all the allozyme sublines used in the experiment were karyotyped with respect to chromosome arm *2L* in order to take any effect due to inversions [especially *In(2L)t*] into account during the analysis. Permanent mounts of larval salivary gland chromosomes, stained with lacto-aceto-orcein, were prepared from the F_1 offspring generated by crossing each subline to a line homozygous for the standard gene arrangement. Inversion heterozygotes were compared to photographs in ASHBURNER and LEMEUNIER (1976).

GPDH enzyme assay: GPDH activity was estimated for each individual fly in the study. A single fly was homogenized on ice in 60 μl of 10 mM potassium phosphate buffer, pH 7.4, containing 1 mM EDTA, and was centrifuged for 2 min at 4° in a microcentrifuge. Forty microliters of the supernatant were added to 1 ml of substrate solution (40 mM glycine-NaOH, pH 9.5, 13.4 mM α-glycerol-3-phosphate, 2.26 mM NAD^+), and changes in OD_{340} were recorded every 6 sec for 2 min on a Gilford 250 spectrophotometer with the sample compartment maintained at 29°. One unit of activity is expressed as nanomoles of NAD^+ reduced per minute per fly, using a molar absorbancy of 6.22×10^3 for NADH.

Flight and wing morphology measurements: The flight and wing morphology variables are described in detail in LAURIE-AHLBERG *et al.* (1985). Table 1 gives an abbreviated list of these variables as specifically discussed in this report. The basic procedures for measurement of these variables are described by CURTSINGER and LAURIE-AHLBERG (1981). However, certain modifications were incorporated, and these are detailed in the following summary of the techniques. Individual males were lightly etherized, weighed on a microgram balance and tethered. A tethered fly was observed at 16× through a stereomicroscope while the wingbeat frequency (*WBF*) was measured to the nearest 100 min^{-1} with a stroboscope and the wing amplitude (*WA*) was measured to the nearest 5 degrees with a camera lucida and protractor. Measurements were taken on each fly at each of three temperatures (15, 22 and 30°), and at each temperature the following order of recording was followed: WBF_1, left *WA*, right *WA* and WBF_2. The averages of the two *WBF* and *WA* measurements at each temperature were used as the observations in subsequent analyses and in the power calculations. Three rectangular, water-jacketed glass chambers, connected to constant temperature circulating water baths, were con-

structed for maintaining the three temperatures. There was no observable variation in temperature within the chambers as monitored continuously by calibrated mercury thermometers anchored in one end. Preliminary tests with tethered flies showed that there was less than one degree of variation caused by refraction in *WA* measurements made within *vs.* outside the chambers. Overhead illumination was provided by high intensity dissecting microscope lamps. Each individual fly was measured at all three temperatures in the order 15, 22 and 30° before going to the next individual. Flies were given 20–30 sec (usually less than 10 sec were required) for equilibration to each temperature before measurements were started. The apparatus was contained in a 25° constant-temperature room.

Subsequent to the flight measurements, one wing was removed, mounted, magnified 50× and traced. The wing outline tracing was digitized by taking transects perpendicular to the long axis of the wing at one-half-inch intervals, which divides it into 8–14 trapezoids, depending on the size of the wing. The wing moments were estimated as the sum over trapezoids of the definite integral defined for each moment in Table 1 (and table 2 in LAURIE-AHLBERG *et al.* 1985). The wing chord (width) c at a perpendicular distance r from the origin is assumed to be the width of the trapezoid at that point. After removal of a wing, the flies were individually placed in 250 μl polypropylene tubes on dry ice and were frozen at −70° for later use in the GPDH enzyme assay.

Power calculations: The estimation of mechanical power output during tethered flight is detailed in LAURIE-AHLBERG *et al.* (1985). Originally, the formulations of WEIS-FOGH (1972, 1973) and ALEXANDER (1977) for aerodynamic and inertial powers and their combination in an explicit calculation for total mechanical power output during hovering flight, as presented in LAURIE-AHLBERG *et al.* (1985), were used. Following the publication of the series of papers by ELLINGTON (1984a–f), the data were reanalyzed using the formulas from ELLINGTON (1984f) for profile power (P_{pro}), induced power (P_{ind}) and inertial power (P_{acc}) and their combinations to give total aerodynamic power as the sum of P_{pro} and P_{ind} and total mechanical power output as one-half the sum of P_{pro}, P_{ind} and P_{acc}. The specific formulas for calculating these powers are referenced in detail in LAURIE-AHLBERG *et al.* (1985), as are the values of constant parameters used in the WEIS-FOGH and ELLINGTON power calculations. Two of these parameters necessarily vary in the present experiment because their values are temperature dependent. The values for the mass density of air, ρ, are 1.23, 1.20 and 1.16 kg m^{-3} at 15, 22 and 30°, respectively, and the corresponding values for the kinematic viscosity of air, η, are 1.46×10^{-5}, 1.52×10^{-5} and 1.60×10^{-5} m^2 sec^{-1}. The analyses of both WEIS-FOGH's and ELLINGTON's methods for estimating the total mechanical power output are provided in order to compare the results of the two methods and also to provide continuity with the previous papers in this series.

Experimental design: There were 15 measurement days within each of two blocks, corresponding to the 5 weekdays over a 3-week span. Block 2 is simply a duplication of block 1, where measurement day 1 of block 2 began 10 days after day 15 of block 1. In this situation, days normally would be nested within blocks, but not in this design, because a unique set of nine genotypes was measured on a particular day in each block, giving a total of 135 unique genotypes over the 15 days.

The relationships among the 135 unique genotypes are outlined in Table 2. Each day represents a particular set of three genetic backgrounds, generally one from each geographic location, and each of the three allozymic genotypes (allotypes) occurs on each genetic background, giving the nine unique genotypes for a particular day. These genotypes were constructed in the following way. As mentioned previously, each isofemale line sampled gave rise to a pair of autozygous lines, one *S* and one *F*. Each unique genotype represents a cross between a particular pair of these autozygous lines. Within each day, each of the three sets of allotypes was generated by making crosses between the autozygous sublines from a specific pair of isofemale lines (allelic subscripts in Table 2 index the isofemale line source). Isofemale lines were paired at random within a geographic location. Thus, autozygous sublines F_{Ai} and S_{Ai} from the ith Austra-

TABLE 2

The number of *Gpdh* genotypes from each location used in the experiment and their origin from the isofemale lines

	Location								
Day	Australia			Europe			North America		
1	$F_{A1}F_{A2}$	$F_{A1}S_{A2}$	$S_{A1}S_{A2}$	$F_{E1}F_{E2}$	$F_{E1}S_{E2}$	$S_{E1}S_{E2}$	$F_{N1}F_{N2}$	$F_{N1}S_{N2}$	$S_{N1}S_{N2}$
2	$F_{A3}F_{A4}$	$F_{A3}S_{A4}$	$S_{A3}S_{A4}$	$F_{E3}F_{E4}$	$F_{E3}S_{E4}$	$S_{E3}S_{E4}$	$F_{N3}F_{N4}$	$F_{N3}S_{N4}$	$S_{N3}S_{N4}$
.		.			.			.	
.		.			.			.	
.		.			.			.	
k	$F_{Ai}F_{Aj}$	$F_{Ai}S_{Aj}$	$S_{Ai}S_{Aj}$	$F_{Ei}F_{Ej}$	$F_{Ei}S_{Ej}$	$S_{Ei}S_{Ej}$	$F_{Ni}F_{Nj}$	$F_{Ni}S_{Nj}$	$S_{Ni}S_{Nj}$
.		.			.			.	
.		.			.			.	
.		.			.			.	
12	$F_{A23}F_{A24}$	$F_{A23}S_{A24}$	$S_{A23}S_{A24}$	$F_{E23}F_{E24}$	$F_{E23}S_{E24}$	$S_{E23}S_{E24}$	$F_{N23}F_{N24}$	$F_{N23}S_{N24}$	$S_{N23}S_{N24}$
13	$F_{A25}F_{A26}$	$F_{A25}S_{A26}$	$S_{A25}S_{A26}$				$F_{N25}F_{N26}$	$F_{N25}S_{N26}$	$S_{N25}S_{N26}$
	$F_{A27}F_{A28}$	$F_{A27}S_{A28}$	$S_{A27}S_{A28}$						
14	$F_{A29}F_{A30}$	$F_{A29}S_{A30}$	$S_{A29}S_{A30}$	$F_{E25}F_{E26}$	$F_{E25}S_{E26}$	$S_{E25}S_{E26}$			
	$F_{A31}F_{A32}$	$F_{A31}S_{A32}$	$S_{A31}S_{A32}$						
15	$F_{A33}F_{A34}$	$F_{A33}S_{A34}$	$S_{A33}S_{A34}$	$F_{E27}F_{E28}$	$F_{E27}S_{E28}$	$S_{E27}S_{E28}$			
	$F_{A35}F_{A36}$	$F_{A35}S_{A36}$	$S_{A35}S_{A36}$						

For example, autozygous allozyme sublines F_{A1} and S_{A1} from isofemale line $A1$ were crossed with F_{A2} and S_{A2} from $A2$ to give the $F_{A1}F_{A2}$, $F_{A1}S_{A2}$ and $S_{A1}S_{A2}$ genotypes.

lian isofemale line were crossed with sublines F_{Aj} and S_{Aj} from the jth Australian line to give the experimental flies $S_{Ai}S_{Aj}$, $S_{Ai}F_{Aj}$ and $F_{Ai}F_{Aj}$, which were measured on day k. Each of the genotypes that share a common pair of subscripts (say Ai,Aj) consequently share a common genetic background (provided by two isofemale lines) regardless of allotype. In addition, this method of genotype construction restores approximately the original heterozygosity in genetic background that existed in the isofemale lines before inbreeding (and to the same extent regardless of allotype).

