

## Roles of hierarchical and metabolic regulation in the allometric scaling of metabolism in Panamanian orchid bees

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### Summary

Assessment of the relative importance of variation in enzyme concentration [E] and metabolic regulation in accounting for interspecific variation in metabolic rates is an unrealized area of research. Towards this end, we used metabolic flux rates during hovering and enzymatic flux capacities ( $V_{\max}$  values, equal to  $[E] \times k_{\text{cat}}$ , where  $k_{\text{cat}}$  is catalytic efficiency) in flight muscles measured *in vitro* from 14 orchid bee species ranging in body mass from 47 to 1065 mg. Previous studies revealed that, across orchid bee species, wingbeat frequencies and metabolic rates decline in parallel with increasing body mass.  $V_{\max}$  values at some enzymatic steps in pathways of energy metabolism decline with increasing mass while, at most other steps,  $V_{\max}$  values are mass-independent. We quantified the relative importance of 'hierarchical regulation' (alteration in  $V_{\max}$ , indicative of alteration in [E]) and 'metabolic regulation' (resulting from variation in substrate, product or modulator concentrations) in accounting for

interspecific variation in flux across species. In addition, we applied the method of phylogenetically independent contrasts to remove the potentially confounding effects of phylogenetic relationships among species. In the evolution of orchid bees, hierarchical regulation completely accounts for allometric variation in flux rates at the hexokinase step while, at other reactions, variation in flux is completely accounted for by metabolic regulation. The predominant role played by metabolic regulation is examined at the phosphoglucosomerase step using the Haldane relationship. We find that extremely small variation in the concentration ratio of [product]/[substrate] is enough to cause the observed interspecific variation in net flux at this reaction in glycolysis.

Key words: allometry, metabolism, glycolysis, hovering flight, metabolic scaling, orchid bee.

### Introduction

Orchid bees (Apidae: Euglossini) belong to a diverse clade consisting of more than 190 species belonging to five genera that vary in body mass by about 20-fold. Pioneering work conducted by Casey and colleagues (Casey and Ellington, 1990; Casey et al., 1992, 1985) showed that wingbeat frequencies and mass-specific metabolic rates during flight decline with increasing body mass. Inspired by this work, we examined allometric scaling patterns in greater detail, and in the context of phylogenetic relationships among species (see accompanying papers, Darveau et al., 2005a,b). We found evidence supporting the idea that the allometric scaling of wingbeat frequency, one of the determinants of muscle volume-specific power output (Pennycuik and Rezende, 1984), drives the scaling of hovering metabolic rate (Darveau et al., 2005a). Orchid bees oxidize carbohydrate as the main energy source for flight (Suarez et al., 2005), so we also conducted interspecific studies of enzymatic flux capacities through carbohydrate-oxidizing pathways and examined how

these relate to metabolic flux rates during flight. We found that flux capacities ( $V_{\max}$  values) at one step, i.e. hexokinase, scale against body mass with an allometric exponent almost identical to that of wingbeat frequency and metabolic rate. In contrast,  $V_{\max}$  values at other steps either decline with lower allometric exponents than hexokinase or are mass-independent, i.e. scale isometrically (Darveau et al., 2005b).

Within an individual bee, >90% of the whole body metabolic rate during hovering flight is due to O<sub>2</sub> consumption by flight muscle mitochondria (Rothe and Nachtigall, 1989; Suarez, 2000). Therefore, rates of whole-body CO<sub>2</sub> production ( $\dot{V}_{\text{CO}_2}$ ) or O<sub>2</sub> consumption ( $\dot{V}_{\text{O}_2}$ ) can be used to estimate flux rates through catabolic pathways (Suarez et al., 1996) or through mitochondrial respiratory chain enzymes (Suarez et al., 1999, 2000). These fluxes represent steady-state rates whose control can be analyzed quantitatively, at least in principle, through metabolic control analysis (Fell, 1997). On the other hand, interspecific variation in flux can be considered

within the framework proposed (but, originally, in an intraspecific context) by ter Kuile and Westerhoff (2001). At any step in metabolism, such variation in flux may be due to 'hierarchical regulation', i.e. variation in enzyme concentration, [E], across species. An alternative is that [E] at a given step is constant across species and variation in flux at this step is due to variation in the concentrations of substrates, products or allosteric effectors, i.e. 'metabolic regulation'. A third possibility is that variation in flux may result from a combination of hierarchical and metabolic regulation.

