# GENETIC VARIABILITY OF FLIGHT METABOLISM IN *DROSOPHILA MELANOGASTER*. II. RELATIONSHIP BETWEEN POWER OUTPUT AND ENZYME ACTIVITY LEVELS

C. C. LAURIE-AHLBERG, P. T. BARNES,[1] J. W. CURTSINGER,[2] T. H. EMIGH, B. KARLIN, R. MORRIS, R. A. NORMAN[3] AND A. N. WILTON[4]

*Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695*

Manuscript received November 18, 1984
Revised copy accepted August 8, 1985

ABSTRACT

The major goal of the studies reported here was to determine the extent to which genetic variation in the activities of the enzymes participating in flight metabolism contributes to variation in the mechanical power output of the flight muscles in *Drosophila melanogaster*. Isogenic chromosome substitution lines were used to partition the variance of both types of quantitative trait into genetic and environmental components. The mechanical power output was estimated from the wingbeat frequency, wing amplitude and wing morphology of tethered flies by applying the aerodynamic models of WEIS-FOGH and ELLINGTON. There were three major results. (1) Chromosomes sampled from natural populations provide a large and repeatable genetic component to the variation in the activities of most of the 15 flight metabolism enzymes investigated and to the variation in the mechanical power output of the flight muscles. (2) The mechanical power output is a sensitive indicator of the rate of flight metabolism (*i.e.*, rate of oxygen consumption during tethered flight). (3) In spite of (1) and (2), no convincing cases of individual enzyme effects on power output were detected, although the number and sign of the significant enzyme-power correlations suggests that such effects are not totally lacking.

THIS is the second report on a series of studies, the goal of which is to determine how naturally occurring variation in the structure or regulation of flight metabolism enzymes contributes to variation in the mechanical power output of the flight muscles of *Drosophila melanogaster*. Because of extensive background information summarized below, the flight system of a Dipteran such as Drosophila provides an excellent opportunity to investigate the physiological effects of genetic variation detected at the biochemical/molecular level. Furthermore, the power output of the flight muscles is, at least potentially, a fitness-related phenotype. Since flight behavior is an integral part of feeding, mating, dispersal and oviposition, it is likely that variation in power

[1] Present address: Zoology Department, Connecticut College, New London, Connecticut 06320.
[2] Present address: Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108.
[3] Present address: Department of Zoology, Arizona State University, Tempe, Arizona 85287.
[4] Present address: Department of Clinical Immunology, Royal Perth Hospital, Perth 6001, Western Australia.

output is ultimately related to variation in reproductive success. Therefore, these studies provide one type of approach to understanding the evolutionary significance of enzyme variability, a long-standing problem in population genetics (LEWONTIN 1974; KIMURA 1983). Related approaches involving insect flight have been used by WATT and collaborators to understand the effects of the allozymes of phosphoglucose isomerase on flight behavior of Colias in the field (WATT 1983; WATT, CASSIN and SWAN 1983) and by CLARK *et al.* (1983) to investigate the effects of *sn*-glycerol-3-phosphate dehydrogenase allozymes on glycolytic intermediates in *Drosophila mercatorum.*

Dipteran flight metabolism has been the subject of extensive physiological investigation, so the biochemical pathw .ys by which energy reserves are transformed into ATP for sustaining flight are well defined (SACKTOR 1975; CRABTREE and NEWSHOLME 1975). Several lines of evidence lead to the conclusion that only carbohydrates are used as the fuel for flight in Drosophila, as in most Dipterans (CHADWICK 1947; WIGGLESWORTH 1949; and others reviewed by SACKTOR 1965). The major storage carbohydrates, glycogen and trehalose, are metabolized via glycolysis, the $\alpha$-glycerophosphate cycle, the Krebs cycle and the mitochondrial respiratory chain. The $\alpha$-glycerophosphate cycle constitutes a shuttle system whereby reducing equivalents from the cytosolic pool of NADH pass the mitochondrial barrier, thus regenerating $NAD^+$ for the glyceraldehyde-3-phosphate dehydrogenase reaction. This shuttle and the extremely efficient tracheolar system that delivers oxygen to the flight muscles allow flight metabolism to proceed entirely aerobically for extended periods (see SACKTOR 1975; KAMMER and HEINRICH 1978). Thus, Drosophila does not acquire an oxygen debt even after prolonged flight (CHADWICK 1947, 1953). Furthermore, the oxidative metabolism of insect flight muscle provides the highest metabolic rates known for any tissue in any organism (SACKTOR 1965). These observations have two important consequences for the objectives of this study: (1) The rate of oxygen consumption during flight, which can be measured with relatively simple methods, should be directly proportional to the rate of ATP production (*i.e.*, flux through glycolysis and the Krebs cycle). (2) Since insect flight evidently demands extremely high metabolic rates, those rates may provide a very sensitive measure for discriminating *in vivo* functional differences among enzyme variants.

The biomechanics of insect flight has also been the subject of extensive investigation (see ALEXANDER 1983), which provides a basis for estimation of the mechanical power imparted to the wings during flight. The power budget for a flying animal is outlined diagramatically in Figure 1. Some of the metabolic power input is converted to mechanical power output, which is the total power expended as a result of wing motion, but most of the input is lost as heat due to muscular inefficiency. The mechanical power output is divided into two major parts: (1) inertial power, that required to accelerate and decelerate the wings at the top and bottom of each stroke; and (2) aerodynamic power, that required to move the wings and body through the air. The aerodynamic power is further divided into three parts: (1) profile power, that required to overcome pressure drag and frictional drag of the wing profile;

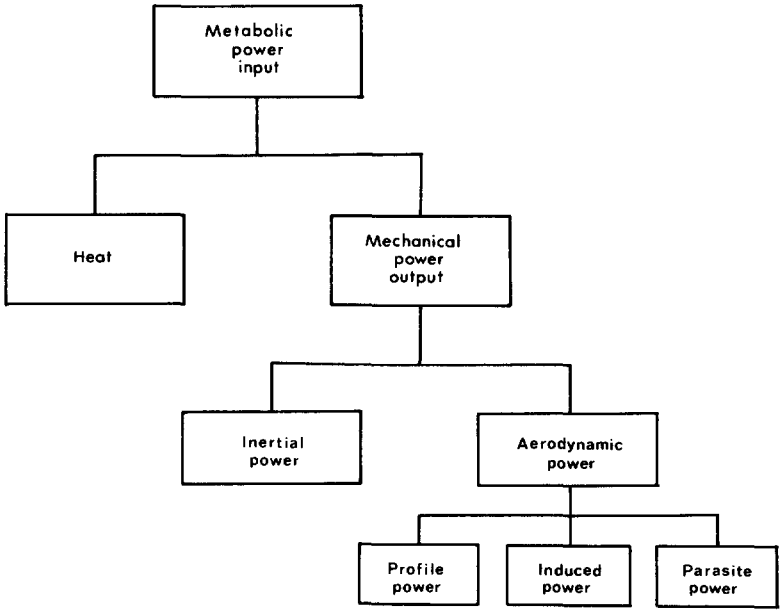


FIGURE 1.—Power expenditure in flapping flight (from CASEY 1981).

(2) induced power, that required to overcome the drag that depends on the induced velocity of the wake associated with circulatory lift; and (3) parasite power, that required to overcome drag on the body as it moves through the air. Parasite drag is generally ignored in analyses of hovering flight. See VOGEL (1981), ALEXANDER (1983) and ELLINGTON (1984f) for further discussion of these concepts.

WEIS-FOGH (1972, 1973) derived formulas for lift and power during hovering flight by using a quasi-steady-state assumption (*i.e.*, ignoring unsteady, rotational effects) and by further assuming that the wings exhibit simple harmonic motion. His results indicated that the simplified model adequately satisfies the lift requirements of many insects, including *Drosophila virilis*, but he noted that unsteady effects were certainly not ruled out. In fact, WEIS-FOGH was the first to discuss the implications of the clap, flip and fling movements that occur during pronation and supination in many insects, including Drosophila. Recently, ELLINGTON (1984a–f) has improved on WEIS-FOGH's approach by including some unsteady effects in the analysis and by obtaining kinematic data for several insects. ELLINGTON's approach leads to substantial improvements in the lift analysis, but the power requirements are only slightly affected. In this paper we use both WEIS-FOGH's and ELLINGTON's power formulas and compare them with respect to their relationship to the rate of oxygen consumption during flight (*i.e.*, the power input). As expected, the correlations between oxygen consumption and each of the various types of power output estimates are very similar.

The first paper of this series (CURTSINGER and LAURIE-AHLBERG 1981) provided a characterization of aerodynamic and inertial powers of *D. melanogaster*

using WEIS-FOGH's methods. The powers were calculated from the wingbeat frequency (*WBF*) and wing amplitude (*WA*) of tethered flies and from the size and shape of the wings. There were three main results: (1) The flight variables are subject to genetic variation due to differences among chromosomes derived from natural populations, (2) the line differences are affected very little by age, ambient temperature and duration of flight and (3) the wingbeat frequency, which is the primary determinant of variation in power output, is highly correlated with the rate of oxygen consumption. These results encouraged us to extend the study to investigate the relationship between power output and enzyme variability.

