GENETIC VARIABILITY OF FLIGHT METABOLISM IN *DROSOPHILA MELANOGASTER*. I. CHARACTERIZATION OF POWER OUTPUT DURING TETHERED FLIGHT[1]

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ABSTRACT

The mechanical power imparted to the wings during tethered flight of *Drosophila melanogaster* is estimated from wing-beat frequency, wing-stroke amplitude and various aspects of wing morphology by applying the steady-state aerodynamics model of insect flight developed by WEIS-FOGH (1972, 1973). Wing-beat frequency, the major determinant of power output, is highly correlated with the rate of oxygen consumption. Estimates of power generated during flight should closely reflect rates of ATP production in the flight muscles, since flies do not acquire an oxygen debt or accumulate ATP during flight. In an experiment using 21 chromosome *2* substitution lines, lines were a significant source of variation for all flight parameters measured. Broad-sense heritabilities ranged from 0.16 for wing-stroke amplitude to 0.44 for inertial power. The variation among lines is not explained by variation in total body size (*i.e.*, live weight). Line differences in flight parameters are robust with respect to age, ambient temperature and duration of flight. These results indicate that characterization of the power output during tethered flight will provide a sensitive experimental system for detecting the physiological effects of variation in the structure or quantity of the enzymes involved in flight metabolism.

A fundamental tenet of modern evolutionary theory is that natural selection directs the process of gene frequency change only indirectly, by discriminating among phenotypes, rather than genotypes. The relationship between genotypic, phenotypic and fitness variation is obscure; in fact, it is seldom possible to describe the effect of a single gene substitution on a fitness-related quantitative character. Here, we present the first of a series of studies, the ultimate goal of which is to describe the way that variation in the structure and quantity of some specific gene products, enzymes involved in flight metabolism, causes variation in a fitness-related metrical phenotype, the power output of flight muscles.

The flight metabolism of *Drosophila melanogaster* is a desirable experimental system for the detection of physiological effects of variation in enzyme structure and regulation.

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(1) The metabolic pathways are well characterized, and the specific functions of many enzymes are known. Of the more than 30 enzymes with well-defined roles in generating ATP for flight in Diptera (SACKTOR 1964, 1970, 1974, 1975; CRABTREE and NEWSHOLME 1975), 12 have been assayed and genetically mapped in *D. melanogaster* (O'BRIEN and MACINTYRE 1978; VOELKER *et al.* 1978; OLIVER, HUBER and WILLIAMSON 1978).

(2) The many biophysical investigations of insect flight provide a basis for estimating the mechanical power imparted to the wings during flight. WEIS-FOGH (1972, 1973) presented formulae for the power output of the flight muscles that derive from a model of the hovering or slow forward flight practiced by many small insects, including Drosophila. The relevant parameters are listed in Table 1.

(3) The flight muscles of Diptera, including Drosophila, exhibit metabolic rates that are among the highest known in any tissue (SACKTOR 1965). As a result, the rate of ATP production in flight muscles is expected to be quite sensitive to functional differences among enzyme variants.

(4) 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, though not demonstrated, that variation in power output is ultimately related to variation in reproductive success.

The bulk of this paper is devoted to describing our methods of characterizing the power output of the flight muscles. We also present evidence that the measured flight variables reflect the rate of ATP production in flight muscles and are subject to genetic modification. These are preliminary (but critical) steps towards relating genetic variation in enzyme activity and structure to the flight phenotype.

A brief description of some of this work has appeared elsewhere (CURTSINGER and LAURIE-AHLBERG 1980).

TABLE 1

Parameters for the computation of the power output of the flight muscles

Measurements obtained	Parameter	Symbol	Units (SI)
From tethered individuals:	Wingbeat frequency	WBF	s^{-1}
	Wing-stroke amplitude	Θ	Radians
From wing tracings:	Wing area	A	m^2
	Wing length	L	m
	Wing width	$W5$	m
	Second moment of area	S	m^4
	Third moment of area	T	m^5
Computed from above:	Aerodynamic power	P_a	W (Watt)
	Inertial power	P_i	W (Watt)
	Total power	P_t	Arbitrary
From groups of 10 males:	Live weight	Wt	kg
Constants:	Coefficient of drag	C_d	Dimensionless
	Air density	ρ_a	$kg\ m^{-3}$
	Wing density	ρ_w	$kg\ m^{-3}$
	Wing thickness	D	m

MATERIALS AND METHODS

Experimental stocks: Flies used were from isogenic chromosome *2* substitution lines, the origin and construction of which was described by LAURIE-AHLBERG *et al.* (1980). The isogenic lines are homozygous for the same *X* and third chromosomes, but are homozygous for different second chromosomes derived from natural populations in Kansas, Wisconsin, Rhode Island and North Carolina. Stocks were routinely maintained at 25° on cornmeal-molasses medium.

