

# Correlation between enzyme activities and routine metabolic rate in *Drosophila*

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*Drosophila*;  
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## Abstract

To determine whether enzyme activity is correlated with physiological performance, we analysed the relationship between routine metabolic rate and published data on activity of 12 enzymes from nine species of *Drosophila*. The enzymes are involved in several aspects of intermediary metabolism including glycolysis. Multiple regression on phylogenetically independent contrasts revealed significant and positive correlations between *in vitro* enzyme activity and routine metabolic rate. The regression analysis included body size and locomotor activity level as covariates. This result suggests that there may be energetic costs associated with increased enzyme capacity.

## Introduction

*Drosophila* species show substantial variation in levels of enzyme activity and evidence for coregulation of some enzymes involved in intermediary metabolism (Clark & Keith, 1989; Clark & Wang, 1994). An important extension of studies of cellular processes is to determine how they influence properties of the whole organism. Links between organismal performance and enzyme activity are important for at least two reasons. First, correlations between enzyme activity and organismal performance are required to determine if there is an opportunity for selection on enzyme activity. Second, these correlations might provide evidence for tradeoffs between enzyme activity and other aspects of performance.

Correlations have been found between the activities of several individual enzymes and organismal performance. For example, succinate dehydrogenase activity and metabolic rate are positively correlated in the brine shrimp *Artemia salina* (Packard & Taylor, 1968) and, in *Colias* butterflies, flight performance and fitness are correlated with population differences in phosphoglucose isomerase (PGI) activity (Watt, 1992). In vertebrates, swimming endurance in *Fundulus heteroclitus* varies between genotypes differing at the LDH-b (lactate dehydrogenase) locus (DiMichele & Powers, 1982; Powers & Schulte,

1996); maximal levels of oxygen consumption and LDH activity are positively correlated in lizard hearts (Garland, 1984); and studies of fish and mammals have examined the relationship between the activities of enzymes involved in glycolytic and oxidative pathways and body size and metabolic rate (Childress & Somero, 1979; Emmett & Hochachka, 1981).

The above studies analyse one or a few enzymes. However, populations and species usually display differences in the activities of many enzymes, and some theoretical work suggests that a number of enzymes can be important in controlling the dynamics of a metabolic pathway (Kacser & Burns, 1973, 1995). Thus, focusing on only a few enzymes could give misleading answers about the relationship between enzyme activity and performance. Few studies have yet addressed the connection between performance and activities of suites of interconnected enzymes involved in linked metabolic pathways (Clark & Wang, 1994). One exception is the work of Laurie-Ahlberg *et al.* (1985); they report scant evidence for correlations between activities of 15 enzymes and energy expenditures during flight in *Drosophila melanogaster*. Interspecific comparisons could increase the power of tests for correlations between enzyme activity and organismal traits by increasing the amount of variation available.

Clark & Wang (1994) analysed activities of 12 enzymes in males from nine species of *Drosophila*. To examine correlations between enzyme activity and organismal performance we measured routine metabolic rate, body mass and activity levels of individual males and females

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from the same nine species. We then used phylogenetically independent contrasts to test for correlations between metabolic rate and a composite measure of enzyme activity in males. We examined a composite measure of enzyme activity because the activities of the enzymes studied by Clark and Wang showed significant correlations. Attempts to identify a single important enzyme with multiple regression or other statistical techniques could be misleading because of the problem of multicollinearity.

The nine species of *Drosophila* studied included: *affinis*, *mauritanica*, *melanogaster*, *pseudoobscura*, *sechellia*, *simulans*, *teissieri*, *virilis* and *yakuba*. The *D. pseudoobscura* were from A. Clark at University of Pennsylvania and the *D. melanogaster* were from a laboratory population originally collected near Davis, CA, by L. Harshmann and M. Turelli in 1990. The remaining species were obtained from the National *Drosophila* Species Stock Center at Bowling Green. All species were reared for two generations prior to our measurements at moderate densities on corn meal, molasses, yeast, agar, tegosept media. The flies were reared at 25 °C on a 12 h:12 h L:D photoperiod.