The study reported in this paper deals primarily with quantitative genetic variation of enzyme activity levels, which, in many cases, is due to variation of modifier genes that are not linked to the structural locus of the affected enzyme (LAURIE-AHLBERG *et al.* 1980, 1982). Our basic approach to quantifying the amount of genetic variation of enzyme activity in natural populations is to view activity as a quantitative trait and to partition its variance into genetic and environmental components with standard biometrical methods. This partitioning is facilitated by the use of isogenic lines, so that genotypes can be replicated over environments. In order to localize activity variants, two sets of homozygous lines were constructed in which either second or third chromosomes from natural populations were substituted into an isogenic background. Within the set of second chromosome substitution lines, for example, all *X* and third chromosome loci are constant, but second chromosome loci vary. This design permits detection of activity variants that are not linked to the structural locus of the affected enzyme and can therefore easily identify one type of modifier locus. So far we have screened our 50 second- and 50 third-chromosome lines for variation in the activities of 26 different enzymes and have found a significant genetic component to the variation of all 26 in one or both sets of lines. Of the 22 enzymes for which the structural gene has been localized, 18 have shown clear evidence for genetic variation of unlinked modifiers (LAURIE-AHLBERG *et al.* 1980, 1982; LAURIE-AHLBERG 1982). Of the 26 enzymes analyzed to date, 23 are included in the present study (Table 1). These include 15 enzymes clearly involved in flight metabolism as well as eight other "control" enzymes. The major purpose of the present study is to determine whether genetic variation in the activity levels of these enzymes (measured under optimal conditions *in vitro*) contributes to variation in the mechanical power output during tethered flight.

Five experiments are reported here. Experiment I was designed to investigate the relationship between the rate of oxygen consumption during flight and various different types of power output estimates, as mentioned above. Experiment II was a pilot experiment involving power output estimates and four enzyme activities on 21 isogenic second-chromosome lines. A description of the flight parameter measurements and power estimates from this experiment has been published (CURTSINGER and LAURIE-AHLBERG 1981). Experiments IIIa and b were much larger experiments involving power output estimates and 23 enzyme activities on 48 second- and 48 third-chromosome iso-

TABLE 1

*Enzymes assayed in this study*

| Enzymes | Abbreviation | EC No. | Map position[a] |
|---|---|---|---|
| I. Flight Metabolism | | | |
| A. Conversion of carbohydrate reserves to glucose-6-phosphate | | | |
| 1. Trehalase | TRE | 3.2.1.28 | *2R* |
| 2. Hexokinase | HEX | 2.7.1.1 | *1*-29.2,*2*-75.3 |
| 3. Phosphoglucomutase | PGM | 2.7.5.1 | *3*-43.4 |
| B. Glycolysis | | | |
| 4. Phosphoglucose isomerase | PGI | 5.3.1.9 | *2*-58.7 |
| 5. Phosphofructokinase | PFK | 2.7.1.11 | ? |
| 6. Aldolase | ALD | 4.1.2.13 | *3R* |
| 7. Triose-phosphate isomerase | TPI | 5.3.1.1 | *3*-101.3 |
| 8. Glyceraldehyde-3-phosphate dehydrogenase | G3PD | 1.2.1.12 | *2R* |
| 9. Phosphoglycerokinase | PGK | 2.7.2.3 | *2*-7.6 |
| C. α-Glycerophosphate cycle | | | |
| 10. α-Glycerophosphate dehydrogenase | GPDH | 1.1.1.8 | *2*-20.5 |
| 11. α-Glycerophosphate oxidase | GPO | 1.1.99.5 | *2R* |
| D. Krebs cycle | | | |
| 12. NAD-isocitrate dehydrogenase | IDD | 1.1.1.41 | ? |
| 13. Succinate dehydrogenase | SDH | 1.3.99.1 | ? |
| 14. Fumarase | FUM | 4.2.1.2 | *1*-19.9 |
| 15. NAD-malate dehydrogenase | MDH | 1.1.1.37 | *3*-62.8 |
| II. Others | | | |
| 16. Glucose-6-phosphate dehydrogenase | G6PD | 1.1.1.49 | *1*-63 |
| 17. 6-phosphogluconate dehydrogenase | 6PGD | 1.1.1.44 | *1*-0.64 |
| 18. Transaldolase | TA | 2.2.1.2 | ? |
| 19. NADP-malic enzyme | ME | 1.1.1.40 | *3*-53.1 |
| 20. NADP-isocitrate dehydrogenase | IDH | 1.1.1.42 | *3*-27.1 |
| 21. Arginine kinase | AK | 2.7.3.3 | *3L* |
| 22. Alcohol dehydrogenase | ADH | 1.1.1.1 | 2-50.1 |
| 23. Aldehyde oxidase | AOX | 1.2.3.1 | *3*-56.6 |
| III. Protein content and weight | | | |
| 24. Mitochondrial protein | MPRO | | |
| 25. Cytosolic protein | CPRO | | |
| 26. Cytosolic and mitochondrial protein | PROT | | |
| 27. Weight | WT | | |

[a] O'BRIEN and MACINTYRE (1978); VOELKER *et al.* (1978); OLIVER, HUBER and WILLIAMSON (1978); FU and COLLIER (1981).

genic lines, respectively. Results concerning the enzyme activity variation in these two experiments have been published (LAURIE-AHLBERG *et al.* 1982; WILTON *et al.* 1982). Finally, experiment IV was done to evaluate the repeatability of certain correlations found significant in one or another of the previous experiments; it involved power output estimates and seven enzyme activities on 48 second-chromosome lines.

TABLE 2

*Flight and wing-morphology variables with values for a typical adult male*

| Variable | Value | SI units | Abbreviation |
|---|---|---|---|
| Wingbeat frequency, $n$ | 217 | Cycles $sec^{-1}$ | *WBF* |
| Wing amplitude, $\phi$ | 2.32 | Radians | *WA* |
| Wing morphology: | | | |
| Chord, $c$ (average) | $0.60 \times 10^{-3}$ | m | |
| Length, $R$ | $2.07 \times 10^{-3}$ | m | |
| Mechanical moments: | | | |
| $\int_0^R c\, dr$ (area) | $1.25 \times 10^{-6}$ | $m^2$ | |
| $\int_0^R c\, r^2\, dr$ (second) | $1.90 \times 10^{-12}$ | $m^4$ | *S* |
| $\int_0^R c\, r^3\, dr$ (third) | $2.80 \times 10^{-15}$ | $m^5$ | *T* |
| $\int_0^R c^2\, dr$ | $0.87 \times 10^{-9}$ | $m^3$ | |
| $\int_0^R c^2\, r\, dr$ | $1.02 \times 10^{-12}$ | $m^4$ | |
| $\int_0^R c^2\, r^2\, dr$ | $1.35 \times 10^{-15}$ | $m^5$ | |
| $\int_0^R c^3\, r\, dr$ | $0.77 \times 10^{-15}$ | $m^5$ | |
| $\int_0^R c^{3/2}\, r^{5/2}\, dr$ | $1.89 \times 10^{-15}$ | $m^5$ | |
| Body mass | $0.59 \times 10^{-6}$ | kg | |

## MATERIALS AND METHODS

*Experimental stocks*: Two sets of isogenic chromosome substitution lines were used in this study: 50 second-chromosome lines, $i_1/i_1$; $+_2/+_2$; $i_3/i_3$, and 50 third-chromosome lines, $i_1/i_1$; $i_2/i_2$; $+_3/+_3$, where $i$ refers to a chromosome from a highly inbred line, *Ho-R*, and + refers to a chromosome from one of four natural populations in the United States—Kansas, North Carolina, Rhode Island, Wisconsin. The +, but not the $i$, chromosomes vary within a set of lines, and each line is homozygous for all three major chromosomes. The construction and electrophoretic analysis of these lines have been described (LAURIE-AHLBERG *et al.* 1980). All flies were reared at 25° on cornmeal-molasses medium.

*Enzyme and general protein assays*: Except for minor modifications in some cases, all of the assays were done according to published procedures. For experiment II, GPDH was assayed by the reverse reaction method of BEWLEY, RAWLS and LUCCHESI (1974), GPO was measured by the 2,6-dichloroindophenol method of O'BRIEN and MACINTYRE (1972) and FUM and SDH were assayed by procedures described in STAM and LAURIE-AHLBERG (1982). For experiments IIIa, IIIb and IV, all assay procedures are described by STAM and LAURIE-AHLBERG (1982).