The original plan called for deriving one genetic background per day from each of the three geographic locations. However, this goal was achieved only for 12 of the 15 days; on each of the three remaining days only two geographic locations are represented (see Table 2). So for days 1–12, each day-by-location combination identifies a particular genetic background, and days are nested within locations since genetic backgrounds within one location have no relationship to genetic backgrounds within another location. The data from days 1–12 for each variable were first analyzed with an ANOVA model including locations (L) and days nested within locations [$D(L)$] as main effects. This model allows a straightforward test of the location effect, but the day-within-location effect actually confounds genetic backgrounds-within-location and the true measurement day effect. For most variables, location was not a significant source, so the model was altered by ignoring location, which allows analysis of all 15 days without introducing imbalance. In the altered model, day (D) is a main effect and genetic backgrounds are nested within days [$G(D)$]. In this model the test of genetic backgrounds-within-days is straightforward, while the day effect actually represents differences among the sets of three genetic backgrounds measured together on one day along with the true measurement day effect.

Each autozygous subline potentially represents a unique allele at the *Gpdh* locus, so the number of independent alleles in the experiment is the number of sublines, 90 S

and 90 *F*. Each genotype in the experiment (say $F_{A1}F_{A2}$) is not only a unique combination of genetic background and allotype, but may also be unique at the *Gpdh* locus itself if there is within-allozyme allelic heterogeneity in the population. In the ANOVA model that ignores geographic location, the allotype-by-genetic-background-within-day effect [$A \times G(D)$] represents both the true interaction between allotype and genetic background, as well as within-allozyme variation due to factors within or closely linked to the *Gpdh* locus (*i.e.*, the region made autozygous). Thus, any within-allozyme heterogeneity at the *Gpdh* locus and/or any interaction between allotype and genetic background should be manifested as significance of this effect.

In the crosses between autozygous sublines generating the experimental flies, one isofemale line source was randomly designated as maternally contributing, the other paternally contributing. The same arrangement was used in both blocks for a particular pair of sublines. In order to check for maternal effects in the *S/F* heterozygotes, crosses in days 1–7 had the *F* allele contributed maternally, whereas those in days 8–15 had the *F* allele contributed paternally.

Experimental flies of each genotype were reared at each of three temperatures, 15, 22, and 30°. Two males of the same genotype reared together at the same temperature in the same vial (*i.e.*, replicates) were analyzed with respect to flight variables at each of three test temperatures—again, 15, 22 and 30°. Live weight, wing morphology and GPDH activity measurements were made on these same individuals. A total of 1620 flies were tested.

The specific ANOVA models vary somewhat according to whether or not flight temperature is involved and whether or not geographic location is included. The specific models for each case can be deduced from the sources given in the tables and from the following consideration of fixed *vs.* random effects. Random effects are blocks (*B*), measurement days (*D*) and genetic backgrounds (*G*). Fixed effects are rearing temperature (*R*), flight temperature (*F*), *Gpdh* allotype (*A*) and geographic location (*L*). Error mean squares and SATTERTHWAITE's *F* tests were calculated according to methods described by SEARLE (1971, p. 401) and NETER and WASSERMAN (1974, p. 664). Differences among means for specified sources were tested for significance, using either the least significant difference (LSD) method (SNEDECOR and COCHRAN 1967) or TUKEY's multiple comparison method (NETER and WASSERMAN 1974), depending on whether the *F*-test was or was not significant, respectively (SNEDECOR and COCHRAN 1967). For tests of hypotheses involving specific means for which the direction of the difference had been predicted, LSDs using one-tailed *t*-values were employed.

Rearing conditions: In order to measure, on the same day, flies with the same genotype but different rearing temperatures, it was necessary to stagger the starting times for the sets of each cross at each rearing temperature. Autozygous sublines were set up in large (16-dram) vials at 25°, using 15 pairs of flies which were cleared after 3 days. Virgin females and males were collected 11 days later and were aged separately for 3 days. Five pairs of flies were crossed in 8-dram vials and were allowed to mate and oviposit for 3 days. Two vials for each cross were established at this time to reduce the probability of loss of a specific cross during subsequent development, although only flies from one of the vials were used for measurement. The adults were cleared and the vials placed in an incubator at the appropriate temperature on a 12 hr–12 hr light-dark cycle. These vials were cleared of emergent offspring after the scheduled period and were returned to the incubator; 24 hr later, five males were collected from the freshly emerged flies. These males were returned to the same incubator, aged for the appropriate number of days and then used in the measurements described above. The developmental periods and adult male aging times, respectively, at the three temperatures were as follows: 15°, 29 days and 12 days; 22°, 14 days and 6 days; 30°, 9 days and 4 days. All males were transferred to fresh food in vials 2 days before measurement, and in addition, males aged at 15° were transferred 5 days after collection.

RESULTS

Karyotype and maternal effects: The karyotypic analysis showed that five of the Australian and one each of the European and North American $Gpdh^F$ autozygous lines contained *In(2L)t*. In addition, one $Gpdh^S$ line from Europe contained an apparently unique inversion on *2L*, having breakpoints at approximately *30A* and *34A*. All inversions existed as heterozygotes in the actual experiment, and ANOVAs indicated no effect on any of the flight variables or activity data. Because of the small number of heterokaryotypes involved, the power of the tests is low; nevertheless, it was accepted that there were no measurable effects due to inversions, and karyotype was ignored in further analysis. Similarly, no maternal effect on the $Gpdh^F/Gpdh^S$ individuals was indicated, so the parental origin of the alleles was ignored in further analysis.

Power output analysis: ANOVAs for both types of power output estimation (WEIS-FOGH; ELLINGTON) are summarized in Table 3. The model for the ANOVA presented here ignores geographic location, since an earlier analysis showed that source not to be significant. Both fixed environmental treatments [rearing (R) and flight (F) temperatures] as well as their interaction ($R \times F$) are significant. These effects are depicted in Figure 2 using total power calculated with ELLINGTON's method. Flies raised at intermediate temperature had higher power output than those raised at either extreme temperature. There was a positive association between flight temperature and power output. The interaction effect indicates some physiological adaptation on the part of the flies as seen, for example, in the fact that flies raised at 15° had the highest power output at the 15° flight temperature, but were clearly the poorest fliers at 30°.

Of the main effects due to genetic causes of variation, allotype (A), genetic backgrounds [$G(D)$] and the interaction $A \times G(D)$, only the genetic background showed a significant effect on power output. These genetic background effects were subject to environmental modification, as indicated by the significant interaction with flight temperature. Although allotype did not have a significant overall effect on power output, its interactions with rearing temperature ($R \times A$) and flight temperature ($A \times F$) were either significant or marginally so, depending on the source of the power calculations. These two interaction effects are shown in Figure 3 (ELLINGTON's method). In the allotype-by-rearing temperature interaction, the $Gpdh^S$ homozygote shows significantly higher power output than the $Gpdh^F$ homozygote at low temperature. The difference is small, however, amounting to only about a 3% increase in power for the $Gpdh^S$ homozygote over the $Gpdh^F$ homozygote, and may be compared to the 54% difference in power output found for the highest over the lowest of the background genotype means for flies raised at 15°. Because of the *a priori* hypothesis that flight at low temperature would favor the $Gpdh^S$ allele and flight at high temperature would favor the $Gpdh^F$ allele, a one-tailed test of significance was used with the allotype-by-flight temperature interaction. The direction of the interaction effects at the two extremes was in accord with the prediction, although the difference between the two homozygotes was not significant at 30°. Similar differences among the allotypes existed at 22° as at