Flight observations: Unless otherwise stated, subjects for flight observation were 6-day-old males reared at 25° on a 12–12 light-dark cycle and observed within 4 hr of incubator "dawn". Flies were lightly etherized and tethered to a fine syringe-cleaning wire, approximately 0.10 mm in diameter, with Permount histological mounting medium (WIGGLESWORTH 1949). The wire was attached to the mesonotum perpendicular to the long axes of the body and the extended wing, and did not appear to interfere with wing movement in that position. Approximately 85% of the several thousand tethered individuals observed began beating the wings spontaneously after recovering from the ether.

Tethered "flight" was observed in a temperature-controlled stock rom, 25°, relative humidity 61–68%. The long axis of the body was positioned horizontally in the viewing field of a 16× dissecting microscope. Wingbeat frequency was measured by adjusting the flashing rate of a variable speed stroboscope (General Radio Model GR 1531-AB "Strobotac") until a single stationary image of the wings was observed. Any integral submultiple flashing rate of the stroboscope relative to the true wing-beat frequency results in a single wing image. The true wing-beat frequency is estimated as the fastest flashing rate of the stroboscope that gives a single wing image. The stroboscope was calibrated with a crystal-controlled frequency counter and found to be accurate within 0.1% in the range of 115 s^{-1} to 250 s^{-1} (beats per second). Wing-beat frequencies were recorded to the nearest 1.67 s^{-1}.

Under constant illumination, the movement of the wings creates an envelope of blurred motion, the boundaries of which are clearly visible under slight magnification from a frontal view. Wing tips invariably meet at the top of the wing stroke, generally directly above the thorax, but the lower boundary varies among individuals. The wing-stroke amplitude, which is approximately the angle corresponding to the arc described by the wing tip during a complete stroke, was measured on both sides with a camera lucida and protractor, to the nearest 5°. Flight observations were always completed within one hr from the time the individual was tethered.

Wing measurements: After the flight observations, flies were removed from the tethering wire and one wing was clipped off at the thorax with microscissors. Each wing was mounted between two microscope slides, sealed with transparent tape, magnified 50× with a profile projector (Ehrenreich Photo-Optical Industries Model LP-2) and traced. The coordinates of the points of intersection of the wing tracing and several transects taken perpendicular to the long axis of the wing at ½″ intervals were entered into a minicomputer with a Tektronics 4956 Graphics Tablet. The transects divided the wing into two triangles and 6–8 trapezoids (as shown in Figure 1). The height of the distal triangle was between 1/10 and 2/10 inch. The height of the distal trapezoid, which was usually less than ½ inch, was determined by the length of the wing. The origin or point of rotation of the wing was assumed to lie inside the thorax, 1/10″ from the basal termination of the wing membrane on the tracing. The total wing length, *L*, is the distance from the origin to the tip. Wing width, *W5* in Tables 1 and 2, is the width of the fifth transect.

An approximate expression for the wing cord at a perpendicular distance r from the origin, $c(r)$, can be obtained by linear interpolation as the width of the trapezoid or triangle bounded by the wing chords $c(R_i)$ and $c(R_{i-1})$ (where $R_{i-1} < r < R_i$; see Figure 1). Then, the area (A) and the second and third moments of the area (S and T) can be calculated for each segment by solving the following integral in closed form, where y equals 1, r^2 or r^3 for A, S or T, respectively:

$$\int_{R_{i-1}}^{R_i} c(r)\, y\, dr \quad . \tag{1}$$

The sum over segments then gives the area and the moments for the whole wing.

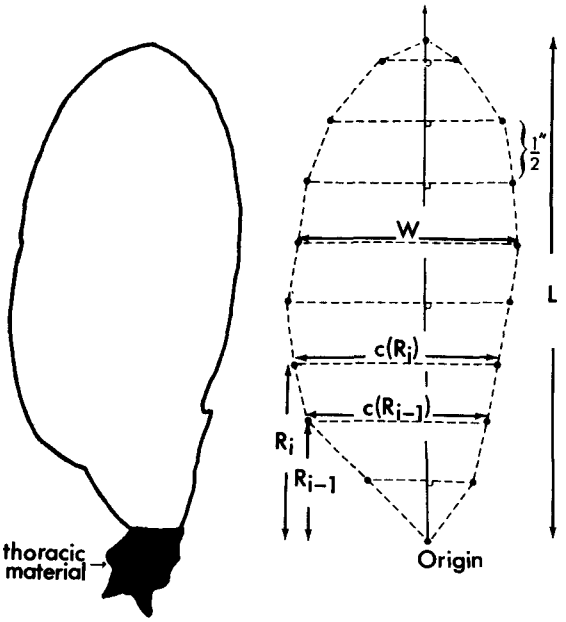

FIGURE 1.—A typical wing tracing on the left; on the right, the transects that define the points used for estimating wing length, width, area and the second and third moments of area.

Respirometry: Oxygen consumption rates were measured on single tethered flies with a horizontal capillary differential syringe manometer (Roger Gilmont Industries, Inc., Model W-4200), modified as described by PETERSON, FREUND and GILMONT (1967). The tethering wire was mounted in a cork that fit snugly in the well of a reaction vessel. Manometric fluid was 0.2% Liquinox in distilled water. CO_2 absorption medium was 10% KOH on Whatman #40 filter paper. Measurements were taken in a water bath at 25°. With slight magnification, it is possible to observe the movement of the wings in the submerged reaction vessel, thus allowing simultaneous measurement of oxygen consumption rate and wing-beat frequency.