*Flight and wing morphology measurements*: The flight and wing morphology measurements are described in Table 2, where numerical values for a typical individual are also given. The procedures for measurement of these variables are described in detail by CURTSINGER and LAURIE-

AHLBERG (1981). Briefly, a tethered fly is observed at 16× through a stereomicroscope while the wingbeat frequency is measured with a stroboscope and the wing amplitude is measured with a camera lucida and protractor. Then one wing is removed, mounted, magnified and traced. The wing outline tracing is digitized by taking transects perpendicular to the long axis of the wing, which divides it into a number of trapezoids. For all but experiment I, each wing was divided into 8–12 trapezoids, depending on its size. For experiment I, the wings were at first divided into 10–12 trapezoids, as usual, but later they were digitized again at much smaller intervals, resulting in 40–47 trapezoids. The wing moments are estimated as the sum over trapezoids of the definite integral defined for each moment in Table 2 (*i.e.*, the wing chord, $c$, at a perpendicular distance $r$ from the origin is assumed to be the width of the trapezoid at that point). The reanalysis of the experiment-I wings using approximately four times the usual number of transects had little effect on the wing moments, but as expected, all moments were slightly lower using the smaller number of transects. For example, for the individual fly in Table 2, wing area was 0.6% lower, the second moment 0.5% lower and the third moment 2.7% lower.

*Power calculations*: WEIS-FOGH (1973, eq. 19) provides an explicit formula for "uncorrected" aerodynamic power, $PA$. This uncorrected $PA$ is actually just the profile power, as defined by ELLINGTON (1984f). WEIS-FOGH's correction for $PA$ (WEIS-FOGH 1973, p. 194) was intended to include the induced power, but ELLINGTON (1984f, p. 171) finds an error in this correction procedure. Here we present the uncorrected $PA$ as WEIS-FOGH's aerodynamic power. ALEXANDER (1977, eq. 10.9) provides an explicit formula for inertial power, $PI$, which follows directly from WEIS-FOGH's expression for the inertial bending moment (WEIS-FOGH 1973, eq. 20). WEIS-FOGH (1972, p. 91 and eq. 17) gives the total mechanical work for a half-stroke as the integral of the sum of aerodynamic and inertial bending moments from the beginning of a half-stroke to the point at which the sum equals zero. Here we use a closed form solution of that integral with bending moments provided by WEIS-FOGH's (1973) equations 15 and 20 to calculate the total mechanical power, $PT$.

$$PT = 2n\,[(-1/2)KX^2 + C\phi^2X - (4/3)CX^3 + (1/8)K\phi^2 + (1/3)C\phi^3]$$

where

$$X = 2C\phi^2/[K + (K^2 + 16C^2\phi^2)^{1/2}]$$

$$K = 8\pi^2n^2\rho_w h \int_0^R cr^2\,dr$$

$$C = \rho C_D\pi^2n^2 \int_0^R cr^3\,dr$$

The constants $C_D$, $\rho$, and $\rho_w h$ are defined below; the other symbols are defined in Table 2.

ELLINGTON (1984f, eq. 29) provides an explicit formula for the profile power, $P_{pro}$, which exactly equals WEIS-FOGH's uncorrected aerodynamic power, under the assumption that the wings exhibit simple harmonic motion (an assumption we use in all the power calculations). He also provides an explicit formula for inertial power, $P_{acc}$ (eq. 39), which is the sum of two parts. The first part, which is the power required to accelerate the wing mass during the first half of each half-stroke, exactly equals WEIS-FOGH's inertial power under the simple harmonic motion assumption. The second part is the corresponding power requirement for the virtual wing mass, which is the mass of air accelerated by the wing (see ELLINGTON 1984b). ELLINGTON's (1984f) equation 20 gives the induced power, $P_{ind}$, corrected for temporal and spatial unsteady effects. He gives two spatial correction factors (eq. 24 and 25), of which we use the average here. ELLINGTON (1984f, p. 171) gives the total aerodynamic power as the sum of $P_{ind}$ and $P_{pro}$, and he gives the total mechanical power output as one-half the sum of $P_{ind}$, $P_{pro}$ and $P_{acc}$ (p. 172).

Several variables appear in both the WEIS-FOGH and ELLINGTON power equations, which we have assumed constant. First, we assume a constant mass density along the long axis of the wing so that the moment of inertia, $I$, of the wing is estimated as the product of the mass per unit area of the wing, $\rho_w h$, and the second mechanical moment

$$I = \rho_w h \int_0^R cr^2\,dr.$$

TABLE 3

*WEIS-FOGH and ELLINGTON'S power estimates for the individual in Table 2*

| Variable | Value (microwatts) | Abbreviation |
|---|---|---|
| WEIS-FOGH'S | | |
| Aerodynamic power | 3.37[a] (4.36)[b] | *PA* |
| Inertial power | 1.66[c] (7.69)[d] | *PI* |
| Total power | 3.59[a,c] | *PT* |
| ELLINGTON'S | | |
| Profile power | 4.36[b] | $P_{pro}$ |
| Induced power | 3.49 | $P_{ind}$ |
| Inertial power | 10.43[d] (7.69)[d,e] | $P_{acc}$ |
| Aerodynamic power | 7.86[b] | $P_a$ |
| Total power | 9.14[b,d] | $P_t$ |

[a] Using $C_D = 0.6$
[b] Using $C_D = 0.776$
[c] Using $\rho_w h = 4.0 \times 10^{-4}$
[d] Using $\rho_w h = 1.85 \times 10^{-3}$
[e] Using only the wing-mass part of ELLINGTON'S $P_{acc}$, which equals WEIS-FOGH'S *PI*.

We previously used an estimated value of $\rho_w h = 4.0 \times 10^{-4}$ kg m$^{-2}$ (CURTSINGER and LAURIE-AHLBERG 1981), but after seeing values for $\rho_w$, the mass density of the wing and standardized values of $h$, the average wing thickness, for various insects in ELLINGTON (1984b), we realized this value appeared too small. Therefore, we repeated the measurements giving this estimate (the mass and area of 20 wings) and found an average value of two replicates of $1.85 \times 10^{-3}$ kg m$^{-2}$, which is much more consistent with ELLINGTON's data for other species. Here, we use $\rho_w h = 4.0 \times 10^{-4}$ for the WEIS-FOGH power estimates, since these were calculated and analyzed for all the experiments before ELLINGTON's paper appeared, and we use $\rho_w h = 1.85 \times 10^{-3}$ for the ELLINGTON power estimates. Second, we assumed that the average drag coefficient, $C_D$, is 0.6 for calculating the WEIS-FOGH powers, but for the ELLINGTON powers, $C_D$ was calculated from equations 27 and 28 (ELLINGTON 1984b). Finally, the mass density of air, $\rho$, at 25° is 1.18 kg m$^{-3}$, and the kinematic viscosity of air, $\eta$, at 25° is $1.55 \times 10^{-5}$ m$^2$ sec$^{-1}$. Table 3 gives the power estimates using these constants and the wing beat frequency, wing amplitude and wing morphology values in Table 2.

*Experiment I design and oxygen consumption methods*: Eight second chromosome isogenic lines were selected to span the range of wingbeat frequencies observed among the set of 50 lines. Flies were reared at 25° on cornmeal-molasses and were aged for 6 days posteclosion. The following measurements—live weight, oxygen consumption, wingbeat frequency, wing amplitude and the wing morphology variables described above—were made on a total of 75 individuals (ten from each of seven lines, five from those remaining). One individual was an extreme outlier with respect to oxygen consumption, perhaps indicating a leak in the apparatus, so it was excluded from the analysis presented here. The measurements were made on 17 days over a 1-month span of time.

Oxygen consumption of individual flies was measured in a horizontal capillary differential syringe manometer (Roger Gilmont Industries, Inc., Model W-4200; PETERSON, FREUND and GILMONT 1967), modified to permit concurrent measurement of wingbeat frequency and wing amplitude (*i.e.*, a flat-sided glass respiration cell of 18 ml volume was constructed). The entire apparatus was submerged in a waterbath held at 24°, resulting in a temperature of 25° measured by Tele-Thermometer (YSI, model 42SC) at the location of the tethered fly since the experimental cell (like the reference cell) was illuminated with a microscope lamp to allow wing amplitude measurement. The manometric fluid was 1.0% Liquinox in distilled water, colored with methylene blue. Carbon dioxide was absorbed by 2.0 ml of 0.01 M $Ba(OH)_2$. The reference cell contained 8

ml of distilled water. The micrometer accuracy and the $CO_2$-absorbing capacity of the $Ba(OH)_2$ solution were checked by injection of pure $CO_2$ from a syringe into the experimental cell.

Oxygen consumption was measured only during periods of continuous "flight" (wing motion), which varied in length among individuals from 2–79 minutes, with an average of 19.6 minutes. During this time, the volume displacement, wingbeat frequency and wing amplitude were measured periodically (from 2–11 times). The volume displaced over time was linear even for the longest flights. The rate of oxygen consumption was estimated as the slope of the regression of volume on time, for which the average $R^2$ for those flights with more than two observations was 0.99. These rates are compared with mechanical powers estimated from the average wingbeat frequency and wing amplitude for each fly.