TABLE 3

ANOVAs for total power output and wing amplitude during tethered flight

Source[a]	d.f.	Total power output: Weis-Fogh F[b]	Total power output: Weis-Fogh significance level[c]	Total power output: Ellington F[b]	Total power output: Ellington significance level[c]	Wing amplitude F[b]	Wing amplitude significance level[c]
B	1	4.86	*	1.62		18.65	****
R	2	26.50	****	18.23	****	5.59	**
$B \times R$	2	1.31		2.15		1.09	
D	14	1.11		1.24		1.49	*
$B \times D$	14	1.12		1.14		1.04	
$R \times D$	28	0.97		1.06		1.14	
$B \times R \times D$	28	1.13		1.08		0.65	
A	2	1.16		0.97		1.10	
$B \times A$	2	0.76		0.75		1.01	
$R \times A$	4	1.56	(0.06)	1.49	*	0.88	
$B \times R \times A$	4	0.63		0.32		1.06	
$D \times A$	28	1.10		1.21	(0.08)	1.17	(0.05)
$B \times D \times A$	28	0.94		0.84		0.71	
$R \times D \times A$	56	0.85		0.91		1.29	*
$B \times R \times D \times A$	56	1.00		0.97		0.58	
$G(D)$	30	1.51	**	1.54	*	1.35	(0.05)
$B \times G(D)$	30	2.14	***	2.65	****	4.00	****
$R \times G(D)$	60	1.28	(0.06)	1.19		1.52	**
$B \times R \times G(D)$	60	2.52	****	3.39	****	1.97	****
$A \times G(D)$	60	0.99		1.18		1.06	
$B \times A \times G(D)$	60	2.07	****	3.05	****	1.79	****
$R \times A \times G(D)$	120	1.23		1.23		0.85	
$B \times R \times A \times G(D)$	120	2.17	****	2.70	****	2.55	****
F	2	112.38	****	177.79	****	5.20	*
$B \times F$	2	6.49	**	5.40	**	4.84	**
$R \times F$	4	48.41	****	63.07	****	12.66	****
$B \times R \times F$	4	2.08	(0.06)	1.90	(0.08)	1.25	
$D \times F$	28	0.92		0.96		0.87	
$B \times D \times F$	28	1.53	**	1.63	***	1.56	**
$R \times D \times F$	56	0.94		1.02		1.14	(0.09)
$B \times R \times D \times F$	56	1.47	*	1.46	*	0.89	
$A \times F$	4	1.36	*	1.25	(0.07)	1.10	
$B \times A \times F$	4	0.66		0.77		0.82	
$R \times A \times F$	8	1.07		1.14		0.94	
$B \times R \times A \times F$	8	0.96		1.04		0.71	
$D \times A \times F$	56	0.95		1.04		1.03	
$B \times D \times A \times F$	56	0.93		1.01		0.80	
$R \times D \times A \times F$	112	0.82		0.91		1.00	
$B \times R \times D \times A \times F$	112	1.02		0.87		0.86	
$F \times G(D)$	60	1.69	****	1.92	****	1.67	****
$B \times F \times G(D)$	60	0.97		0.79		0.96	
$R \times F \times G(D)$	120	1.56	**	1.51	*	1.44	*
$B \times R \times F \times G(D)$	120	1.01		0.91		0.86	
$A \times F \times G(D)$	120	1.00		1.03		1.06	
$B \times A \times F \times G(D)$	120	1.06		0.88		0.92	
$R \times A \times F \times G(D)$	240	1.07		0.89		0.91	
Error	2670	.		.		.	
Total	4859	.		.		.	

[a] Letter codes refer to: block (B), rearing temperature (R), measurement day (D), allotype (A), genetic background (G) and flight temperature (F).

[b] Satterthwaite's approximation for a mixed effects model; see text.

[c] Values in parentheses are probability levels for marginally significant terms ($0.05 < P < 0.10$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

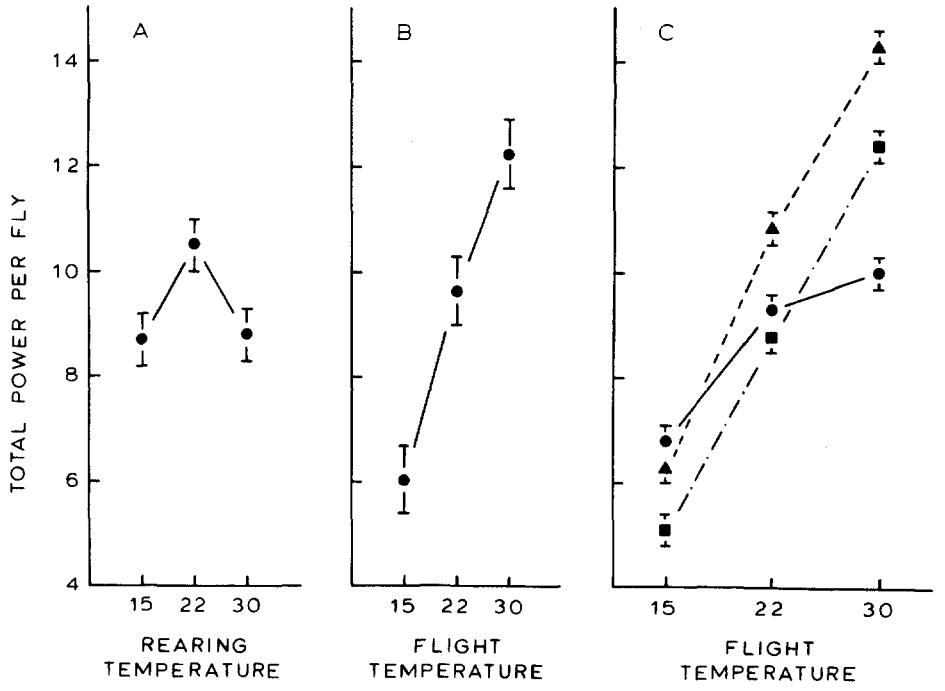

FIGURE 2.—The effects of environmental temperature on total power output (microwatts) using ELLINGTON's model. A, Rearing temperature; N = 1620 per mean. B, Flight temperature; N = 1620 per mean. C, Rearing-by-flight temperature interaction, where rearing temperatures are symbolized as 15° (●), 22° (▲) and 30° (■); N = 540 per mean. The 95% confidence intervals (C.I.) are shown (two-tailed test).

15°, suggesting that, at least in this temperature range, the $Gpdh^S$ homozygote exhibited a slight but significantly superior flight phenotype as measured by total mechanical power output of the flight muscles. The difference between the two homozygotes is small, amounting to only about a 2% increase in power for the $Gpdh^S$ homozygote for flight at 15°. This difference between allotypes can be compared to the 40% difference in power output during flight at 15° between the highest and lowest of the genetic backgrounds.

The analysis of power output using WEIS-FOGH's method is virtually identical to that discussed for ELLINGTON's method. The temperature-dependent effects of the allotype ($R \times A$ and $A \times F$) on power output under WEIS-FOGH's method show an approximate 4% increase in power for the $Gpdh^S$ homozygote over the $Gpdh^F$ homozygote for both interactions at 15°. Almost all the other interaction effects in both analyses that are significant, especially those involving the genetic background effect [$G(D)$], contain the block term, which itself is significant under the WEIS-FOGH model. Apparently there was some difference between the blocks that led to changes in the expression of the genetic background effects represented by the isofemale lines.

GPDH activity analysis: The ANOVAs for GPDH activity and for live weight are summarized in Table 4. Geographic location is not significant for these variables, so the model presented ignores that source. For GPDH activity,

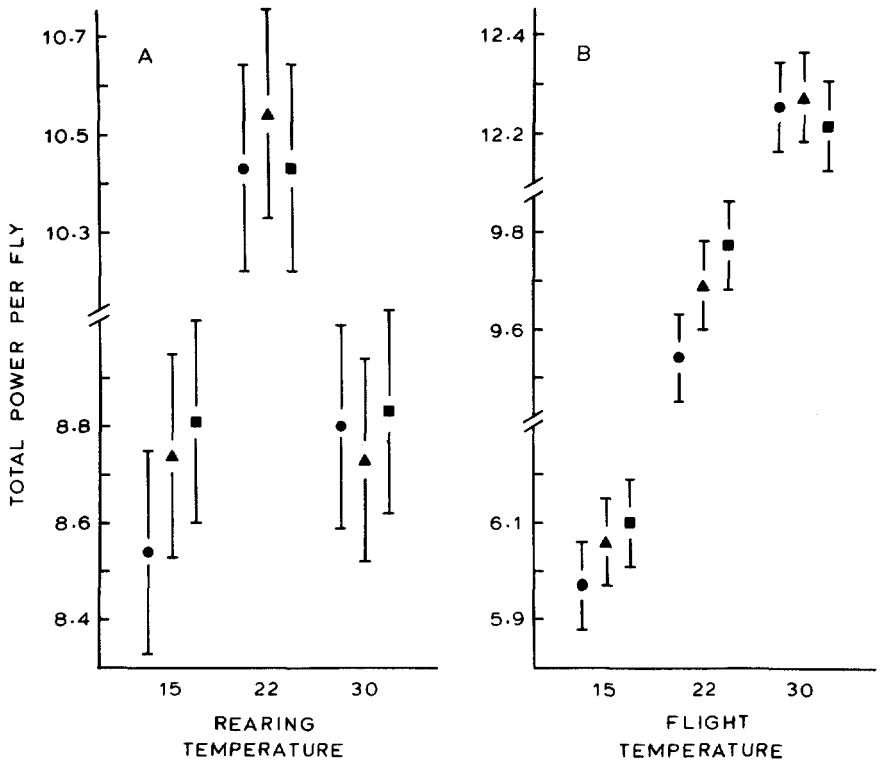

FIGURE 3.—Allotype-by-temperature interactions affecting total power output (microwatts) using ELLINGTON's model. A, Allotype-by-rearing temperature. B, Allotype-by-flight temperature. N = 540 per mean. Allotypes are symbolized as $Gpdh^F/Gpdh^F$ (●), $Gpdh^F/Gpdh^S$ (▲) and $Gpdh^S/Gpdh^S$ (■). The 95% C.I. are shown (two-tailed test in A and one-tailed test in B).

all three sources of genetic variation, allotype (A), genetic background [$G(D)$] and the interaction $A \times G(D)$ are significant. Also, rearing temperature (R) was a significant source of variation affecting activity level. The allotype and rearing temperature effects are shown in Figure 4. The $Gpdh^S$ homozygote showed the highest level of *in vitro* activity, whereas the $Gpdh^F$ homozygote showed the lowest and the heterozygote was intermediate. The rearing temperature effects showed an inverse relationship with *in vitro* activity; flies raised at 15° had the highest, those raised at 30° the lowest and those raised at 22° had intermediate levels. The ranking of activity levels among the three allotypes did not change across rearing temperatures (see Table 5). As discussed earlier, the $A \times G(D)$ effect could represent either a true allotype-by-genetic background interaction and/or within-allozyme variation closely linked to or within the *Gpdh* locus.