We estimated the metabolic rate of individual adult flies using flow-through respirometry to detect the CO<sub>2</sub> produced by individuals. Flow-through respirometry provides an indirect but reliable estimate of metabolic rate (Lighton, 1991). We assume equivalent respiratory quotients for all the species. In brief, we used a Sable Systems TR-3 respirometry apparatus for the measurements. This consists of a Licor 6262 infra-red CO<sub>2</sub> detector together with a computer-controlled baselining device, flow controller and A/D board. We placed individual flies in a small glass tube in the CO<sub>2</sub>-free airstream of this system and after a 4–6-min equilibration period, recorded the CO<sub>2</sub> output for 2–4 min (more details in Lighton, 1991; Berrigan & Partridge, 1997). Note that CO<sub>2</sub> outputs were very stable for periods of 10–20 min using this set-up. Metabolic rates declined after this, possibly as a response to the dry conditions. Activity was monitored using a cool infra-red motion detector. We calculated CO<sub>2</sub> outputs according to Lighton (1991) based on flow rates of 30–60 mL min<sup>-1</sup>. After a set of measurements we sexed and weighed the flies to the nearest 0.01 mg. We converted our measurements of activity to units of cm sec<sup>-1</sup> by multiplying the number of times the infra-red detector was tripped times the length of the glass tube (5.5 cm) and dividing by the total time of the recording. When the flies were active, they walked back and forth fairly regularly (visual observations and see Berrigan & Partridge, 1997).

We present the data in two ways. First, we summarize the data on metabolic rate for the nine species using descriptive statistics. We report data for both males and females. Second we analyse the relationship between metabolic rate and enzyme activity using the independent contrasts method of Felsenstein (1985). This method attempts to account for the nonindepend-

dence of related species. To compare metabolic rate data to estimates of enzyme activity reported in Clark & Wang (1994) we used principal components analysis to generate aggregate measures of enzyme activity in the nine species of *Drosophila*. Clark and Wang analysed enzyme activity in male flies. Therefore, we used data from males to calculate contrasts for the measures of performance used here (but we have included the data on females for investigators who are interested in further examining these correlations). The statistical analysis described here was performed using the program JMP (SAS Institute inc., CARY, NC, USA) and Compare (E. Martins, 1995, University of Oregon; Martins, 1996). Metabolic rate and mass were log<sub>10</sub> transformed prior to analysis.

## Results and discussion

Metabolic rates estimated in this study (Table 1) were comparable to past studies of *Drosophila* (Hunter, 1964; Giesel *et al.*, 1989; Berrigan & Partridge, 1997). As expected, metabolic rate increased with mass and females were larger and had higher metabolic rates than males. Linear regression on log transformed metabolic rate vs. log transformed mass gives an estimated slope of 0.657 (95% CI 0.56–0.75,  $n = 122$ ,  $r = 0.78$ ). Separate regressions for the sexes result in virtually identical slopes.

The activities of the 12 enzymes considered here were estimated from crude activity levels (nmoles of substrate reduced per minute per fly) *in vitro* (Clark & Wang, 1994); these data do not allow separate analyses of  $K_m$  and  $V_{max}$  of the enzymes (Clark & Keith, 1989). We summarized the activities of the 12 enzymes by performing a principal components analysis on these activity levels (from Table 1A in Clark and Wang). Table 2 gives the eigenvalues and vectors for the first three components. Note that these components explain about 90% of the variation between species in enzyme activity levels. Furthermore, the first component loads positively on activities of most of the enzymes. The negative loadings for fatty acid synthetase (FAS) and glucose-6-phosphate dehydrogenase (G6PD) and the near zero loading for 6-phosphogluconate dehydrogenase (PGD) are interesting because these three enzymes are involved in fat synthesis whereas the remaining enzymes are important for glycolysis and other components of intermediary metabolism (Fig. 1 of Clark & Wang, 1994; A. G. Clark, personal communication). The first component explains about 60% of the variation in enzyme activity. The remaining two components show a mix of positive and negative loadings and explain much less of the variation in activity levels. For the above reasons, we include only principal component 1 (PC1) in our analysis of the relationship between metabolic rate and enzyme activity.

To control for the potential nonindependence of data points from different species in our regression analysis (Felsenstein, 1985), we calculated phylogenetically

**Table 1** Mean values of mass, metabolic rate (MR) and walking speed for nine species of *Drosophila*. The values in parentheses are one standard error.