*Design of experiments II–IV*: Each of these experiments were conducted with a randomized block design, where "block" refers to a period of time during which flies from each of the isogenic lines were reared and sampled in random order. The standard rearing conditions consisted of placing 50 pairs of parents for 2 days in a half-pint bottle containing cornmeal-molasses medium. The newly eclosed male progeny were split into two groups, those for flight measurement and those for enzyme assay, and were aged for 5–8 days. The number of flies sampled per line per block and the within-block sampling structure varied among experiments.

The relationship between enzyme activity and power output could not be investigated on a single fly basis, since we are interested in many enzymes, some of which require multiple fly homogenates for assay. Therefore, the enzymes were assayed from mass homogenates of contemporaneous siblings of the individuals who were tethered and used for flight parameter measurement. This design prevents estimation of correlations between microenvironmental effects, but does not affect the estimation of genetic correlations, with which we are mainly concerned.

The design of experiment II with respect to the flight variables has been described (CURTSINGER and LAURIE-AHLBERG 1981). Within each of four blocks, samples from each of 21 second-chromosome lines were obtained on each of 4 days. Each sample consisted of two sets of ten 6-day-old males (one for live weight and the assay of GPDH and GPO, the other for live weight and the assay of SDH and FUM) and from one to four individual males were used for flight and wing measurements. This design resulted in 16 observations per line for each enzyme, and 46–63 individuals per line gave complete sets of flight data (wing morphology measurements and three replicate observations each of *WBF* and *WA*).

The design of experiments IIIa and b with respect to enzyme activities has been described (LAURIE-AHLBERG *et al.* 1982). Within each of three blocks, samples from each of 48 second (IIIa) or third (IIIb) chromosome isogenic lines were obtained on each of 2 days. Each sample consisted of one set of 100 males (25 each of 5-, 6-, 7-, and 8-day-old males) for the assay of 23 enzymes, live weight and general protein concentration, and one each of 5-, 6-, 7- and 8-day-old males for wing morphology and flight measurements (two replicates each of *WBF* and *WA*). This design resulted in six observations per line for each enzyme activity, live weight and protein content and in 24 observations per line for the flight variables.

Within each of the five blocks of experiment IV, samples from each of 46 second-chromosome lines were obtained on each of 4 days. Each sample consisted of 30 males (15 each of 6- and 7-day-old males) for live weight and the assay of ADH, FUM, GPDH, GPO, G6PD, HEX, PGI and general protein concentration and one each of 6- and 7-day-old males for wing morphology and flight measurements (two replicates each of *WBF* and *WA*). This design resulted in 20 observations per line for each enzyme activity, live weight and protein and in 40 observations per line for the flight variables.

## RESULTS

Experiment I was designed to explore the relationship between metabolic power input, estimated by the rate of oxygen consumption during flight, and the mechanical power output, estimated from analysis of the wing motion and aerodynamic principles. A numerical comparison between the two types of power can be made using the standard conversion of 20 J chemical energy per

TABLE 4

*Line means of specific powers and the aerodynamic efficiency from experiment I*

| Line | Specific power input (from $O_2$ rate) (W $N^{-1}$) | Specific power output (W $N^{-1}$) | | | | | Muscular efficiency (%) $E_a$ |
|---|---|---|---|---|---|---|---|
| | | $P_{pro}$ | $P_{ind}$ | $P_a$ | $P_{acc}$ | $P_t$ | |
| KA16 | 14.9 | 0.33 | | 0.33 | 0.63 | 0.84 | |
| KA25 | 22.7 | 0.87 | 0.59 | 1.45 | 2.07 | 1.76 | 6.4 |
| KA33 | 24.8 | 0.84 | 0.63 | 1.47 | 2.12 | 1.80 | 6.1 |
| WI05 | 23.7 | 0.72 | 0.68 | 1.40 | 1.75 | 1.58 | 6.2 |
| NC11 | 21.3 | 0.78 | 0.60 | 1.38 | 1.77 | 1.57 | 6.8 |
| RI02 | 22.2 | 0.68 | 0.74 | 1.41 | 1.73 | 1.57 | 6.5 |
| RI03 | 25.6 | 0.91 | 0.60 | 1.51 | 2.19 | 1.85 | 6.0 |
| *Ho-R* | 19.4 | 0.71 | 0.65 | 1.37 | 1.72 | 1.55 | 7.3 |

milliliter of $O_2$ for aerobic metabolism (ELLINGTON 1984f). Average specific powers (calculated by ELLINGTON's formulas), as well as the aerodynamic muscular efficiencies ($P_a/P_{input}$) are shown in Table 4 for each line. The distribution of line means is approximately continuous except for the line KA16, as noted previously (CURTSINGER and LAURIE-AHLBERG 1981). This line has a power output so low that it cannot support its body weight in free flight. Therefore, the induced power is not presented for KA16 because the method of calculation assumes that the fly is supporting its body weight. No such assumption is made for any of the other powers. The data in Table 4 show that the inertial power ($P_{acc}$) is roughly the same magnitude as the aerodynamic power ($P_a$) for *D. melanogaster*, and consequently, total power is only slightly greater than aerodynamic power. This result is consistent with WEIS-FOGH's (1972) analysis of VOGEL's (1967) data for *D. virilis*, but differs from the situation for most insect species, including other Dipterans, for which inertial power may be several times higher than aerodynamic power (WEIS-FOGH 1973; ELLINGTON 1984f; CASEY 1981). WEIS-FOGH (1973) suggests this difference is due to the fact that *Drosophila* has an unusually low wingbeat frequency for its size. Table 4 also shows that the aerodynamic muscular efficiencies are 6.0 to 7.3%, which is very similar to the values for other insects: 6% for Bombus, 5% for Apis and 8% for Eristalis (ELLINGTON 1984f). Finally, the average power input estimated from the oxygen consumption data (excluding the line KA16) is 22.8 W $N^{-1}$, which is only about half as great as the average from our earlier experiment (CURTSINGER and LAURIE-AHLBERG 1981). The reasons for this difference are not clear, although the apparatus and $CO_2$-absorbing solutions used were different (*i.e.*, NaOH *vs.* $Ba(OH)_2$). The lower rates in the present experiment are not due to a lower $CO_2$-absorbing capacity of $Ba(OH)_2$, since we determined that the capacity of that solution was far in excess of the amounts produced by the flies (see MATERIALS AND METHODS). The $Ba(OH)_2$ experiment value of 22.8 W $N^{-1}$ is more similar to the estimates for other Drosophila species by CHADWICK (1947) and CHADWICK and GILMOUR (1940): approximately 12 W $N^{-1}$ for *D. repleta, D. americana* and *D. virilis.*

The most important goal of experiment I was to determine the extent of

correlation between the power input and power output estimates, particularly with respect to the variation among lines. Although the mechanical power output is an appropriate *functional* measure of the level of flight metabolism, the rate of oxygen consumption is clearly more directly related to the rate of flux through the metabolic pathways (but is much more difficult to measure). Table 5 shows the partial correlation (with live weight fixed) between rate of oxygen consumption and each of the different types of power output estimates, and Figure 2 shows the relationship between *PT* and $O_2$ consumption. The correlations among individuals within lines (averaged over lines), which is due to variation of environmental causes, is distinguished from the correlation over line means, which is primarily due to genetic variation. Both types of correlation are highly significant for inertial, aerodynamic and total powers, but the correlations over line means are considerably higher: 0.94 and 0.96 for WEIS-FOGH's and ELLINGTON's total power estimates, respectively. Particularly since we are primarily concerned with the physiological consequences of *genetic* differences among lines, these results clearly indicate that the mechanical power output is a very good indicator of the actual rate of flight metabolism. Wingbeat frequency alone, which we had previously shown to be highly correlated with rate of oxygen consumption (CURTSINGER and LAURIE-AHLBERG 1981), appears to be a somewhat less-accurate indicator, having a line mean correlation of 0.87.

A secondary goal of experiment I was to compare the WEIS-FOGH and ELLINGTON power estimates with respect to their correlation with oxygen consumption. ELLINGTON's (1984 a–f) treatment is more complete than WEIS-FOGH's, but very similar in several ways, as noted earlier. We had calculated and analyzed powers by WEIS-FOGH's method for all the experiments covered by this paper before ELLINGTON's paper was published. Since Table 5 shows that ELLINGTON's power estimates do not provide a significantly better indication of the rate of flight metabolism than WEIS-FOGH's (in fact the correlations are almost identical), we have not reanalyzed all the data in order to utilize ELLINGTON's improvements in the aerodynamic analysis. Therefore, the discussions of experiments II–IV deal entirely with WEIS-FOGH's power estimates.

Experiments II–IV were designed to determine the extent of genetic variation affecting the flight and enzyme activity variables and, particularly, to determine the degree of correlation between the genetic effects on enzyme activity and on the mechanical power output during flight.

Table 6 shows the broad-sense heritabilities for the flight-related variables from experiments II, III and IV. These heritabilities were calculated from the raw data for individual flies (excluding the outlier line KA16 described above). In every case the heritabilities are highly significantly greater than zero (*i.e.*, $P < 0.0001$ for the $F$-test of the line effect in the analysis of variance). All but one of the values are above 0.20 and many are above 0.30, indicating a very substantial genetic contribution to the variation in most cases.