Relationship between GPDH activity and power output: Since the genetic background effects were highly significant for both power output and GPDH activity level, the relationship between them was investigated. Table 6 shows the partial correlations (with live weight fixed) between power and GPDH activity over the 45 background genotype means. None of the values are

TABLE 4

ANOVAs for GPDH activity and for live weight

Source[a]	d.f.	GPDH activity: Raw F[b]	GPDH activity: Raw Significance level[c]	GPDH activity: Specific F[b]	GPDH activity: Specific Significance level[c]	Weight F[b]	Weight Significance level[c]
B	1	13.68	***	9.86	**	1.67	
R	2	15.34	**	11.60	***	7.73	*
B × *R*	2	3.56	*	0.79		3.62	*
D	14	0.83		0.77		0.96	
B × *D*	14	4.77	****	4.81	****	1.14	
R × *D*	28	0.94		0.79		1.07	
B × *R* × *D*	28	1.76	*	1.17		1.09	
A	2	6.30	**	6.69	**	0.61	
B × *A*	2	1.38		1.37		1.51	
R × *A*	4	1.36		1.34		0.63	
B × *R* × *A*	4	0.59		0.15		1.15	
D × *A*	28	1.05		1.14		0.96	
B × *D* × *A*	28	1.10		0.52		0.80	
R × *D* × *A*	56	1.32	*	1.13		0.92	
B × *R* × *D* × *A*	56	1.03		1.04		1.71	**
G(D)	30	2.34	****	2.89	****	2.68	****
B × *G(D)*	30	0.90		0.97		2.77	****
R × *G(D)*	60	1.19		1.79	*	1.25	
B × *R* × *G(D)*	60	0.89		1.03		2.58	****
A × *G(D)*	60	3.34	****	2.06	**	2.88	****
B × *A* × *G(D)*	60	1.28	(0.08)	1.45	*	2.19	****
R × *A* × *G(D)*	120	1.02		1.14		1.80	****
Error	930	·		·		·	
Total	1619	·		·		·	

[a] Letter codes refer to: block (*B*), rearing temperature (*R*), measurement day (*D*), allotype (*A*), genetic background (*G*) and flight temperature (*F*).

[b] SATTERTHWAITE'S approximation for a mixed effects model; see text.

[c] Values in parentheses are probability levels for marginally significant terms ($0.05 < P < 0.10$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

significantly different from zero. This lack of correlation does not imply that any variation in GPDH activity has no effect, since this procedure averages over the *Gpdh* allele effects. It shows primarily that variation in GPDH *in vitro* activity caused by genetic variation in modifier loci is not strongly correlated with flight ability. It was indicated that *Gpdh* allotype has a significant effect on activity levels, but not on power output. However, inspection of the means (Table 5 and Figure 4A) show that the rank order of allotypes in power output is identical to that for activity levels. This comparison suggests a relationship between allotype activity levels and power output that the current experimental design was not powerful enough to demonstrate as significant.

Effect of weight on GPDH activity and power output: Previous work has shown that weight is often correlated with both power and enzyme activity levels (CURTSINGER and LAURIE-AHLBERG 1981; LAURIE-AHLBERG *et al.* 1985).

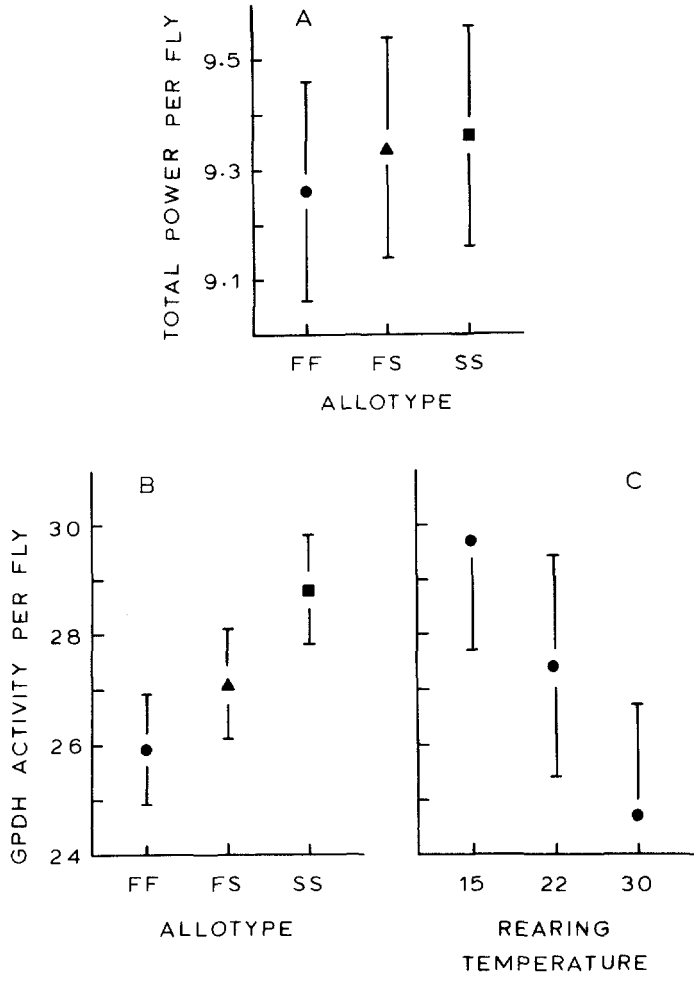

FIGURE 4.—The effects of allotype on total power output (microwatts) using ELLINGTON's model and of allotype and rearing temperature on *in vitro* GPDH activity (nanomoles NAD^+/min fly). A, Allotype and power; $N = 1620$ per mean. B, Allotype and activity; $N = 540$ per mean. C, Rearing temperature and activity; $N = 540$ per mean. The 95% C.I. are given (two-tailed test).

In those experiments, the raw data were adjusted for weight by the linear regression over the isogenic line means (see LAURIE-AHLBERG *et al.* 1980). However, this technique could not be used in the present study because of the complicating structure imposed by the rearing and flight temperature treatments. The regression of weight on power, using the 45 background genotype means, showed significant heterogeneity of slopes among temperature levels within the rearing temperature treatment, within the flight temperature treatment, as well as within their combination. This same problem existed also for the activity data among rearing temperatures. Simply dividing the raw power or activity data by their corresponding weight values (giving specific power or

TABLE 5

Means for live weight, GPDH activity and power output associated with rearing temperature (*R*), allotype (*A*) and their interaction (*R* × *A*)

Effect	Rearing temperature	Allotype	Weight[a]	GPDH activity		Total power output[b]	
				Raw[c]	Specific[d]	WEIS-FOGH	ELLINGTON
R	15°		7.42	29.7	39.6	3.14	8.70
	22°		7.00	27.4	38.7	3.93	10.47
	30°		6.75	24.7	36.0	2.80	8.79
			*	*	*	*	*
A		*FF*	7.02	25.9	36.5	3.25	9.26
		FS	7.05	27.1	37.9	3.30	9.34
		SS	7.09	28.8	39.9	3.32	9.36
				*	*		
R × *A*	15°	*FF*	7.40	28.4	38.0	3.04	8.54
	15°	*FS*	7.44	29.7	39.4	3.16	8.74
	15°	*SS*	7.42	31.3	41.3	3.23	8.82
	22°	*FF*	6.93	25.6	36.5	3.92	10.43
	22°	*FS*	7.01	27.4	38.7	3.96	10.54
	22°	*SS*	7.06	29.2	40.8	3.91	10.43
	30°	*FF*	6.74	23.9	34.9	2.80	8.80
	30°	*FS*	6.71	24.3	35.6	2.78	8.73
	30°	*SS*	6.79	26.0	37.5	2.83	8.83
						(*)	*

[a] Micronewtons (10^{-6} kg m/s^2).
[b] Microwatts.
[c] Nanomoles/min fly.
[d] Nanomoles/min mg.
* Significant effect in ANOVA; (*)marginally significant.

TABLE 6

Partial correlations (with weight fixed) between total power and GPDH activity over background genotype means

Flight temperature	Rearing temperature		
	15°	22°	30°
15°	−0.23	−0.01	0.18
	−0.22	−0.14	−0.11
22°	−0.10	−0.08	0.01
	−0.06	−0.09	−0.04
30°	−0.02	0.14	0.10
	−0.03	0.13	0.07

There are 45 background genotype means per estimate. The upper number in each pair is based on WEIS-FOGH's formula, the lower number on that of ELLINGTON.

activity) is not an acceptable adjustment method either, because it often does not eliminate the correlation or it may impose a different correlational structure on the data. For instance, with the activity data in this experiment, sig-

nificant negative correlations are generated between the weight and activity means of the 45 background genotype means within each rearing temperature, when the so-called specific activity adjustment is made. These difficulties make the usefulness of specific weight adjustment questionable.