Power calculations: Aerodynamic, inertial, and total power were calculated as described by WEIS-FOGH (1972, 1973), with some simplifications, as follows:

Using the symbols defined in Table 1, we may express the aerodynamic power, which is the power required to overcome drag on the wing, as

$$P_a = (2/3)\, \rho_a\, C_d\, T\, \Pi^2\, WBF^3\, \Theta^3 \tag{2}$$

[see WEIS-FOGH 1973, equation (19)]. Here, we assume that ρ_a and C_d are constants, where $\rho_a = 1.17$ kg m^{-3} is the density of moist air at 25° and $C_d = 0.6$. The estimate of C_d, which depends on shape, comes from the work of VOGEL (1967b) on *D. virilis*. All other variables in equation (2) are estimated from observations on tethered flies and wing measurements.

The general expression for inertial power, which is the power required to decelerate and accelerate the wing mass at the top and bottom of each stroke, follows directly from WEIS-FOGH's (1973) equation (20) and was explicitly derived by ALEXANDER (1977):

$$P_i = 2\, I\, \Pi^2\, WBF^3\, \Theta^2\ . \tag{3}$$

I, the mass moment of inertia, is defined by equation (4), where ρ_w is the mass density of the wings, $c(r)$ is the wing chord at a distance r from the axis of rotation, $D(r)$ is the thickness of the wing at that distance r and L is the total length of the long axis of the wing.

$$I = \rho_w \int_0^L c(r)\, D(r)\, r^2\, dr\ . \tag{4}$$

We assume that $\rho_w D$ is a constant, estimated to be 4.4×10^{-4} kg m^{-2} from the weight and area of twenty wings. It then follows from the definition of the second moment of the wing area that $I = \rho_w DS$. Thus, the equation used to estimate inertial power is

$$P_i = 2\,\rho_w\, D\, S\, \Pi^2\, WBF\, \Theta^2 \ . \tag{5}$$

Total power, which includes both aerodynamic and inertial components, is estimated from WEIS-FOGH's (1973) equations (23) and (33). The approximation of total power gives a measure in arbitrary units and depends on N, the ratio of maximum aerodynamic and inertial bending moments:

$$N = \frac{4\,\rho_w\, D\, S}{\rho_a\, C_d\, T\, \Theta} \ . \tag{6}$$

The total power imparted by the thorax to the wings is

$$P_t = WBF\left[\frac{6N^2 + 13N + 14}{12(N+1)}\right] \ . \tag{7}$$

RESULTS

Characteristics of tethered flight

Long flights: Continuous flights of 6-day-old males can last up to four hours, as shown in Figure 2. Flies assume a characteristic posture in tethered flight, with the prothoracic and mesothoracic legs tucked against the body, and the metathoracic legs extended behind and parallel to the abdomen.

WBF (wing-beat frequency) is relatively constant during the first hour of long flights. Rates of change of *WBF* during that period have been observed to lie in the range of -2×10^{-3}s^{-2} to 7×10^{-4}s^{-2}, based on observation of 25 males from six isogenic lines at 5-minute intervals. In no cases were the regression coefficients of *WBF* on time significantly different from zero for the first hour of tethered flight. A similar constancy of *WBF* has been reported for *D. funebris* (WILLIAMS,

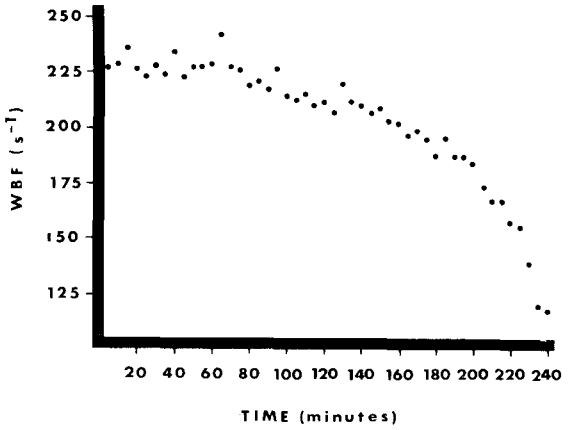

FIGURE 2.—Wing-beat frequency recorded at 5-minute intervals during a continuous 4-hour flight of an individual male. Long flights are characterized by relatively constant *WBF* during the first hour, then gradual decline to cessation. Wing-stroke amplitude remains relatively constant during long flights.

BARNES and SAWYER 1943), but other species of Drosophila show a rapid decline of *WBF* during tethered flight (CHADWICK and GILMOUR 1940; CHADWICK 1947).

Following the initial period of relative constancy, *WBF* gradually declines to about 120 s^{-1}, at which point wing beating stops. WIGGLESWORTH (1949) showed that flies in this state of "exhaustion" can be induced to beat the wings briefly by feeding specific carbohydrates, suggesting that depletion of metabolic reserves is a limiting factor in the later stages of long flights.