Nearly all of the flight and enzyme activity variables are significantly correlated with live weight, so both types of variables have been adjusted by their

TABLE 5

*Partial correlations (with weight fixed) between rate of oxygen consumption and mechanical power output estimates*

| | Weis-Fogh | | | | Ellington | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *WBF* | *PI* | *PA* | *PT* | $P_{acc}$ | $P_{pro}$ | $P_{ind}$[a] | $P_a$ | $P_t$ |
| Over individuals within lines[b] | 0.49*** | 0.74*** | 0.75*** | 0.75*** | 0.74*** | 0.75*** | −0.47* | 0.68*** | 0.74*** |
| Over line means | 0.87* | 0.95*** | 0.94** | 0.94** | 0.95*** | 0.94** | −0.29 | 0.96*** | 0.96*** |

[a] The line KA16 was excluded from the $P_{ind}$ correlations.
[b] Averaged over lines by Fisher's Z-transformation method.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

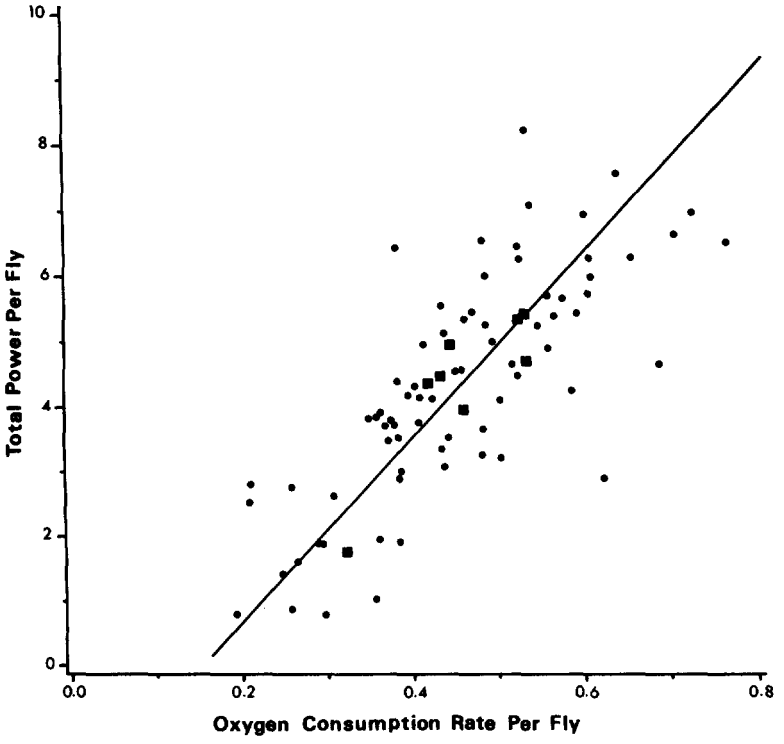


FIGURE 2.—Total power (in microwatts) *vs.* rate of oxygen consumption (in microliters per minute) from experiment I. The points marked by circles are individual flies, and the points marked by squares are line means. The line for regression of power on oxygen consumption rate over the line means is shown.

TABLE 6

*Broad-sense heritabilities for the flight-related variables*

| | Chromosome 2 | | | Chromosome 3 |
|---|---|---|---|---|
| Variable | Experiment II | Experiment IIIa | Experiment IV | Experiment IIIb |
| *WBF* | 0.39 | 0.37 | 0.31 | 0.42 |
| *MWA* | 0.16 | 0.27 | 0.23 | 0.22 |
| *S* | 0.28 | 0.40 | 0.27 | 0.59 |
| *T* | 0.27 | 0.39 | 0.26 | 0.59 |
| *PA* | 0.39 | 0.40 | 0.24 | 0.29 |
| *PI* | 0.44 | 0.40 | 0.25 | 0.29 |
| *PT* | 0.40 | 0.39 | 0.24 | 0.28 |

Raw data on individual flies (excludes KA16); $P < 0.0001$ for all variables and experiments.

regression (over lines) on live weight in order to correct for genetic effects and correlations due to overall body size variation (see CURTSINGER and LAURIE-AHLBERG 1981; LAURIE-AHLBERG *et al.* 1982). Live weights were not obtained for individual flies but, rather, for the sets of flies homogenized in mass

TABLE 7

K *values for weight-adjusted flight data*

| | Chromosome *2* | | | Chromosome *3* |
|---|---|---|---|---|
| Variable | Experiment II | Experiment IIIa | Experiment IV | Experiment IIIb |
| *WBF* | 0.51 | 0.46 | 0.45 | 0.66 |
| *MWA* | 0.35 | 0.30 | 0.39 | 0.42 |
| *S* | 0.42 | 0.52 | 0.36 | 0.68 |
| *T* | 0.40 | 0.51 | 0.35 | 0.67 |
| *PA* | 0.55 | 0.42 | 0.30 | 0.38 |
| *PI* | 0.58 | 0.45 | 0.32 | 0.43 |
| *PT* | 0.55 | 0.42 | 0.30 | 0.53 |

The line KA16 is excluded; $P < 0.0001$ for all variables and experiments.

for enzyme activity measurement. Thus, the adjustments were made to a flight variable averaged over the set of individuals who were reared and collected together with those providing the live weight measurement. Since the term "heritability" should be reserved for measurements on single individuals, we define an analogous variance component ratio, $K$, which is the proportion of variation among the observations on a set of individuals that is attributable to differences among lines. See CURTSINGER and LAURIE-AHLBERG (1981) and LAURIE-AHLBERG (1982) for the precise formulas used in calculating the $K$'s for experiments II and for IIIa and b, respectively. These $K$ values for the flight variables are given in Table 7, which reveals that the values are as high as or higher than the corresponding broad-sense heritabilities, in spite of the weight adjustment. Clearly, overall body size variation does not account for the high genetic component to the variation in the flight variables.

The $K$ values for the weight-adjusted enzyme activities are given in Table 8, along with the significance level for the $F$-test of lines in the analysis of variance. Nearly all the enzyme activities have a highly significant genetic contribution to their variation, as previously reported for experiments IIIa and b (LAURIE-AHLBERG *et al.* 1982). These $K$ values are very similar to those reported previously for enzyme activities adjusted for both weight and protein content (LAURIE-AHLBERG *et al.* 1982). As for the flight variables, we conclude that there is highly significant genetic variation affecting most of the enzyme activities, independent of overall body size variation.

Experiments II, IIIa and IV, which involved various overlapping subsets of the 50 second-chromosome lines, provide an opportunity to investigate the repeatability of the line effects over time. Experiment II was performed in 1979, experiment IIIa in 1980 and experiment IV in 1982. Table 9 shows the partial correlations (with live weight fixed) between experiments for each of the variables included in two or more of them. The correlations are positive and highly significant for each of the flight variables except *WA*, indicating a high degree of repeatability and, hence, stability over time of the genetic effects. Similarly the enzymes ADH, GPDH and GPO show highly repeatable

TABLE 8

*K values for weight-adjusted enzyme activities and protein*

| Variable | Chromosome 2 | | | Chromosome 3 |
|---|---|---|---|---|
| | Experiment II | Experiment IIIa | Experiment IV | Experiment IIIb |
| ADH | | 0.93**** | 0.77**** | 0.54**** |
| AK | | 0.43**** | | 0.33**** |
| ALD | | 0.32**** | | 0.35**** |
| AOX | | 0.17*** | | 0.65**** |
| FUM | 0.52**** | 0.16** | 0.30**** | 0.08* |
| GPDH | 0.69**** | 0.52**** | 0.55**** | 0.46**** |
| GPO | 0.75**** | 0.27**** | 0.71**** | 0.17** |
| G3PD | | 0.09* | | 0.03 |
| G6PD | | 0.36**** | 0.41**** | 0.61**** |
| HEX | | 0.46**** | 0.22**** | 0.39**** |
| IDH | | 0.85**** | | 0.51**** |
| MDH | | 0.07* | | 0.17** |
| IDD | | 0.20*** | | 0.15** |
| PFK | | 0.32**** | | 0.10* |
| PGD | | 0.42**** | | 0.34**** |
| PGI | | 0.21**** | 0.26**** | 0.53**** |
| PGK | | 0.38**** | | 0.31**** |
| PGM | | 0.46**** | | 0.40**** |
| SDH | 0.56**** | 0.14* | | 0.27**** |
| TA | | 0.65**** | | 0.35**** |
| TPI | | 0.10* | | 0.17** |
| TRE | | 0.23**** | | 0.07** |
| ME | | 0.59**** | | 0.83**** |
| MPRO | | 0.09** | | 0.11* |
| CPRO | | 0.34**** | | 0.42**** |
| PROT | | | 0.27**** | |

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

genetic effects, but the other five enzymes do not show significant correlation between experiments.