In order to assess the value of the ratio adjustment procedure, the ANOVAs for both raw and specific activities, as well as weight, are compared in Table 4. Very little difference is found between the two analyses for activity, particularly in the major effects of interest such as rearing temperature (*R*), allotype (*A*) and genetic background [*G*(*D*)]. In contrast, the analysis for weight is quite different and shows no significant effect for allotype. Weight has a significant genetic component associated with the genetic background, as does activity, but the expression of these differences among genetic backgrounds is strongly affected by interaction with the environmental components of the experiment. The means for particular effects that showed significance for one or more of the variables (weight, total power or GPDH activity) are shown in Table 5. The means for weight do not predict either those for power among rearing temperatures or those for activity among allotypes. There is no consistency in comparisons among means for the rearing temperature-by-allotype interaction involving weight or any of the other variables, but there is for power and activity at 15°. Thus, although live weight may account for some of the variation in activity and power output, it clearly does not account for a major part of it. In addition, previous work with isogenic lines has shown that overall body size variation cannot account for the high genetic component to the variation in enzyme activity levels or flight variables (LAURIE-AHLBERG *et al.* 1980, 1985).

Analysis of wingbeat frequency, wing size and wing amplitude: Total mechanical power output of the flight muscles is calculated from the component flight variables indicated in Table 1. Of these variables, the ANOVAs for wing amplitude (Table 3) and wingbeat frequency and wing area (Table 7) are given. The only source of genetic variation that contributed to variation in *WA* came from the genetic background [*G*(*D*)], and it was only marginally significant. *WA* appeared to be highly susceptible to environmental sources of variation, as blocks (*B*), rearing temperatures (*R*) and flight temperatures (*F*) were significant effects. It is not surprising, then, that several of the interaction effects involving the genetic backgrounds and these treatments were significant as well.

WBF and the wing size/shape variables (*AR*, *l*, *S* and *T*), of which wing area (*AR*) is used representatively, were of particular interest because they had highly significant geographic location (*L*) effects (Table 7). The means for each geographic location of origin for *WBF* and *AR*, as well as the total power output, are shown in Figure 5. The European population had significantly larger wings than the other two locations and had a correspondingly lower *WBF*. The differences between *WBF* and wing size/shape appeared to complement each other in determining the power output, such that all three locations had essentially similar power production levels (the mean for North America

TABLE 7

ANOVAs for wing beat frequency and wing area

Source[a]	d.f.	*WBF*		Area	
		F^b	Significance level[c]	F^b	Significance level[c]
B	1	0.56		2.02	
R	2	344.28	****	647.90	****
B × *R*	2	0.39		2.01	
L	2	6.28	***	11.67	****
B × *L*	2	0.63		0.32	
R × *L*	4	0.93		3.97	**
B × *R* × *L*	4	0.58		0.94	
A	2	0.69		0.43	
B × *A*	2	2.67	(0.05)	1.78	
R × *A*	4	1.19		0.55	
B × *R* × *A*	4	2.15	(0.08)	1.52	
L × *A*	4	1.31		0.63	
B × *L* × *A*	4	0.51		1.38	
R × *L* × *A*	8	1.09		1.20	
B × *R* × *L* × *A*	8	0.44		1.96	*
D(L)	33	2.52	****	4.60	****
B × *D(L)*	33	3.53	****	2.93	****
R × *D(L)*	66	1.78	***	2.09	**
B × *R* × *D(L)*	66	2.73	****	2.13	****
A × *D(L)*	66	1.45	**	3.38	****
B × *A* × *D(L)*	66	1.77	***	1.80	***
R × *A* × *D(L)*	132	1.13		1.74	****
B × *R* × *A* × *D(L)*[d]	132	2.30	****	.	
F	2	667.57	****		
B × *F*	2	6.42	***		
R × *F*	4	222.33	****		
B × *R* × *F*	4	1.83			
L × *F*	4	2.68	*		
B × *L* × *F*	4	1.45			
R × *L* × *F*	8	1.42			
B × *R* × *L* × *F*	8	1.62			
A × *F*	4	1.36	(0.08)		
B × *A* × *F*	4	1.79			
R × *A* × *F*	8	1.61	*		
B × *R* × *A* × *F*	8	0.60			
L × *A* × *F*	8	1.32			
B × *L* × *A* × *F*	8	1.04			
R × *L* × *A* × *F*	16	1.98	**		
B × *R* × *L* × *A* × *F*	16	0.50			
F × *D(L)*	66	2.28	****		
B × *F* × *D(L)*	66	0.95			
R × *F* × *D(L)*	132	1.63	**		
B × *R* × *F* × *D(L)*	132	0.90			
A × *F* × *D(L)*	132	1.43	*		
B × *A* × *F* × *D(L)*	132	0.69			
R × *A* × *F* × *D(L)*	264	0.78			
Error[d]	2208	.			
Total[d]	3887	.			

[a] Letter codes refer to: block (*B*), rearing temperature (*R*), location (*L*), allotype (*A*), measurement day (*D*) and flight temperature (*F*).

[b] SATTERTHWAITE's approximation for a mixed effects model; see text.

[c] Values in parentheses are probability levels for marginally significant terms ($0.05 < P < 0.10$).

[d] The Error term for wing area is *B* × *R* × *A* × *D(L)*, which has 780 d.f.; and the Total d.f. = 1295.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

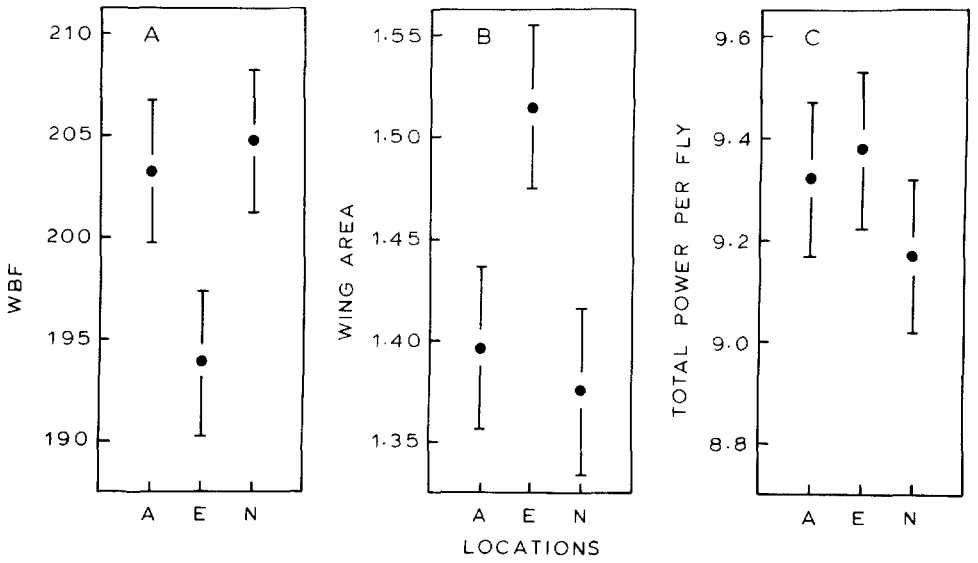

FIGURE 5.—The effect of location (continent) of origin. A, Wingbeat frequency (sec^{-1}); N = 1296 per mean. B, Wing area (mm^2); N = 432 per mean. C, Total power output (microwatts) using ELLINGTON's model; N = 1296 per mean. Locations are noted as Australia (A), Europe (E) and North America (N). The 95% C.I. are given (two-tailed test).

is not significantly different from the other two locations, based on TUKEY's minimum significant difference of 0.25 microwatts).

This complementation of *WBF* and wing size/shape could be the result of selection favoring an intermediate optimum in flight phenotype, such that genetic variation causing larger wing size is offset by selection for genetic variation decreasing *WBF*. Alternatively, this effect could be due to the wings and flight muscle acting as a mechanical oscillator (GREENEWALT 1960), such that the physical constraints on the flight mechanism would automatically adjust the *WBF* according to the size of the wings, irregardless of the genetic basis for either character. A somewhat similar effect was noted by LAURIE-AHLBERG *et al.* (1985) for *WBF* and *WA*, in that a significant negative correlation existed between these two variables across isogenic chromosome substitution line means. It was suggested that these two variables could provide a certain trade-off in the power budget, such that some homeostasis is provided with respect to total power output. However, *WBF* remained highly positively correlated with total power across line means, whereas *WA* did not. CURTSINGER and LAURIE-AHLBERG (1981) demonstrated a within-line negative correlation between *WBF* and *WA* as well. This effect could represent physiological homeostasis, because there is no genetic variation within these lines. Perhaps the flies always put out the maximum possible power, so that, when one of the flight components changes, the others must compensate. In the latter study, there was no correlation between these two variables across lines. In neither of the two cases were wing size/shape and *WBF* negatively related, which argues against the mechanical oscillator explanation, whereas both studies

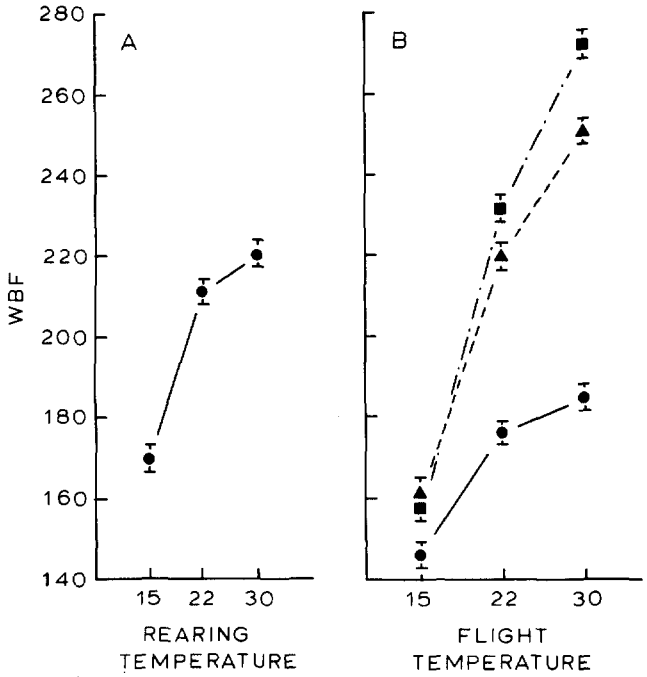

FIGURE 6.—The effects of environmental temperature on wingbeat frequency (sec^{-1}). A, Rearing temperature; N = 1296 per mean. B, Rearing-by-flight temperature interaction, where rearing temperatures are symbolized as 15° (●), 22° (▲) and 30° (■); N = 432 per mean. The 95% C.I. are shown (two-tailed test).

showed a significant genetic component affecting *WBF* and wing size/shape as in this study, supporting the intermediate optimum explanation.