The total wing-stroke amplitude does not vary measurably during long flights, even during the period of *WBF* decline, suggesting that *WBF* and wing-stroke amplitude are independently controlled (CHADWICK 1953). However, the amplitude on the left or right side can change during a long flight, possibly as a result of attempts to turn on the tethering wire.

Ontogeny of WBF: A total of 172 males from six isogenic lines were collected at emergence, aged under standard conditions for one to eight days and then tethered for three replicated *WBF* observations. The line means and overall means for each age group are shown in Figure 3. *WBF* increases during the first and second days after emergence, but is otherwise independent of age over the range tested. Analysis of variance omitting days one and two showed a highly significant line effect ($F = 38.1$, $p < 0.001$), but no statistically significant effect of age or line × age interaction.

Temperature dependence: *WBF* of thirty-eight individuals from three isogenic lines all reared at 25° were observed at each of three temperatures, with three replications per individual per temperature. Flies were allowed at least one minute to acclimate to temperature shifts, but the *WBF* change was almost immediate. Analysis of variance showed a highly significant line effect and temperature ef-

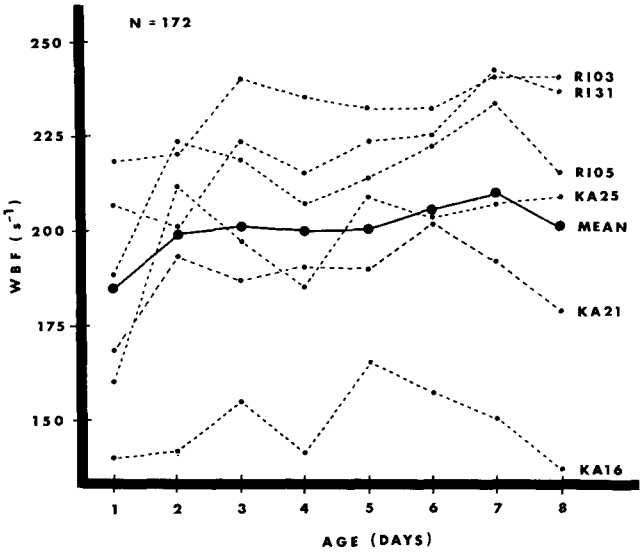

FIGURE 3.—Ontogeny of wing-beat frequency. *WBF* increases during the first 2 days after emergence, but is otherwise independent of age over the range tested.

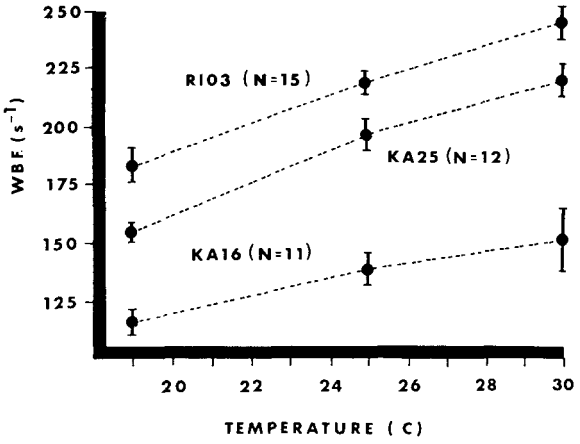

FIGURE 4.—Wing-beat frequency as a function of ambient temperature. Individuals were observed three times at each temperature. Vertical bars indicate the standard error of line means.

fect, but no significant line × temperature interaction. The results are shown in Figure 4. In a similar experiment with outbred flies, 259 individuals from 40 isofemale lines were each observed at 15°, 20°, 25° and 30°, with three replications per individual per temperature. Many of these flies would not beat their wings below about 15°. Analysis of variance again showed highly significant line and temperature effects, but no significant line × temperature interaction.

Oxygen consumption rate: *WBF* and oxygen consumption rates were measured on 28 individuals from four isogenic lines. The results are shown in Figure 5, where each point represents the average of at least two *WBF* observations and

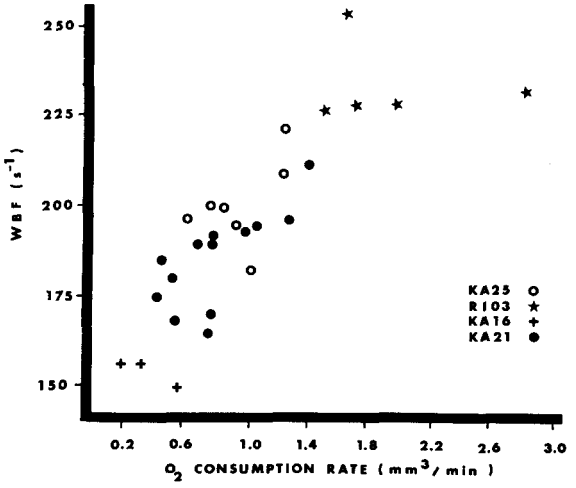

FIGURE 5.—Correlation between oxygen consumption rate and wing-beat frequency. Each point represents the average of at least two simultaneous *WBF* and consumption measurements on an individual tethered inside a respirometer. The product-moment correlation coefficient is highly significant ($r = 0.82$, $p < 0.001$).