Species	<i>n</i>	Mass (mg)	MR (ml CO <sub>2</sub> h <sup>-1</sup> ) (×10 <sup>-3</sup> )	Speed (cm s <sup>-1</sup> )
Females				
<i>affinis</i>	6	1.12 (0.022)	4.72 (0.368)	0.10 (0.064)
<i>mauritania</i>	7	0.97 (0.066)	3.45 (0.405)	0.06 (0.045)
<i>melanogaster</i>	6	1.10 (0.038)	4.60 (0.361)	2.45 (0.769)
<i>pseudoobscura</i>	6	1.55 (0.043)	5.55 (0.463)	0.08 (0.034)
<i>sechellia</i>	7	0.85 (0.030)	3.87 (0.291)	0.45 (0.152)
<i>simulans</i>	9	1.32 (0.039)	3.22 (0.210)	0.03 (0.034)
<i>teissieri</i>	7	0.78 (0.017)	2.83 (0.123)	0.30 (0.099)
<i>virilis</i>	6	2.70 (0.069)	7.19 (0.271)	0.43 (0.138)
<i>yakuba</i>	8	0.72 (0.042)	2.72 (0.153)	0.83 (0.198)
Males				
<i>affinis</i>	8	0.71 (0.017)	3.80 (0.255)	0.40 (0.154)
<i>mauritania</i>	6	0.68 (0.057)	2.28 (0.234)	0.25 (0.139)
<i>melanogaster</i>	7	0.73 (0.018)	3.36 (0.169)	2.40 (0.686)
<i>pseudoobscura</i>	6	1.01 (0.021)	2.92 (0.401)	0.30 (0.196)
<i>sechellia</i>	7	0.69 (0.022)	3.72 (0.257)	0.50 (0.198)
<i>simulans</i>	5	0.74 (0.025)	2.60 (0.222)	0.30 (0.127)
<i>teissieri</i>	7	0.63 (0.022)	2.31 (0.151)	0.04 (0.028)
<i>virilis</i>	7	2.37 (0.068)	6.60 (0.317)	0.65 (0.183)
<i>yakuba</i>	7	0.45 (0.014)	2.15 (0.121)	1.19 (0.404)

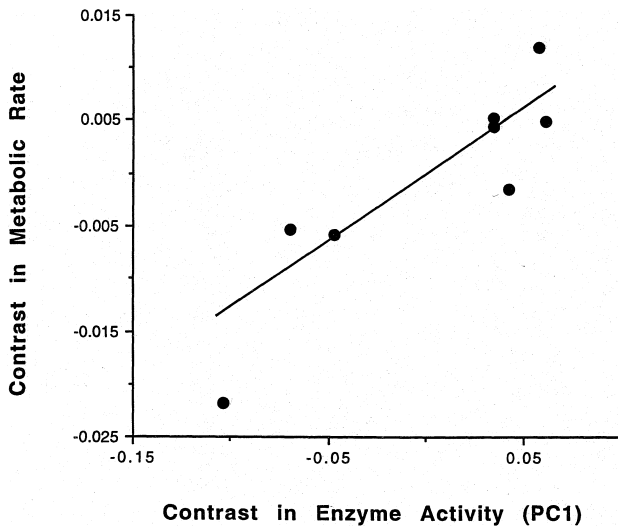
independent contrasts between log mass, log metabolic rate, log speed and the value of the first principal component from our analysis of enzyme activity. The standardized contrasts were obtained using a phylogeny for the nine species of *Drosophila* (Fig. 6 of Clark & Wang, 1994), with branch lengths estimated from molecular distance data (A. G. Clark, personal communication). We chose not to perform contrasts using mass-specific metabolic rate because the enzyme activity data were

recorded on a per fly basis, and Clark & Wang (1994) reported significant differences in the relationship between size and enzyme activity for different enzymes. Including mass in the multiple regression should control for the effects of mass on enzyme activity.