The correlation between the genetic effects on different weight-adjusted variables was estimated for each experiment as the standardized covariance component for lines, obtained from an analysis of cross-products. We do not know of any test for significance of these genetic correlations, but MODE and ROBISON (1959) provide a method for computing the standard error. Here, we assume that any genetic correlation estimate more than twice its standard error is probably statistically significant at roughly the 5% level. This criterion is very consistent with significance levels of the product-moment correlations between line means (data not shown).

The genetic correlations between flight-related variables are given in the upper section of Table 10. Total power is highly correlated with wingbeat frequency and with the second and third mechanical moments of the wing, which was expected since these variables are used in computing *PT*. However, the relationship between wing amplitude, which also enters into the equation,

TABLE 9

*Partial correlations (with weight fixed) between line means from different experiments*

| Variable | Experiments II and IIIa ($n$ = 20) | Experiments II and IV ($n$ = 19) | Experiments IIIa and IV ($n$ = 44) |
|---|---|---|---|
| *WBF* | 0.73*** | 0.83*** | 0.74*** |
| *MWA* | 0.44 | 0.21 | 0.42** |
| *S* | 0.75*** | 0.61** | 0.55*** |
| *T* | 0.74*** | 0.62** | 0.55*** |
| *PA* | 0.55* | 0.59* | 0.64*** |
| *PI* | 0.62** | 0.75*** | 0.68*** |
| *PT* | 0.56* | 0.61** | 0.64*** |
| ADH | | | 0.98*** |
| FUM | 0.28 | 0.39 | 0.01 |
| GPDH | 0.94*** | 0.90*** | 0.87*** |
| GPO | 0.63** | 0.86*** | 0.66*** |
| G6PD | | | 0.19 |
| HEX | | | 0.16 |
| PGI | | | 0.15 |
| SDH | 0.29 | | |

$n$ = number of lines in common.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

and power is not consistently significant. Wingbeat frequency and wing amplitude are highly negatively correlated in three of the four experiments. These two variables represent a trade-off situation in the total power budget. A certain constant level of power can be achieved by various different combinations of *WBF* and *WA*, but the lower the *WBF*, the higher the *WA* and vice versa. Evidently, some of the genetic variation in *WBF* is partially compensated by variation in *WA*, which should provide some homeostasis with respect to the power output. However, the extent of genetic variability affecting *PT* is only slightly lower than that for *WBF* (Table 7).

The genetic correlations between enzyme activities measured in more than one of the second chromosome experiments are shown in the lower section of Table 10. Many of these correlations appear highly significant, particularly for the third-chromosome lines. The interrelationships among the enzymes studied in experiments IIIa and b are discussed in detail by WILTON *et al.* (1982).

The genetic correlations between *PT* and the enzyme activity variables are shown in Table 11. The following observations are important. (1) Of the 57 correlation estimates, 11 (or 19%) appear to be significant (*i.e.*, the estimate is more than twice its standard error). This is a greater percentage than expected by chance. (2) All but one of the "significant" correlations are positive. However, the one negative correlation is between *PT* and PFK, classically considered to be the rate-limiting step of glycolysis. (3) Not all of the significant correlations involve enzymes that are directly involved in flight metabolism. In fact, three of seven nonflight enzymes and six of 16 flight enzymes show significant correlation. However, it must be noted that many of the nonflight

TABLE 10

*Genetic correlations ± standard error for weight-adjusted data*

| | Chromosome 2 | | | | Chromosome 3 |
|---|---|---|---|---|---|
| Variable Pair | Experiment II | Experiment IIIa | Experiment IV | Mean[a] | Experiment IIIb |
| *WBF, MWA* | 0.20 ± 0.24 | −0.57 ± 0.14* | −0.70 ± 0.80* | −0.41 | −0.59 ± 0.12* |
| *WBF, S/T* | 0.23 ± 0.23 | 0.00 ± 0.18 | −0.13 ± 0.16 | 0.03 | −0.46 ± 0.13* |
| *WBF, PT* | 0.80 ± 0.10* | 0.67 ± 0.10* | 0.41 ± 0.13* | 0.65 | 0.35 ± 0.15* |
| *MWA, S/T* | −0.26 ± 0.22 | 0.06 ± 0.18 | 0.09 ± 0.16 | −0.04 | 0.24 ± 0.16 |
| *MWA, PT* | 0.68 ± 0.13* | 0.00 ± 0.19 | 0.13 ± 0.16 | 0.31 | 0.25 ± 0.17 |
| *S/T, PT* | 0.38 ± 0.21 | 0.60 ± 0.12* | 0.65 ± 0.10* | 0.55 | 0.48 ± 0.13* |
| ADH, FUM | | −0.11 ± 0.24 | 0.18 ± 0.15 | 0.04 | −0.10 ± 0.28 |
| ADH, GPDH | | −0.06 ± 0.16 | 0.07 ± 0.15 | 0.01 | 0.66 ± 0.11* |
| ADH, GPO | | 0.01 ± 0.18 | 0.25 ± 0.14 | 0.13 | −0.01 ± 0.22 |
| ADH, G6PD | | 0.16 ± 0.17 | 0.44 ± 0.15* | 0.31 | 0.72 ± 0.09* |
| ADH, HEX | | 0.19 ± 0.16 | 0.14 ± 0.16 | 0.17 | 0.85 ± 0.07* |
| ADH, PGI | | 0.06 ± 0.19 | 0.35 ± 0.14* | 0.21 | 0.86 ± 0.06* |
| FUM, GPDH | 0.13 ± 0.23 | 0.04 ± 0.26 | 0.30 ± 0.15* | 0.16 | 0.24 ± 0.26 |
| FUM, GPO | 0.38 ± 0.20 | 0.57 ± 0.19* | 0.37 ± 0.14* | 0.45 | 0.97 ± 0.19* |
| FUM, G6PD | | 0.25 ± 0.29 | 0.25 ± 0.18 | 0.25 | −0.10 ± 0.26 |
| FUM, HEX | | 0.04 ± 0.27 | −0.20 ± 0.17 | −0.08 | −0.17 ± 0.29 |
| FUM, PGI | | 0.58 ± 0.29* | 0.73 ± 0.09* | 0.66 | 0.05 ± 0.27 |
| GPDH, GPO | 0.16 ± 0.22 | 0.25 ± 0.19 | 0.07 ± 0.15 | 0.16 | 0.11 ± 0.22 |
| GPDH, G6PD | | 0.10 ± 0.18 | 0.05 ± 0.18 | 0.08 | 0.50 ± 0.13* |
| GPDH, HEX | | 0.42 ± 0.15* | −0.01 ± 0.16 | 0.22 | 0.69 ± 0.10* |
| GPDH, PGI | | 0.09 ± 0.20 | 0.26 ± 0.15 | 0.18 | 0.72 ± 0.08* |
| GPO, G6PD | | 0.43 ± 0.20* | 0.29 ± 0.17 | 0.36 | −0.17 ± 0.21 |
| GPO, HEX | | 0.20 ± 0.19 | 0.08 ± 0.16 | 0.14 | −0.19 ± 0.23 |
| GPO, PGI | | 0.40 ± 0.22 | 0.35 ± 0.14* | 0.38 | 0.00 ± 0.22 |
| G6PD, HEX | | 0.43 ± 0.15* | 0.36 ± 0.18* | 0.40 | 0.88 ± 0.06* |
| G6PD, PGI | | 0.27 ± 0.21 | 0.56 ± 0.14* | 0.43 | 0.84 ± 0.06* |
| HEX, PGI | | 0.34 ± 0.18 | 0.14 ± 0.17 | 0.24 | 0.96 ± 0.04* |

[a] The mean by Fisher's Z-transformation. Its standard error is not known because the three chromosome-2 experiments do not represent independent samples.

* Correlation estimates greater than twice their standard error.

enzymes show strong genetic correlation with the flight enzymes (Table 10 and WILTON *et al.* 1982). (4) Unlike many of the flight variable pairs and enzyme activity pairs in Table 10, there is almost no consistency among the different experiments in terms of which correlations are significant. Only FUM shows a significant correlation with *PT* in more than one of the experiments. (5) FUM is a mitochondrial enzyme, so its relationship with power output may be a nonspecific effect of the density of mitochondria in the flight muscles (see PENNYCUICK and REZENDE 1984). The significant relationship between general mitochondrial protein concentration (MPRO) and power in experiment IIIb supports this idea, but the lack of correlation between power and the other mitochondrial enzymes (GPO, MDH, IDD, SDH) does not. These observations suggest that genetic variation in the activities of some flight metabolism enzymes affects the power output, but the effects are so small and the enzymes