two corresponding O_2 consumption rates measured on single individuals for 5-minute or longer intervals. The relatively small sample size is due to frequent difficulty in stimulating flight once the fly is mounted in an air-tight reaction vessel submerged in a water bath. CHADWICK and GILMOUR (1940) and REED, WILLIAMS and CHADWICK (1942) constructed simple models of flight energetics that predict a positive correlation between WBF^2 and O_2 consumption (or work) per wing stroke. If the work or oxygen consumption per stroke is proportional to the second power of *WBF*, then the work or consumption per unit time should vary with WBF^3. That prediction has been verified in observations on *D. virilis*, *D. americana* and *D. repleta* (CHADWICK and GILMOUR 1940; CHADWICK 1947). Our observations on *D. melanogaster* show proportionality between rates of O_2 consumption and *WBF*, WBF^2 and WBF^3 as equally acceptable hypothesis: all three product-moment correlation coefficients lie in the range of 0.82 to 0.84 ($p < 0.001$).

We were unable to obtain reliable estimates of wing-stroke amplitude on flies tethered in the respirometer. Consequently, it is not possible at this time to test hypotheses concerning the relationship between power output and O_2 consumption.

A multiple-line study

Design: A set of observations consisted of tethering one 6-day-old male from each of 21 isogenic chromosome *2* substitution lines, recording *WBF* and wing-stroke amplitude of those that spontaneously started wing beating, and recording the size and shape parameters from one wing from each of the fliers. *WBF* and wing-stroke amplitude were recorded with three replications at one-minute intervals for each flier. These measurements were averaged to obtain one value per variable per fly for the analyses reported here; 64 sets were observed, four per day, four days a week, for four weeks. The flies for each week of observations were reared in a different set of four half-pint bottles. The flies used on each of the four days within each week came from the same set of four bottles, but differed in time of emergence from those bottles. Because of variations in the number of spontaneous fliers, the resulting data structure is unbalanced.

Analyses of variance: A total of 1166 fliers were observed, with 46 to 63 fliers per line. Line means are shown in Table 2, where the symbols and units are as defined in Table 1. The wing-stroke amplitude reported in Table 2 is the average of the left and right sides, in radians. The live weights reported in Table 2 are based on 10 males reared simultaneously with the flies tethered for flight observations, with 16 replications (one for each day of flight observations). The distribution of line means for each of the variables is approximately continuous, except for *WBF* and the three powers that depend on it, because of a single outlier (Figure 6). The *WBF* outlier, KA16, has been excluded from all the analyses involving *WBF*, P_a, P_i and P_t.

The model equation for analysis of the multiple line study is:

$$Y_{ijklm} = \mu_i + \alpha_{ij} + \beta_{ik} + (\alpha\beta)_{ijk} + \gamma_{il} + (\alpha\gamma)_{ijl} + (\beta\gamma)_{ikl} + (\alpha\beta\gamma)_{ijkl} + \varepsilon_{ijklm} ,$$

where Y corresponds to the value of the ith variable in the jth week, kth day, lth

TABLE 2

Line means of flight parameters from the multiple line study

Line*	*WBF* (s^{-1})	θ (rads)	*Wt*† (10^{-6}kg)	*L* (10^{-3}m)	*A* ($10^{-6}m^2$)	*W5* (10^{-3}m)	*S* ($10^{-12}m^4$)	*T* ($10^{-15}m^5$)	P_a (10^{-7}W)	P_i (10^{-7}W)	P_t
KA06	217	2.26	6.68	1.90	1.12	0.80	1.42	1.92	10.6	5.8	280
KA16	155	2.28	6.71	1.90	1.14	0.81	1.43	1.94	4.2	2.3	201
KA21	203	2.33	7.26	1.89	1.14	0.81	1.42	1.91	9.7	5.2	262
KA25	213	2.22	6.85	1.97	1.28	0.87	1.73	2.43	12.0	6.5	274
KA27	220	2.17	7.29	1.89	1.13	0.80	1.41	1.89	9.9	5.7	289
KA33	238	2.32	7.71	1.96	1.22	0.84	1.64	2.29	18.4	9.6	305
WI08	223	2.14	7.46	1.95	1.22	0.85	1.63	2.27	11.4	6.6	291
WI12	228	2.12	7.95	1.96	1.27	0.86	1.69	2.35	12.4	7.1	297
NC12	233	2.45	7.41	1.93	1.22	0.85	1.59	2.19	19.0	9.5	296
NC14	210	2.13	7.29	1.98	1.27	0.86	1.73	2.43	10.1	5.7	271
NC16	230	2.19	7.48	1.94	1.21	0.85	1.60	2.21	13.3	7.4	297
NC35	220	2.23	7.17	1.92	1.20	0.84	1.55	2.11	11.7	6.5	286
RI03	242	2.20	7.15	1.94	1.23	0.85	1.63	2.24	16.1	8.9	313
RI05	232	2.29	7.91	2.00	1.29	0.87	1.77	2.50	17.7	9.2	295
RI08	207	2.36	7.32	1.91	1.15	0.82	1.47	2.00	11.1	5.8	266
RI16	217	2.22	7.79	1.95	1.23	0.85	1.64	2.27	12.2	6.7	281
RI23	210	2.29	6.30	1.90	1.15	0.81	1.44	1.94	10.2	5.6	272
RI28	223	2.14	7.03	1.92	1.17	0.81	1.49	2.03	10.7	6.2	292
RI31	223	2.20	7.61	1.96	1.24	0.85	1.66	2.31	12.6	7.0	287
RI36	238	2.36	7.45	1.97	1.24	0.85	1.68	2.35	19.2	9.9	304
RI46	212	2.28	7.12	1.94	1.17	0.81	1.51	2.07	11.0	6.0	272
Mean	222	2.25	7.24	1.94	1.20	0.84	1.58	2.17	12.5	6.8	282