Figure 1 shows the relationship between the standardized contrasts of metabolic rate and enzyme activity. Multiple regression analysis of the standardized contrasts indicates significant effects of enzyme activity on meta-

	PC1	PC2	PC3
EigenValue	7.2096	2.3322	1.3726
Per cent	60.0800	19.4346	11.4383
Cumulative per cent	60.0800	79.5146	90.9529
Enzymes	Eigenvectors		
Alcohol dehydrogenase	0.23031	-0.27877	0.52595
Fatty acid synthetase	-0.12822	0.45632	-0.26667
Glucose-6-phosphate dehydrogenase	-0.02342	0.55503	0.32453
Glycogen phosphorylase	0.35476	0.07810	-0.03025
α-Glycerol-3-phosphate dehydrogenase	0.35151	0.09419	0.14547
Glycogen synthase	0.31918	-0.15846	0.27283
Hexokinase	0.34783	-0.02000	-0.29031
Malic enzyme	0.34397	0.15862	0.12652
6-phosphogluconate dehydrogenase	0.06887	0.56482	0.21536
Phosphoglucose isomerase	0.28619	0.08245	-0.49477
Phosphoglucomutase	0.36473	0.10245	-0.01987
Trehalase	0.34598	-0.06357	-0.24203

**Table 2** Principal components analysis of the activities of 12 enzymes involved in intermediary metabolism in nine *Drosophila* species. These data were collected by Clark & Wang (1994).



**Fig. 1** Standardized contrasts in metabolic rate ( $\log \text{ mL CO}_2 \text{ h}^{-1} \text{ CO}_2$ ) vs. standardized contrasts of enzyme activity (PC1 from Table 2) for data obtained from males of nine species of *Drosophila*. The slope ( $b = 0.126$ ,  $\text{SE} = 0.036$ ) of the regression line was obtained from a multiple regression analysis including mass and speed as independent variables (see text). Note the regression is fit through the origin because the data are standardized contrasts.

bolic rate ( $F_{1,8} = 12.5$ ,  $P = 0.017$ ), and no significant effects of mass ( $F_{1,8} = 3.4$ ,  $P = 0.12$ ), or speed ( $F_{1,8} = 0.37$ ,  $P = 0.57$ ). The full model has an  $r^2 = 0.90$  and explains a significant amount of variation in the contrasts in metabolic rate ( $F_{3,5} = 15.9$ ,  $P = 0.005$ ). Note that regression analyses of contrasts must be fit through the origin (Felsenstein, 1985). Removing speed from the analysis results in significant mass and enzyme effects. Thus, the association of metabolic rate and enzyme activity is not caused by differences in mass or speed. This also indicates that the significantly greater locomotor activity of *D. melanogaster* is not responsible for the observed pattern. Male vs. female values for metabolic rate, mass and speed were highly correlated among species. Similar results were obtained from an analysis of contrasts assuming equal branch lengths (not shown).

We found significant correlations between routine metabolic rate and enzyme activity among the nine species of *Drosophila*. These data were obtained from individuals reared at different times in different laboratories. Thus the correlation is robust to the effect of environmental conditions. Measurements of enzyme activity and power output during flight in *D. melanogaster* have failed to find correlations between enzyme activity and energy output with either individual enzymes or principal component scores (Laurie-Ahlberg *et al.*, 1985). The present study may have found significant correlations because the use of different species increased the amount of variation present in both traits. We do not test for the influence of individual enzymes on metabolic rate because of the small number of

species in this study and because the activities of different enzymes are correlated with each other (Clark & Wang, 1994). Our results could also differ from those of Laurie-Ahlberg *et al.* because of differences in the measure of performance or in the enzymes analysed. Laurie-Ahlberg *et al.* studied energy expenditures during flight and a larger set of enzymes.

The observed correlation between enzyme activity levels and routine metabolic rate could reflect a tradeoff between the costs of protein production and maintenance and the ability to achieve high levels of performance. Several of the enzymes in this study are involved in flight metabolism (Laurie-Ahlberg *et al.*, 1985), and *Drosophila* flight requires very high levels of energy expenditure (Vogel, 1967); thus, the increase in routine metabolic rate may be a necessary consequence of being able to achieve the high energy output needed for flight. However, more detailed studies of the links between suites of enzymes and organismal performance are needed to determine if the costs of enzyme production and/or maintenance are responsible for the differences in routine metabolic rate between these species.

This note represents an example of a comparative study of organismal performance and its relationship to the activities of suites of enzymes involved in several important metabolic pathways. In general, links between enzyme activity and maximal power output, and between routine metabolic rate and maximal metabolic rate, are an active area of research (Bennett, 1991; Diamond & Hammond, 1992; Suarez, 1996); and comparative studies are a powerful tool for generating testable hypotheses about such links (Pierce & Crawford, 1997).

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