TABLE 11

*Genetic correlations ± standard error between* PT *and enzyme activities for weight-adjusted data*

| | Chromosome *2* | | | | Chromosome *3* |
|---|---|---|---|---|---|
| Enzyme | Experiment II | Experiment IIIa | Experiment IV | Mean[a] | Experiment IIIb |
| ADH | | −0.03 ± 0.17 | −0.23 ± 0.15 | −0.13 | 0.24 ± 0.17 |
| AK | | 0.25 ± 0.18 | | | 0.01 ± 0.19 |
| ALD | | 0.10 ± 0.20 | | | 0.48 ± 0.16* |
| AOX | | −0.07 ± 0.23 | | | 0.00 ± 0.17 |
| FUM | 0.40 ± 0.20* | 0.55 ± 0.27* | 0.22 ± 0.16 | 0.40 | 0.57 ± 0.27* |
| GPDH | 0.35 ± 0.21 | 0.16 ± 0.17 | 0.03 ± 0.16 | 0.18 | 0.36 ± 0.16* |
| GPO | 0.37 ± 0.20 | 0.26 ± 0.20 | −0.03 ± 0.16 | 0.21 | 0.25 ± 0.22 |
| G3PD | | −0.25 ± 0.29 | | | 0.56 ± 0.57 |
| G6PD | | 0.25 ± 0.18 | −0.15 ± 0.19 | 0.05 | −0.01 ± 0.18 |
| HEX | | 0.32 ± 0.17 | −0.05 ± 0.17 | 0.14 | 0.23 ± 0.18 |
| IDH | | 0.36 ± 0.15* | | | 0.24 ± 0.17 |
| MDH | | 0.62 ± 0.29* | | | −0.00 ± 0.23 |
| IDD | | 0.35 ± 0.20 | | | 0.17 ± 0.23 |
| PFK | | −0.41 ± 0.18* | | | 0.20 ± 0.27 |
| PGD | | 0.49 ± 0.15* | | | −0.08 ± 0.19 |
| PGI | | 0.12 ± 0.21 | −0.09 ± 0.17 | 0.02 | 0.27 ± 0.17 |
| PGK | | 0.38 ± 0.17* | | | 0.17 ± 0.19 |
| PGM | | 0.05 ± 0.18 | | | −0.04 ± 0.19 |
| SDH | 0.15 ± 0.23 | 0.47 ± 0.24 | | 0.32 | 0.37 ± 0.19 |
| TA | | 0.24 ± 0.17 | | | −0.08 ± 0.19 |
| TPI | | 0.65 ± 0.33 | | | 0.26 ± 0.22 |
| TRE | | 0.30 ± 0.19 | | | 0.19 ± 0.33 |
| ME | | 0.22 ± 0.17 | | | 0.41 ± 0.15* |
| MPRO | | 0.27 ± 0.28 | | | 0.57 ± 0.24* |
| CPRO | | 0.14 ± 0.19 | | | 0.30 ± 0.17 |
| PROT | | | −0.19 ± 0.17 | | |

[a] The average by Fisher's *Z*-transformation method. Its standard error is not known because the three chromosome-*2* experiments do not represent independent samples.

* Correlation estimates greater than twice their standard error.

are so intercorrelated among themselves that statistical significance of individual enzyme effects cannot be demonstrated.

Several multivariate analyses were performed to investigate the possibility of synergistic effects among enzymes, or whether certain groups of enzymes work in concert to produce an effect on power. To look for synergistic effects, biplots were constructed (see WILTON *et al.* 1982), and multiple regressions of power on the first five principal components of the enzyme activities were analyzed. Neither of these methods revealed any indication of significant synergistic effects. In addition, variable selection procedures were used in the multiple regression of power on enzyme activities (all possible regressions of enzyme subsets and stepwise regression). These analyses did not reveal any groups of enzymes that are together more highly significant than individual enzymes.

Both empirical data and theoretical considerations suggest a hyperbolic re-

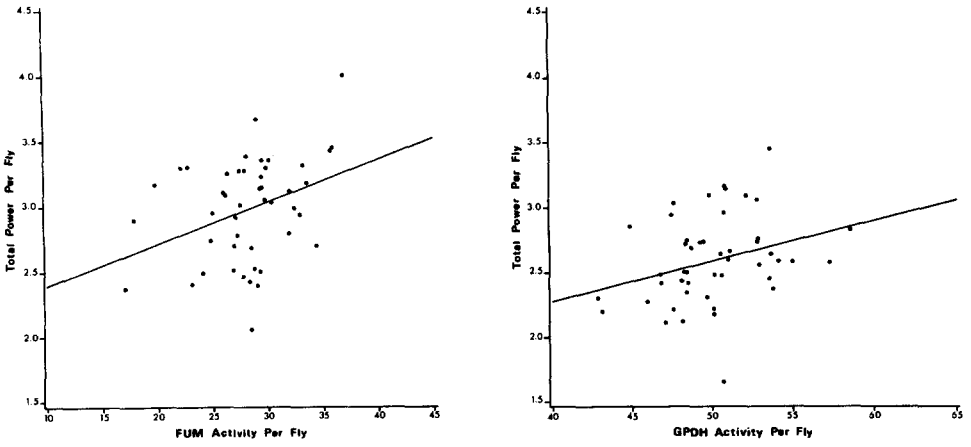


FIGURE 3.—Plots of total power (in microwatts) *vs.* enzyme activity (in nanomoles per minute per fly) for isogenic chromosome substitution line means. The individual observations were adjusted by regression on live weight before averaging (see text). The relationship for FUM is from experiment IIIa (second chromosome), and that for GPDH is from experiment IIIb (third chromosome). In each case the regression line is shown, for which the slope is significantly different from zero at the 5% level.

lationship between level of enzyme activity and rate of flux through a multienzyme metabolic pathway (KACSER and BURNS 1973, 1979, 1981). In this case, the relationship could be nearly linear over a range of low-enzyme activities, but could show little or no correlation over a higher range. Thus, correlation over all the line means in our experiments could give misleadingly low correlation estimates. However, inspection of plots of the line means of power output *vs.* activity does not reveal any clear indications of a hyperbolic curve (see Figure 3). Of course, this observation could mean that activities of all of the lines fall within the high, insensitive range.

Seven of the enzymes that are coded by loci on the second or third chromosome were screened for allozymic variation by starch gel electrophoresis (LAURIE-AHLBERG *et al.* 1982). There was no electrophoretic variation for MDH or ME; however, second-chromosome lines varied for HEX-C, GPDH and ADH, and third-chromosome lines varied for PGM and AOX. There is no significant difference among allozymes with respect to power output for any of these enzymes, but the tests are not powerful for the flight-related enzymes HEX-C, PGM and GPDH, because of very low levels of polymorphism (LAURIE-AHLBERG *et al.* 1982).

## DISCUSSION

There are three major results of the studies reported in this paper. (1) Chromosomes sampled from natural populations of *D. melanogaster* provide a large and repeatable genetic component to the variation in the activities of flight metabolism enzymes and to the variation in the mechanical power generated by the flight muscles. (2) The mechanical power output, estimated from aerodynamic formulas and observations of the wing motion of tethered flies,

is a sensitive indicator of the rate of flight metabolism. (3) In spite of (1) and (2) above, no convincing cases of individual enzyme effects on power output were detected, although the number and sign of the significant enzyme-power correlations suggests that such effects are not totally lacking.

Several possibilities may account for our failure to observe repeatably significant correlation between the activity of individual flight metabolism enzymes and power output, even if such relationships actually exist:

1. An obvious problem is the lack of statistical power. A majority of the genetic correlations in Table 11 have a standard error between 0.15 and 0.30, which means that correlations below about 0.4 to 0.5 cannot be detected reliably. Nearly all of the "significant" cases in Table 11 are within this range, so the inconsistencies among experiments, in terms of which correlations are significant, are to be expected. These rather high standard errors occurred in spite of our efforts to maximize power. For example, experiment IV alone involved a total of 1840 observations for each flight variable and a total of 920 observations for each enzyme activity variable.

2. The enzyme activity measurements in this study were made under optimal reaction conditions (*i.e.*, saturating substrate). Thus, variation among lines in these *in vitro* activities may not reflect the variation *in vivo*. This consideration is of particular concern if the genetic variation affects the catalytic properties of the enzyme rather than its concentration. In most cases we do not know the relative importance of structure *vs.* concentration effects, but for all the enzymes investigated so far (GPDH, G6PD, 6PGD, ME, ADH and catalase) most if not all the variation in activity level is accounted for by variation in enzyme concentration estimated immunologically (LAURIE-AHLBERG *et al.* 1981; MARONI *et al.* 1982; LAURIE-AHLBERG and BEWLEY 1983; BEWLEY and LAURIE-AHLBERG 1984).

3. The nature of the isogenic chromosome substitution lines may limit the range of detectable effects. The effect of variation in any one enzyme is clearly dependent on the activity levels of all the other enzymes in the system. If, for example, one of the enzymes of glycolysis was extremely low due to a mutant allele fixed on the *X* chromosome common to all of the isogenic lines, then the rate of flight metabolism would be very insensitive to variation in any of the other enzymes. If this were the case, the isogenic flies probably would show much lower power output than outbred flies. Although we do not have a direct, simultaneous comparison of inbred and outbred flies, a separate experiment involving recently collected flies analyzed at 25° indicates they are not markedly different with respect to wingbeat frequency (the major determinant of power output). In that experiment, the average *WBF* over 40 isofemale lines was 230 $sec^{-1}$, whereas the averages for the isogenic lines from experiments II, IIIa and IV are 222, 212 and 223, respectively. The somewhat lower *WBF* for isogenic flies is probably accounted for mainly by their lower overall body size (733 $\mu g$ *vs.* 855 $\mu g$ for outbred flies). Nevertheless, the common genetic background of the isogenic lines cannot be excluded as a potential problem.