* The first two letters indicate the state of origin of chromosome *2*.
† Weight per 10 males.

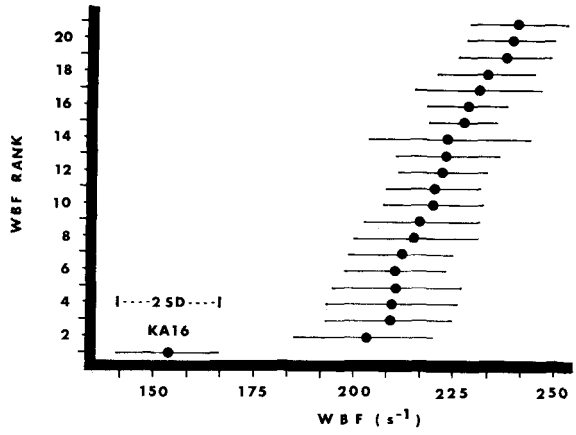

FIGURE 6.—Wing-beat frequency distribution of line means from the multiple-line study. Line means are based on three observations of each of 46 to 63 individuals per line. Horizontal bars indicate two standard deviations. With the exception of the obvious outlier, *WBF* line means are approximately continuously distributed.

line and mth fly; $i = 1, \ldots 10$, corresponding to the ten variables listed in Table 2, other than Wt, j and $k = 1, \ldots 4$, $l = 1, \ldots 21$, and $m = 1, 2, 3$ or 4 (unbalanced). For the analysis of variance of the raw data, the method of unweighted means was used (NETER and WASSERMAN 1974, p. 615).

In addition to the analysis of raw data, weight-adjusted data were also tested in order to remove any general body-size effects. Linear regressions of $\bar{Y}_{ijkl\cdot}$ on weight (Wt_{jkl}) were performed over lines for each of the 16 week × day combinations, and the sums of products were pooled to obtain a single estimate of the regression coefficient for each dependent variable (b_i). Adjusted variables were then obtained by the expression $\hat{Y}_{ijkl} = \bar{Y}_{ijkl\cdot} - b_i(Wt_{jkl})$. θ was not weight-adjusted because no overall correlation between those two variables was observed. All other flight variables were positively and significantly correlated with weight (see below). Analysis of the weight-adjusted variables follows the model equation above, but the $(\alpha\beta\gamma)_{ijkl}$ and ε_{ijklm} effects are, of course, confounded.

Significance tests and variance component ratios are shown in Table 3, for both raw and adjusted data. Because all five wing-morphology variables are very highly correlated (see below), only the analysis for wing area is given. The last columns of Table 3 show two kinds of variance-component ratios: K, defined below, and H^2, the usual broad-sense heritability.

$$H^2 = \hat{\sigma}_l^2/(\hat{\sigma}_l^2 + \hat{\sigma}_{wl}^2 + \hat{\sigma}_{dl}^2 + \hat{\sigma}_{wdl}^2 + \hat{\sigma}_e^2) \ ,$$

where $\hat{\sigma}_l^2$ is the estimated line variance component, $\hat{\sigma}_{wl}^2$, $\hat{\sigma}_{dl}^2$ and $\hat{\sigma}_{wdl}^2$ are the components for the interactions involving lines and $\hat{\sigma}_e^2$ is the variance among individuals within a week × day × line combination.

$$K = \hat{\sigma}_l^2/(\hat{\sigma}_l^2 + \hat{\sigma}_{wl}^2 + \hat{\sigma}_{dl}^2 + \hat{\sigma}_{wdl}^2 + \hat{\sigma}_e^2) \ ,$$