As with all the other variables examined, *WBF* and *AR* show highly significant effects due to genetic background. These genetic background effects are dependent on interaction with environmental factors, as also observed for live weight.

Relationship between wingbeat frequency and power output: *WBF* is usually highly correlated with mechanical power output (CURTSINGER and LAURIE-AHLBERG 1981; LAURIE-AHLBERG *et al.* 1985). This conclusion was drawn from studies utilizing only a single environmental temperature (25°). However, examples showing that *WBF* is not always predictive of the power relationships, especially across large environmental differences, are shown in Figure 6 for the rearing temperature effect and the rearing-by-flight temperature interaction. When compared to the same effects shown in Figure 2 for total power, the conclusions concerning environmental effects and physiological adaptation would be quite different if the wing morphology variables contributing to power output were ignored. In particular, flies raised at 15° always had significantly lower *WBF* than flies raised at 22 or 30°; yet, they clearly generated more power for flight at 15° because of their larger wings. (The wing size/shape variables showed a significant inverse relationship with rearing temperature, similar to weight.) At higher flight temperatures, the larger wings ap-

TABLE 8

Comparison among means for the rearing temperature-by-allotype-by-flight temperature interaction for wingbeat frequency and total power output

Rearing temperature	Flight temperature		
	15°	22°	30°
		WBF	
15°	*SS* > *FF*	NS	*SS* > *FF*
	NS	*FS* > *FF*	*FS* > *FF*
	NS	NS	NS
22°	NS	NS	*SS* < FF
	FS > *FF*	NS	NS
	NS	NS	*FS* > *SS*
30°	NS	NS	*SS* < *FF*
	NS	NS	NS
	NS	NS	NS
		Total power	
15°	*SS* > *FF*	*SS* > *FF*	*SS* > *FF*
	FS > *FF*	*FS* > *FF*	NS
	NS	NS	*FS* < *SS*
22°	*SS* > *FF*	*SS* > *FF*	*SS* < *FF*
	FS > *FF*	NS	NS
	NS	NS	*FS* > *SS*
30°	NS	*SS* > *FF*	*SS* < *FF*
	NS	NS	*FS* < *FF*
	NS	*FS* < *SS*	NS

The LSD method was used for *WBF*, and TUKEY'S method was used for total power. Comparisons are in the order *SS* vs. *FF*, *FS* vs. *FF* and *FS* vs. *SS*. Significant differences between means and their directions are indicated; NS = nonsignificant difference. Powers were calculated according to ELLINGTON'S method (see text).

parently became a detriment, and a higher *WBF* with smaller wings was more advantageous.

Within each temperature level, however, the correlation between *WBF* and total power may still be maintained. For example, although the rearing temperature-by-allotype and flight temperature-by-allotype interactions for *WBF* were not significant, the rank orders of the *WBF* means within the temperature classes of both interactions were the same as for total power (see Figure 3), with one exception. This exception was for flight at 22°, where the heterozygote showed the highest *WBF*. In all these cases, however, none of the differences between means were significant.

Analysis of the three-way interaction among Gpdh allotype, rearing and flight temperatures: Although the rearing temperature-by-allotype-by-flight temperature interaction ($R \times A \times F$) was not significant for power output, the fact that it was for *WBF* prompted an analysis of both sets of means. The most important aspect of this analysis, shown in Table 8, is that, for flies raised at

15° and flown at 15°, the $Gpdh^S$ homozygotes have higher values than the $Gpdh^F$ homozygotes; whereas for flies raised at 30° and flown at 30°, individuals homozygous for the $Gpdh^F$ allele have higher values than the other two allotypes. The results are very consistent between *WBF* and total power, as might be expected because the means are taken over locations and within each of the major environmental temperature classes. In addition, the results are internally consistent within each cell defined by the rearing-by-flight temperature matrix. That is, if the $Gpdh^S$ homozygote has higher *WBF* or power than the $Gpdh^F$ homozygote, the heterozygote never has significantly lower values than the $Gpdh^F$ homozygote. One might expect such examples of apparent heterozygote disadvantage to exist if random variation was causing some of the differences. It is also noteworthy that the only two instances where the $Gpdh^F$ allele shows greater flight ability is at 30° flight temperatures. That the $Gpdh^S$ allele shows higher values at most other temperature combinations is consistent with the generally higher activity levels associated with this allele. Perhaps at high flight temperatures involving flies raised at similarly high temperatures, the *in vivo* functional aspects of the allozymes are different from those simply estimated by *in vitro* activity levels under conditions of substrate saturation.

DISCUSSION

The results of this experiment demonstrate differential effects of the *Gpdh* allozymic genotypes on the mechanical power output of the flight muscles that are dependent on both rearing and flight temperatures. The allotype effects are small in magnitude compared with genetic background effects, amounting to only a few percent difference in the means in even the most significant cases. Nevertheless, they are detectable against a naturally heterozygous genetic background. When flies are reared at 15°, slow homozygotes have significantly greater power output than fast homozygotes at all flight temperatures. At the 22 and 30° rearing temperatures, the direction of the difference between homozygotes varies with flight temperature. In general, the *S/F* heterozygotes have a power output intermediate between the two homozygotes. These results are consistent with the direction of the latitudinal clines and seasonal changes in allozyme frequency in nature, in that the slow homozygote has higher power for flies reared and flown at 15°, whereas the fast homozygote has higher power for flies reared and flown at 30°. However, it should be noted that a direct role of temperature in maintenance of the latitudinal allozyme frequency clines has not been demonstrated (see OAKESHOTT *et al.* 1982) and that temporal changes have been observed only for populations in the northeastern United States (BERGER 1971; CAVENER and CLEGG 1981).

Whether or not the temperature-dependent effects of the allotypes on power output are consistent with their biochemical properties cannot be decided at this time for lack of appropriate data. In this study the GPDH activity measurements (V_{max} estimates) are based on an *in vitro* assay under optimal conditions, including saturating substrate concentration and a constant 29° reaction temperature. Because activities were estimated from single fly homogenates,

there was insufficient material to investigate the effect of different reaction temperatures. Furthermore, it should be emphasized that the substrate concentrations and other assay conditions are not likely to reflect the situation *in vivo*. Nevertheless, the significant main effect of allotype on GPDH activity level demonstrates that the allozymes differ in biochemical properties under some conditions, and some inferences can be made by comparing power output at the 30° flight temperature with the activity data (measured at 29°). Even though the slow homozygote has a higher activity than the fast homozygote at all rearing temperatures, the direction of the difference in power output at the 30° flight temperature varies with rearing temperature. Among flies reared at 15°, the slow homozygote has higher power output than the fast homozygote, whereas the reverse is true for flies reared at 22 or 30°. Thus, the V_{max} estimates obtained in this study do not account for the apparent functional differences *in vivo*, but this is not surprising because there are many arguments against the use of V_{max} for this purpose. See ZERA, KOEHN and HALL (1985) and WATT (1985) for detailed discussions of the problems in predicting *in vivo* function from kinetic parameters estimated *in vitro*.

The significant differences among allotypes in GPDH activity (V_{max}) observed in this study could be due to differences in catalytic efficiency and/or enzyme concentration. The flight temperature-dependent effects of allotype on power output are difficult to account for on the basis of concentation differences alone. Among flies reared at 22 or 30°, the direction of the difference between slow and fast homozygotes varies with flight temperature. Since an individual fly was measured at different flight temperatures within a very short time period (generally less than a minute), the changing effects on power are very unlikely to be due to changing enzyme concentration. Nevertheless, it is important to note that the effect of a fixed enzyme concentration difference may vary over flight temperatures, since the kinetics of other enzymes in the pathway may change. As discussed at length by KACSER and BURNS (1973, 1981), the effect of a change in concentration of one enzyme in a pathway on the flux (its sensitivity coefficient) depends on the kinetic parameters of all other enzymes in the system. In this case, however, a change in the sign of the sensitivity coefficient is required, which seems unlikely. Therefore, the power output results suggest temperature-dependent differences in kinetic parameters between the allozymes.