4. Another possibility for our failure to detect any striking enzyme-power output relationships involves the choice of enzymes. Since we obviously did

not include every enzyme involved in the catabolism of carbohydrate reserves during flight, some key "rate-limiting" enzyme(s) may have been excluded. However, we did include the two enzymes classically considered to be the rate-limiting steps of glycolysis and the Krebs cycle, phosphofructokinase and NAD-isocitrate dehydrogenase, respectively, as well as other enzymes thought to be important in the control of flux through these pathways, trehalase, hexokinase and succinate dehydrogenase (see CRABTREE and NEWSHOLME 1975; SACKTOR 1975). Glycogen phosphorylase is the only enzyme considered important by SACKTOR (1975) that was excluded.

The concept of a "rate-limiting" or "flux-controlling" enzyme for a metabolic pathway segment has recently been challenged by KACSER and BURNS (1973, 1979, 1981), KOHN and CHIANG (1982), RAY (1983) and others. These authors advocate use of a continuous-scale "sensitivity coefficient" to measure the effect that the change in concentration of one enzyme has on flux through a multienzyme pathway. Theoretical analysis of both steady- and nonsteady-state systems indicates that metabolic regulation can be distributed among a number of enzymes in a synergistic way. KACSER and BURNS (1979) and KOHN and CHIANG (1982) emphasize that there need not be a single rate-limiting enzyme, although some may have much larger sensitivity coefficients than the others. KACSER and BURNS (1981) provide empirical data and theoretical results suggesting that the relationship between metabolic flux and enzyme activity is hyperbolic, such that flux is very sensitive to variation at a low range of activity levels, but very insensitive at higher ranges. Furthermore, they predict that "wild-type" activity levels fall in the insensitive range. Our results are certainly consistent with this prediction, but by no means constitute a powerful test of their hypotheses.

Further progress on understanding how much of the genetic variation in mechanical power output is caused by variation of flight metabolism enzymes will require the development of more powerful experimental approaches. In particular, the ability to experimentally manipulate the level of just one enzyme from zero to wild-type activity will be needed to investigate the relevance of the metabolic sensitivity analyses discussed above. Such experiments are now conceivable, with the use of cloned DNA sequences and *P*-element transformation of Drosophila (RUBIN and SPRADLING 1982), which could be used to construct flies with variable doses of a low-activity mutant allele. Attention must also be paid to the role of structural gene variants and specific environmental effects, as in the next paper of this series, which deals with the temperature-dependent effects of the polymorphic GPDH allozymes (P. T. BARNES and C. C. LAURIE-AHLBERG, unpublished results).

We wish to thank SHIRLEY CHAO, KAZUO HIRAIZUMI, FREDERICK IMMERMAN, NAOHIKO MIYASHITA, MONICA SHELTON, LYNN STAM and JUSTINA WILLIAMS for their expert technical assistance. We are also grateful to C. C. COCKERHAM and B. S. WEIR for statistical advice, to CECELIA LAURIE for mathematical advice and to H. E. SCHAFFER for advice on many aspects of the project. This is paper 9539 of the journal series of the North Carolina Agricultural Research Service, Releigh, North Carolina. This work was supported by United States Public Health Service grants GM28455 and GM11546.

## LITERATURE CITED

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., 1983 *Animal Mechanics*, Blackwell Scientific Publications, London.

BEWLEY, G. C. and C. C. LAURIE-AHLBERG, 1984 Genetic variation affecting the expression of catalase in *Drosophila melanogaster*: correlation with rates of enzyme synthesis and degradation. Genetics **106:** 435–448.

BEWLEY, G. C., J. M. RAWLS, JR., and J. C. LUCCHESI, 1974 α-Glycerophosphate dehydrogenase in *Drosophila melanogaster*: kinetic differences and developmental differentiation of the larval and adult isozymes. J. Insect Physiol. **20:** 153–165.

CASEY, T. M., 1981 A comparison of mechanical and energetic estimates of flight cost for hovering Sphinx moths. J. Exp. Biol. **91:** 117–129.

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.

CHADWICK, L. E. and D. GILMOUR, 1940 Respiration during flight in *Drosophila repleta* Wollaston: the oxygen consumption considered in relation to the wing rate. Physiol. Zool. **13:** 398–410.

CLARK, R. L., E. BOERWINKLE, G. J. BREWER and C. F. SING, 1983 Studies of enzyme polymorphism in the Kamuela population of *Drosophila mercatorum*. III. Effects of variation at the αGPD locus and subflight stress on the energy charge and glycolytic intermediate concentrations. Genetics **104:** 661–675.

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., 1984(a–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. Lond. [Biol] **305:** 1–181.

FU, L. J. and G. E. COLLIER, 1981 Cytogenetic localization of the dosage sensitive regions for arginine kinase and glyceraldehyde-3-phosphate dehydrogenase in *Drosophila melanogaster*. Genetics **97** (Suppl): s37–s38.

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

KACSER, H. and J. A. BURNS, 1979 Molecular democracy: who shares the controls. Biochem. Soc. Trans. **7:** 1149–1160.

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.

KOHN, M. C. and E. CHIANG, 1982 Metabolic network sensitivity analysis. J. Theor. Biol. **98:** 109–126.

LAURIE-AHLBERG, C. C., 1982 Genetic, ontogenetic and tissue-specific variation of dipeptidases in *Drosophila melanogaster*. Biochem. Genet. **20:** 407–424.

LAURIE-AHLBERG, C. C. and G. C. BEWLEY, 1983 Naturally occurring genetic variation affecting the expression of sn-glycerol-3-phosphate dehydrogenase in *Drosophila 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.

LAURIE-AHLBERG, C. C., J. H. WILLIAMSON, B. J. COCHRANE, A. N. WILTON and F. I. CHASALOW, 1981 Autosomal factors with correlated effects on the activities of the glucose 6-phosphate and 6-phosphogluconate dehydrogenases in *Drosophila melanogaster*. Genetics **99:** 127–150.

LAURIE-AHLBERG, C. C., A. N. WILTON, J. W. CURTSINGER and T. H. EMIGH, 1982 Naturally occurring enzyme activity variation in *Drosophila melanogaster*. I. Sources of variation for 23 enzymes. Genetics **102:** 191–206.

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

MARONI, G., C. C. LAURIE-AHLBERG, D. A. ADAMS and A. N. WILTON, 1982 Genetic variation in the expression of ADH in *Drosophila melanogaster*. Genetics **101:** 431–446.

MODE, C. J. and H. F. ROBINSON, 1959 Pleiotropism and the genetic variance and covariance. Biometrics **15:** 518–537.

O'BRIEN, S. J. and R. J. MACINTYRE, 1972 The $\alpha$-glycerophosphate cycle in *Drosophila melanogaster*. I. Biochemical and developmental aspects. Biochem. Genet. **7:** 141–161.

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.

OLIVER, M. J., R. E. HUBER and J. H. WILLIAMSON, 1978 Genetic and biochemical aspects of trehalase from *Drosophila melanogaster*. Biochem. Genet. **16:** 927–940.

PENNYCUICK, C. J. and M. A. REZENDE, 1984 The specific power output of aerobic muscle, related to the power density of mitochondria. J. Exp. Biol. **108:** 377–392.

PETERSON, R. N., M. FREUND and K. GILMONT, 1967 Measurement of low rates of oxygen consumption with a horizontal capillary-differential syringe manometer. Proc. Soc. Exp. Biol. Med. **125:** 645–648.

RAY, W. J., JR., 1983 Rate-limiting step: a quantitative definition. Application to steady state enzymic reactions. Biochemistry **22:** 4625–4637.

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.

STAM, L. F. and C. C. LAURIE-AHLBERG, 1982 A semi-automated procedure for the assay of 23 enzymes from *Drosophila melanogaster*. Insect Biochem. **12:** 537–544.

VOELKER, R. A., C. H. LANGLEY, A. J. LEIGH-BROWN and S. OHNISHI, 1978 New data on allozyme loci in *Drosophila melanogaster*. Drosophila Inform. Serv. **53:** 200.

VOGEL, S., 1967 Flight in *Drosophila*. I. Flight performance of tethered flies. J. Exp. Biol. **44:** 567–578.

VOGEL, S., 1981 *Life in Moving Fluids*. Willard Grant Press, Boston, Massachusetts.

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., 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.

WILTON, A. N., C. C. LAURIE-AHLBERG, T. H. EMIGH and J. W. CURTSINGER, 1982 Naturally occurring enzyme activity variation in *Drosophila melanogaster*. II. Relationships among enzymes. Genetics **102:** 207–221.

Communicating editor: W. W. ANDERSON