TABLE 3

Significance tests and variance component estimates for the multiple line study

Variable	Sources of variation							Variance-component ratios	
	Week	Day	W×D	Line	W×L	D×L	W×D×L	K	H^2
WBF	0.04	0.05	−0.04	4.17***	0.52***	0.04	−0.18	0.63	0.39
	−0.02	0.13**	−0.03	2.89***	0.63***	0.13		0.51	
Θ	1.99*	0.11	1.34***	6.47***	2.27**	0.25	1.83	0.32	0.16
A	0.08	0.23	0.30***	2.55***	0 32***	0.25***	−0.00	0.55	0.33
	−0.09	−0.08	0.28***	1.46***	0.02	0.09		0.46	
P_a	0.71	−0.07	0.79***	9.50***	2.36***	0.08	−0.56	0.61	0.39
	0.40	−0.07	0.66***	7.36***	2.28***	−0.00		0.55	
P_i	0.11	−0.00	0.11***	2.16***	0.49***	−0.01	−0.11	0.65	0.44
	0.05	0.01	0.09***	1.57***	0.48***	−0.02		0.58	
P_t	−0.02	0.02	0.01	1.84***	0.21**	0.07	−0.04	0.57	0.33
	−0.03	0.05*	0.02	1.27***	0.28***	0.11		0.46	
Wt	0.03	0.56***	0.03*	2.06***	0.26***	0.11***		0.73	

Analyses of Θ, *A*, and *WT* include all 21 lines; for the other variables, KA16 was excluded. For each variable, the first row represents analysis of raw data, and the second row represents analysis of weight-adjusted data (see text). Variance-component ratios are defined in text. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

where $\hat{\sigma}^2_e$ is the error variance of the week × day × line means. K is the proportion of variance among the week × day × line means that is attributable to lines after correction for week and day effects. K was computed in order to provide a measure of the effect of weight adjustment on the line component of variance.

Lines are a highly significant component of variance for all the flight variables, as well as for weight. Most of the week × line interaction terms are significant, which indicates a line-specific sensitivity to variations in rearing conditions. The main effects for the environmental factors, days and weeks, are significant in only a few cases, but the week × day interaction is significant for most variables. It is important to note that lines remain highly significant when the observations are weight-adjusted. Comparison of the variance-component ratio K for raw and weight-adjusted observations shows that adjustment decreases the line component in all cases, but not to a great extent. Therefore, while most of the flight variables are positively correlated with weight, variation in weight can account for only a small part of the variation observed.

Correlations: The five wing variables A, $W5$, L, S and T are highly intercorrelated, the minimum product-moment correlation being that for L × $W5$ line means ($r = 0.886$, $p < 0.001$). Product-moment correlations over line means between WBF, θ, wing area and the power calculations are shown above the main diagonal in Table 4. The three power measures are highly intercorrelated and also highly correlated with WBF. Because all the variables other than θ are significantly correlated with Wt, the partial correlation coefficients of line means with weight as a constant were computed as shown below the main diagonal in Table 4. It is clear that the $A \times P_a$ and $A \times P_i$ line mean correlations are high because A, P_a and P_i are all highly correlated with Wt; the corresponding partial correlations are not statistically significant. Wing-stroke amplitude is negatively correlated with weight and shows a closer relationship to the power variables when weight effects are held constant. WBF is the variable most closely related to the calculated power variables, especially P_t, for which the partial correlation is very high.

Correlations between line effects, γ_{il}, are shown above the main diagonal in Table 5. These are not true product-moment correlations, but are computed from

TABLE 4

Correlations of line means (above diagonal) and partial correlations of line means with weight as the constant variable (below diagonal)

	WBF	θ	A	P_a	P_i	P_t	Wt
WBF	—	0.05	0.44	0.83***	0.89***	0.98***	0.54*
θ	0.20	—	−0.22	0.51*	0.39	−0.09	−0.21
A	0.16	−0.12	—	0.51*	0.55*	0.40	0.63**
P_a	0.77***	0.70***	0.32	—	0.99***	0.73***	0.46*
P_i	0.85***	0.59**	0.34	0.99***	—	0.81***	0.51*
P_t	0.98***	0.03	0.10	0.64**	0.74***	—	0.54*

Correlations were computed excluding line KA16. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

TABLE 5

Correlations of line (genetic) effects (above diagonal) and average correlations of error effects (below diagonal)

	WBF	θ	*A*	P_a	P_i	P_t	*Wt*
WBF	—	0.03	0.48	0.84	0.90	0.99	0.57
		±0.24	±0.18	±0.07	±0.04	±0.01	±0.16
θ	−0.30	—	−0.25	0.49	0.37	−0.09	−0.24
	***		±0.23	±0.19	±0.21	±0.24	±0.23
A	−0.03	0.05	—	0.52	0.57	0.45	0.62
				±0.17	±0.16	±0.19	±0.14
P_a	0.35	0.65	0.47	—	0.99	0.75	0.48
	***	***	***		±0.01	±0.11	±0.18
P_i	0.54	0.49	0.48	0.97	—	0.83	0.53
	***	***	***	***		±0.08	±0.17
P_t	0.97	−0.50	−0.10	0.13	0.35	—	0.58
	***	***	**	***	***		±0.16

For the correlation of error effects, the significance level of the *z*-test is given; for the correla- of line effects, the standard error is given. The correlations of error effects were averaged over all lines except for $A \times P_i$, for which inclusion of line KA16 ($r = 0.016$) caused significant heterogeneity. The $WBF \times P_t$ error-effects correlations are significantly heterogeneous over lines, ranging from 0.917 to 0.989; each correlation is significant at the 0.001 level. Correlations of genetic effects were computed excluding line KA16. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

the covariance and variance component estimates as follows: if $\sigma_{ii'}$ is the line covariance component estimate and σ_i and $\sigma_{i'}$ are the square roots of the line variance component estimates for variables i and i', then $r_{ii'} = \sigma_{ii'} / \sigma_i \sigma_{i'}$: No significance test is known, but the standard errors can be computed (MODE and ROBINSON 1959). Comparison of Tables 4 and 5 shows that the correlations of line effects are very similar to the correlations of line means.