Temperature-dependent differences in V_{max} and K_m (for dihydroxyacetone phosphate) among the allotypes of GPDH in *D. melanogaster* have been reported by MILLER, PEARCY and BERGER (1975). The differences found are in accord with the direction of the patterns found in nature if variation at the locus is leading to some form of thermal adaptation. However, their estimates were obtained from partially purified adult homogenates and involved a mixture of the two major isozyme forms found in adults (GPDH-1, found in the flight muscle, and GPDH-3, found in the fat body). These two isozymes are known to differ extensively in certain biochemical parameters (BEWLEY and MILLER 1979), and the differences reported by MILLER, PEARCY and BERGER (1975) could have resulted from different mixtures of the two isozymes in the

lines used in the study. More recently BEWLEY, NIESEL and WILKINS (1984) reported no temperature-dependent differences in kinetic properties between the *F* and *S* homodimeric forms of GPDH-1 purified to homogeneity. Although their values for each kinetic parameter were based on only two determinations, the rank order of the values did not change between 15 and 30°. In a preliminary report of possible kinetic differences among allozymes of the larval isozymes (GPDH-3) derived from crude extracts, MCKECHNIE, KOHANE and PHILLIPS (1981) reported an apparent case of heterozygote disadvantage at 28° and a slight heterozygote advantage at 16°, although no standard errors were given or statistical analyses performed. Similar discrepancies among thermal stability studies of the homo- and heterodimeric forms of the enzyme exist. Most studies report no differences among allozymes or allotypes (MILLER, PEARCY and BERGER 1975; BEWLEY 1978; BEWLEY, NIESEL and WILKINS 1984). However, ALAHIOTIS, MILLER and BERGER (1977) reported that the $Gpdh^S$ allozyme had greater thermal stability than the $Gpdh^F$ allozyme, which is in the opposite direction of the pattern of variation observed in nature. Each of these studies used different assay conditions and different degrees of purity of the enzymes. Thus, the disparity of the results is not surprising. Clearly, what is needed is a complete and rigorous analysis of the kinetic properties of the purified allozymes of the GPDH-1 and GPDH-3 isozymes in *D. melanogaster*, under an experimental design supplying some statistical power. Other studies that have combined careful determination of kinetic parameters with a knowledge of the physiological role that the enzyme plays in affecting some measurable phenotypic trait have been quite successful in explaining observed geographic or temporal patterns in allozyme polymorphisms in terms of natural selection (POWERS, DIMICHELE and PLACE 1983; WATT 1983; WATT, CASSIN and SWAN 1983).

The significant effects of genetic background on both GPDH activity and power output are consistent with earlier work using isogenic chromosome substitution lines, in which strong second and third chromosome effects on both variables were observed (LAURIE-AHLBERG *et al.* 1985). Most, if not all, of the GPDH activity variation among both second and third chromosome isogenic lines is accounted for by variation in enzyme concentration estimated immunologically (LAURIE-AHLBERG and BEWLEY 1983), but allozymic variation was not included in that analysis. In this study, there was no correlation between GPDH activity and power output over genetic backgrounds within each temperature regime. The general lack of a significant association between these two variables was also found in the isogenic line experiments. However, the fact that in one of those experiments, using third chromosome lines, there was a significant positive correlation, and the fact that the other experiments with second chromosome lines showed positive correlation estimates, suggests that there may actually be a weak association between GPDH variation caused by modifier genes and power output.

The case for thermal adaptation with respect to the *Gpdh* allozyme polymorphism has been investigated in other studies using adult survival and fertility, larval viability or developmental rate. Long-term exposure to tempera-

tures between 15 and 30° and short-term exposure to extreme heat or cold stress, as well as selection for fast and slow development, have been used. The physiological basis that is assumed to underlie the effects of these experiments is that GPDH may also affect the production of α-glycerophosphate for subsequent lipid biosynthesis or cryoprotectant (glycerol) production. However, the specificity of this function involving GPDH physiologically is not well understood. The results vary from study to study and have shown evidence both for (McKechnie, Kohane and Phillips 1981; Cavener 1983; Oakeshott, Wilson and Parnell 1985) and against (Milkman 1977; Oakeshott 1979; Schenfeld and McKechnie 1979) significant differences among *Gpdh* allotypes. Recently, Oakeshott, Wilson and Parnell (1985) have shown that some of these differences are apparently dependent on the genetic background of the population from which the study material was derived. It is not clear to what extent the inconsistencies among these studies are due to specific interactions of the *Gpdh* locus with background genotype or to linkage disequilibrium with associated loci. In the study reported here, all the phenotypic characters used as variables in calculating total power output, as well as the power output itself, have shown significant genetic background effects. Significant population effects (continental differences) with respect to *WBF* and wing size/shape characters also were found. Nevertheless, this diversity of background variation did not obscure the allotype-by-environmental temperature interaction effects on power output, which appear to be general phenomena associated with allozyme variation at the *Gpdh* locus. Even in this case, though, the effects on power output cannot unequivocally be attributed to the allozyme variation because, as DNA sequence and restriction site variation data at the alcohol dehydrogenase (*Adh*) locus have shown, linkage disequilibrium in *D. melanogaster* can exist on a world-wide basis in the region around a single locus (Kreitman 1983; C. F. Aquadro, S. F. Deese, M. M. Bland, C. H. Langley and C. C. Laurie-Ahlberg, unpublished results).

In conclusion, the results of this study show that the allozymic variation at the *Gpdh* locus contributes what can be considered a minor polygenic effect on quantitative variation in the power output during flight and that genotype-by-environment interaction is an important component of that effect. It is not clear whether such small effects (on the order of a few percent) have any important consequences in terms of the forces that control the allozyme frequencies in nature. Small power differences might be inconsequential, but it is conceivable that they could make the difference between flight *vs.* nonflight at temperature extremes or that they contribute to maneuverability variation in air currents. Thus, even though it is not clear whether the power effects reported here contribute substantially to maintenance of the latitudinal cline or to the temporal variation in allozyme frequencies, the results indicate that *Gpdh* allozyme variation has phenotypic consequences beyond the enzyme level and contributes to the pool of variability that can serve as the material for directional evolutionary changes in flight metabolism. Investigation of the polymorphic *Gpdh* locus and its relevance to adaptation can continue in the laboratory with biochemical studies of the allozymes, as well as by using cloned

DNA sequences (*i.e*, the *Gpdh* structural locus) and *P*-element mediated transformation (RUBIN and SPRADLING 1982) to manipulate experimentally the activity (concentration) of GPDH from zero to "wild-type" levels. It is clear that the laboratory studies will continue to require some complementary studies of the biological relevance of these effects in natural populations.

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LITERATURE CITED

ALAHIOTIS, S., S. MILLER and E. BERGER, 1977 Natural selection at the α-GDH locus in *Drosophila.* Nature **269:** 144–145.

ALEXANDER, R. M., 1977 Flight. pp. 249–278. In: *Mechanics and Energetics of Animal Locomotion,* Edited by R. M. ALEXANDER and G. GOLDSPINK. Chapman & Hall, London.

ALEXANDER, R. M., 1984 *Animal Mechanics.* Blackwell Scientific Publications, Oxford.

ASHBURNER, M. and F. LEMEUNIER, 1976 Relationships within the *melanogaster* species subgroup of the genus *Drosophila* (*Sophophora*). I. Inversion polymorphisms in *Drosophila melanogaster* and *Drosophila simulans.* Proc. R. Soc. Lond. (Biol.) **193:** 137–157.

BERGER, E., 1971 A temporal survey of allelic variation in natural and laboratory populations of *Drosophila melanogaster.* Genetics **67:** 121–136.

BEWLEY, G. C., 1978 Heat stability studies at the α-glycerophosphate dehydrogenase locus in populations of *Drosophila melanogaster.* Biochem. Genet. **16:** 769–775.

BEWLEY, G. C. and S. G. MILLER, 1979 Origin and differentiation of the soluble α-glycerolphosphate dehydrogenase isozymes in *Drosophila melanogaster.* pp. 23–52. In: *Isozymes: Current Topics in Biological and Medical Research,* Vol. 3, Edited by M. C. RATTAZZI, J. G. SCANDALIOS and G. S. WHITT. Alan R. Liss, New York.

BEWLEY, G. C., D. W. NIESEL and J. R. WILKINS, 1984 Purification and characterization of the naturally occurring allelic variants of *sn*-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster.* Comp. Biochem. Physiol. **79B:** 23–32.

CAVENER, D. R., 1983 The response of enzyme polymorphisms to developmental rate selection in *Drosophila melanogaster.* Genetics **105:** 105–113.

CAVENER, D. R. and M. T. CLEGG, 1981 Temporal stability of allozyme frequencies in a natural population of *Drosophila melanogaster.* Genetics **90:** 629–644.

CHADWICK, L. E., 1947 The respiratory quotient of *Drosophila* in flight. Biol. Bull. (Woods Hole) **93:** 229–239.

CHADWICK, L. E., 1953 The motion of the wings. pp. 577–614. In: *Insect Physiology,* Edited by K. D. ROEDER. John Wiley & Sons, New York.

COLLIER, G. E., 1977 Inter- and intraspecific variation of α-glycerophosphate dehydrogenase among *Drosophila.* Isozyme Bull. **10:** 58–60.

COYNE, J. A., W. F. EANES, J. A. M. RAMSHAW and R. K. KOEHN, 1979 Electrophoretic heterogeneity of α-glycerophosphate dehydrogenase among many species of *Drosophila.* Syst. Zool. **28:** 164–175.