The correlations of error effects are shown below the main diagonal in Table 5. These correlations were obtained by computing the sums of squares and products for each week × day × line combination separately and pooling the sums to obtain a product-moment correlation for each line. Theye were then tested for homogeneity and averaged over lines by FISHER's *z*-transform method. Note that the line-effects correlation between θ and *WBF* is quite different from the corresponding error-effects correlation. One possible explanation is that the total power is relatively constant for a given line, such that any change in θ is accompanied by an opposite and compensating change in *WBF*. Between lines there is no constancy of flight power output, and θ and *WBF* vary more or less independently (probably from a variety of causes). The $WBF \times A$ line-effects correlation also differs considerably from the corresponding error-effects correlation, possibly as a result of weight variations over lines. Within lines, the $WBF \times A$ correlation loses statistical significance. The most surprising feature of Table 5 is the significant negative correlation of P_t and θ error effects. It appears that the negative correlation of *WBF* and θ error effects causes increased θ to result in reduced *WBF* and consequently reduced P_t; perhaps the formula

for P_t is inherently more sensitive to variation in *WBF* than in wing-stroke amplitude.

DISCUSSION

The studies presented here have established three points that are fundamental to our goal of relating variation in enzyme structure and quantity to variation in the power output of the flight muscles.

First, wing-beat frequency and wing-stroke amplitude measurements are robust. Within rather broad limits, age, ambient temperature and duration of flight have little effect on line differences in flight parameters. As a result, line effects can be interpreted as differences in flight physiology that persist over a variety of environmental conditions. Tethered "flight" is admittedly artificial, but it is easily standardized, facilitates measurement of the critical flight parameters and probably gives an accurate approximation of the flight variables in free flight (see VOGEL 1966).

Second, the estimated power output of the flight muscles reflects the metabolic rate. Drosophila do not acquire an oxygen debt during flight (CHADWICK 1947), nor do the Diptera accumulate ATP during flight (SACKTOR 1974); it follows that oxygen consumption rates must be directly related to rates of ATP production. Wing-beat frequency is highly correlated with oxygen consumption rates, both within individuals during long flights (REED, WILLIAMS and CHADWICK 1942; CHADWICK and GILMOUR 1940; CHADWICK 1947) and among individuals from different lines, as shown in Figure 5. Further, as seen in Table 4, wing-beat frequency is the major determinant of power output. VOGEL (1967a,b) and GÖTZ (1968) have shown that wingbeat frequency is not altered to achieve control of the relative magnitudes of lift and thrust vectors in Drosophila; the important flight control is provided by varying wing-stroke amplitude and body angle.

Third, flight variables are subject to genetic variation that is attributable to chromosomes derived from natural populations. Lines are a highly significant source of variation for all flight variables in the multiple line study, for both raw and weight-adjusted data. The broad-sense heritabilities range from 0.16 for wing-stroke amplitude to 0.44 for inertial power, as shown in Table 3. The relatively low genetic component for wing-stroke amplitude is possibly due to the effect of behavioral modifications of that variable; it appears that flies alter the stroke amplitude, increasing on one side and decreasing on the other, as part of a turning maneuver (GÖTZ 1968). Attempts to turn on the tethering wire could inflate the error variance and reduce the genetic component.

There are several limitations of our method for establishing the power generated by flight muscles. WEIS-FOGH's power formulae (1972, 1973) are based on assumptions of steady-state aerodynamics, simple harmonic motion of the wings and freely moving wing-hinges (*i.e.*, elastic forces ignored). Furthermore, we have assumed constant wing thickness, wing density and coefficient of drag. Using estimates of flight parameters obtained from several groups of flying insects, including Drosophila, WEIS-FOGH (1972, 1973) concluded that the steady-state models provide an adequate energetic description of flapping flight—the

calculated lift is sufficient to sustain hovering, and the power requirements are consistent with known metabolic rates. To some extent, our work is limited by the fact that the mechanics of insect flight are incompletely understood; in particular, the consequences of the "clap-fling" mechanism of lift generation used by Drosophila and other insects are unknown (WEIS-FOGH 1972, 1973).

In spite of possible inaccuracies and the complexity of total power estimation, we and others have provided strong evidence for a close relationship between metabolic rate, as measured by O_2 consumption, and wing-beat frequency. We have devised measurement techniques that are highly repeatable, feasible for large replication and sufficient for the estimation of power output of the flight muscles. Experiments involving characterization of the activity of some enzymes involved in flight metabolism and their effects on power output are in progress.

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