CRABTREE, B. and E. A. NEWSHOLME, 1975 Comparative aspects of fuel utilization and metabolism by muscle. pp. 405–500. In: *Insect Muscle*, Edited by P. N. R. USHERWOOD. Academic Press, London.

CURTSINGER, J. W. and C. C. LAURIE-AHLBERG, 1981 Genetic variability of flight metabolism in *Drosophila melanogaster*. I. Characterization of power output during tethered flight. Genetics **98:** 549–564.

ELLINGTON, C. P., 1984a–f The aerodynamics of hovering insect flight. (a) I. The quasi-steady analysis. pp. 1–15. (b) II. Morphological parameters. pp. 17–40. (c) III. Kinematics. pp. 41–78. (d) IV. Aerodynamic mechanisms. pp. 79–113. (e) V. A vortex theory. pp. 115–144. (f) VI. Lift and power requirements. pp. 145–181. Philos. Trans. R. Soc. London. [Biol.] **305:** 1–181.

GREENEWALT, C. H., 1960 The wings of insects and birds as mechanical oscillators. Proc. Am. Philos. Soc. **104:** 605–611.

JOHNSON, F. M. and H. E. SCHAFFER, 1973 Isozyme variability in species of the genus *Drosophila*. VII. Gentoype-environment relationships in populations of *D. melanogaster* from the Eastern United States. Biochem. Genet. **10:** 149–163.

KACSER, H. and J. A. BURNS, 1973 The control of flux. Symp. Soc. Exp. Biol. **27:** 65–104.

KACSER, H. and J. A. BURNS, 1981 The molecular basis of dominance. Genetics **97:** 639–666.

KAMMER, A. E. and B. HEINRICH, 1978 Insect flight metabolism. Adv. Insect Physiol. **13:** 133–228.

KIMURA, M., 1983 *The Neutral Theory of Molecular Evolution*. Cambridge University Press, New York.

KOTARSKI, M. A., S. PICKERT, D. A. LEONARD, G. J. LAROSA and R. J. MACINTYRE, 1983 The characterization of α-glycerophosphate dehydrogenase mutants in *Drosophila melanogaster*. Genetics **105:** 387–407.

KREITMAN, M., 1983 Nucleotide polymorphism at the alcohol dehydrogenase locus of *Drosophila melanogaster*. Nature **304:** 412–417.

LAKOVAARA, S. and L. KERÄNEN, 1980 Variation at the *α-Gpdh* locus of drosophilids. Hereditas **92:** 251–258.

LAKOVAARA, S., A. SAURA and P. LANKINEN, 1977 Evolution at the *α-Gpdh* locus in Drosophilidae. Evolution **31:** 319–330.

LAURIE-AHLBERG, C. C., P. T. BARNES, J. W. CURTSINGER, T. H. EMIGH, B. KARLIN, R. MORRIS, R. A. NORMAN and A. N. WILTON, 1985 Genetic variability of flight metabolism in *Drosophila melanogaster*. II. Relationship between power output and enzyme activity levels. Genetics **111:** 845–868.

LAURIE-AHLBERG, C. C. and G. C. BEWLEY, 1983 Naturally occurring genetic variation affecting the expression of *sn*-glycerol-3-phosphate dehydrogenase in *Drosophlia melanogaster*. Biochem. Genet. **21:** 943–961.

LAURIE-AHLBERG, C. C., G. MARONI, G. C. BEWLEY, J. C. LUCCHESI and B. S. WEIR, 1980 Quantitative genetic variation of enzyme activities in natural populations of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **77:** 1073–1077.

LEWONTIN, R. C., 1974 *The Genetic Basis of Evolutionary Change*. Columbia University Press, New York.

MAXWORTHY, T., 1981 The fluid dynamics of insect flight. Annu. Rev. Fluid Mech. **13:** 329–350.

MCKECHNIE, S. W., M. KOHANE and S. C. PHILLIPS, 1981 A search for interacting polymorphic enzyme loci in *Drosophila melanogaster*. pp. 121–138. In: *Genetic Studies of Drosophila Populations*, Edited by J. B. GIBSON and J. G. OAKESHOTT. Australian National University, Canberra.

MILKMAN, R., 1977 Temperature shock selection at three allozyme loci. Drosophila Inform. Serv. **52:** 58.

MILLER, S., R. W. PEARCY and E. BERGER, 1975 Polymorphism at the α-glycerophosphate dehydrogenase locus in *Drosophila melanogaster*. I. Properties of adult allozymes. Biochem. Genet. **13:** 175–188.

NETER, J. and W. WASSERMAN, 1974 *Applied Linear Statistical Models.* Richard D. Irwin, Inc., Homewood, Illinois.

OAKESHOTT, J. G., 1979 Selection affecting enzyme polymorphisms in laboratory populations of *Drosophila melanogaster*. Oecologia (Berl.) **43:** 341–354.

OAKESHOTT, J. G., J. B. GIBSON, P. R. ANDERSON, W. R. KNIBB, D. G. ANDERSON and G. K. CHAMBERS, 1982 Alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase clines in *Drosophila melanogaster* on different continents. Evolution **36:** 86–96.

OAKESHOTT, J. G., S. W. MCKECHNIE and G. K. CHAMBERS, 1984 Population genetics of the metabolically related *Adh*, *Gpdh* and *Tpi* polymorphisms in *Drosophila melanogaster*. I. Geographic variation in *Gpdh* and *Tpi* allele frequencies in different continents. Genetica **63:** 21–29.

OAKESHOTT, J. G., S. R. WILSON and P. PARNELL, 1985 Selective effects of temperature on some enzyme polymorphisms in laboratory populations of *Drosophila melanogaster*. Heredity. In press.

O'BRIEN, S. J. and R. J. MACINTYRE, 1972 The α-glycerophosphate cycle in *Drosophila melanogaster*. II. Genetic aspects. Genetics **71:** 127–138.

O'BRIEN, S. J. and R. J. MACINTYRE, 1978 Genetics and biochemistry of enzymes and specific proteins of *Drosophila*. pp. 396–551. In: *The Genetics and Biology of Drosophila*, Vol. 2a, Edited by M. ASHBURNER and T. R. F. WRIGHT. Academic Press, New York.

O'BRIEN, S. J. and Y. SHIMADA, 1974 The α-glycerophosphate cycle in *Drosophila melanogaster*. IV. Metabolic ultrastructure and adaptive consequences of *αGpdh-1* "null" mutations. J. Cell Biol. **63:** 864–882.

POWERS, D. A., L. DIMICHELE and A. R. PLACE, 1983 The use of enzyme kinetics to predict differences in cellular metabolism, developmental rate and swimming performance between LDH-B genotypes of the fish, *Fundulus heteroclitus*. pp. 147–170. In: *Isozymes: Current Topics in Biological and Medical Research*, Vol. 10, Edited by M. C. RATTAZZI, J. G. SCANDALIOS and G. S. WHITT. Alan R. Liss, New York.

RUBIN, G. M. and A. C. SPRADLING, 1982 Genetic transformation of *Drosophila* with transposable element vectors. Science **218:** 348–353.

SACKTOR, B., 1965 Energetics and respiratory metabolism of muscular contraction. pp. 483–580. In: *Physiology of Insecta*, Vol. II, Edited by M. ROCKSTEIN. Academic Press, New York.

SACKTOR, B., 1975 Biochemistry of insect flight. pp. 3–88. In: *Insect Biochemistry and Function*, Edited by D. J. CANDY and B. A. KILBY. John Wiley & Sons, New York.

SCHENFELD, E. and S. W. MCKECHNIE, 1979 The α-glycerophosphate dehydrogenase (α-Gpdh) polymorphism in *Drosophila melanogaster*: adult survival under temperature stress. Theor. Appl. Genet. **54:** 235–237.

SEARLE, S. R., 1971 *Linear Models.* John Wiley & Sons, New York.

SNEDECOR, G. W. and W. G. COCHRAN, 1967 *Statistical Methods*, Ed. 6. Iowa State University Press, Ames, Iowa.

WATT, W. B., 1983 Adaptation at specific loci. II. Demographic and biochemical elements in the maintenance of the *Colias* PGI polymorphism. Genetics **103:** 691–724.

WATT, W. B., 1985 Allelic isozymes and the mechanistic study of evolution. pp. 89–132. In:

Isozymes: Current Topics in Biological and Medical Research, Vol. 12, Edited by M. C. RATTAZZI, J. G. SCANDALIOS and G. S. WHITT. Alan R. Liss, New York.

WATT, W. B., R. C. CASSIN and M. S. SWAN, 1983 Adaptation at specific loci. III. Field behavior and survivorship differences among *Colias* PGI genotypes are predictable from *in vitro* biochemistry. Genetics **103:** 725–739.

WEIS-FOGH, T., 1972 Energetics of hovering flight in hummingbirds and in Drosophila. J. Exp. Biol. **56:** 79–104.

WEIS-FOGH, T., 1973 Quick estimates of flight fitness in hovering animals, including novel mechanisms for lift production. J. Exp. Biol. **59:** 169–230.

WIGGLESWORTH, V. B., 1949 The utilization of reserve substances in *Drosophila* during flight. J. Exp. Biol. **26:** 150–163.

ZERA, A. J., R. K. KOEHN and J. G. HALL, 1985 Allozymes and biochemical adaptation. pp. 81–112. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 10, Edited by G. A. KERKUT and L. I. GILBERT. Pergamon Press, New York.

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