## Materials and methods

### Theory

We summarize the approach described by ter Kuile and Westerhoff (2001) and our application of it to metabolic scaling in orchid bees as follows:

The hierarchical regulation coefficient  $\rho_h$ , which is a measure of the contribution of enzyme concentration [E], and the metabolic regulation coefficient  $\rho_m$ , which is a measure of the contribution of mechanisms that result in the modulation of the activity of a constant concentration of enzyme, to variation in flux, are related as:

$$\rho_h + \rho_m = 1. \quad (1)$$

At any enzyme-catalyzed step  $i$ ,  $\rho_h$  is a function of the relative change in rate,  $\partial v_i$ , divided by the relative change in enzyme concentration,  $\partial e_i$ , times the ratio of change in  $e_i$  to the change in flux  $J$ . Assuming that  $\partial \ln v_i / \partial \ln e_i$  equals 1 (ter Kuile and Westerhoff, 2001),

$$\rho_h = \frac{\partial \ln v_i}{\partial \ln e_i} \cdot \frac{d \ln e_i}{d \ln J} = \frac{d \ln e_i}{d \ln J}. \quad (2)$$

Similarly, at any step  $i$ , metabolic regulation results from a change in an enzyme-catalyzed rate divided by the change in concentration of its substrate, product or allosteric modulator ( $X$ ), times the ratio of change in  $X$  to change in  $J$ , such that:

$$\rho_m = \sum_X \frac{\partial \ln v_i}{\partial \ln X} \cdot \frac{\partial \ln X}{\partial \ln J}. \quad (3)$$

Orthologous enzymes from organisms of similar body temperature display similar catalytic efficiencies ( $k_{cat}$  values) (Hochachka and Somero, 2002). Because

$$V_{max} = e_i k_{cat}, \quad (4)$$

it follows that interspecific variation in  $V_{max}$  measures variation in  $e_i$ . From Eq. 1, the slope of  $\ln V_{max}$  plotted against  $\ln J$  yields the hierarchical regulation coefficient  $\rho_h$ . It follows that  $1 - \rho_h$  yields the metabolic regulation coefficient  $\rho_m$ .

We note here that this approach was not devised for interspecific comparisons; ter Kuile and Westerhoff (2001) applied it to the analysis of variation in glycolytic rates in protozoa resulting from experimental manipulation. However, the mechanistic bases for and the quantitative relationships that

describe intraspecific variation in flux among protozoa apply equally well to interspecific variation in flux among orchid bees. In a given steady-state situation (in this case, high rates of carbohydrate oxidation during hovering flight), the interspecific variation in flux can be accounted for either by interspecific variation in [E] or by interspecific variation in substrate, product or modulator concentrations. However, the interspecific variation in flux may also be due to variation in other morphological, physiological and biochemical traits besides these. Thus, in the work described herein, the value of slope of  $\ln V_{max}$  plotted against  $\ln J$  is potentially influenced by other traits that covary with the biochemical parameters that we measured.

We address the above problem with the use of Felsenstein's method of phylogenetically independent contrasts (PIC analysis; Felsenstein, 1985; Garland et al., 1992). Briefly, in conventional statistical analyses of interspecific data, it is assumed that species are equally related to each other. In reality, they are related to each other by their phylogenetic relationships. Because closely related species tend to display greater similarity than more distantly related ones, estimates of  $\rho_h$  from slopes of linear regressions may be biased by phylogenetic relationships and correlated traits other than those hypothesized to account for interspecific variation in, as in the present study, flux rates. PIC analysis provides a statistical approach that allows for phylogenetic non-independence and makes possible the application of the approach described by ter Kuile and Westerhoff (2001) to the analysis of interspecific data. In the present work, PIC analysis was conducted using the PDAP (Midford et al., 2003) module in Mesquite (Maddison and Maddison, 2004). The analyses were performed using both gradual and speciation models of character evolution, with branch-lengths obtained from genetic distances inferred from cytochrome *b* partial sequences (Darveau et al., 2005a) or with branch lengths set to 1.

### Sources of data

Glycolytic flux rates were estimated from  $\dot{V}_{CO_2}$  during flight (Darveau et al., 2005a). Enzyme  $V_{max}$  values are from Darveau et al. (2005b). Hypothetical phylogenies used in PIC analysis were generated on the bases of partial sequences for the mitochondrial cytochrome *b* gene (Darveau et al., 2005a).

### Calculations

Mathematical modeling of the phosphoglucoisomerase reaction was performed using Mathcad Professional, version 8 (MathSoft, Inc., Cambridge, MA, USA). Model assumptions, equations and parameters are provided in the text.

## Results and discussion

Glycolytic flux rates, estimated from  $\dot{V}_{CO_2}$  during flight, ranged from 4.64 to 13.0  $\mu\text{mol g}^{-1} \text{min}^{-1}$  across species. Fig. 1 shows  $\ln V_{max}$  plotted against  $\ln J$  with regression slopes equivalent to hierarchical regulation coefficients,  $\rho_h$ . At this level of analysis, completely hierarchical regulation is

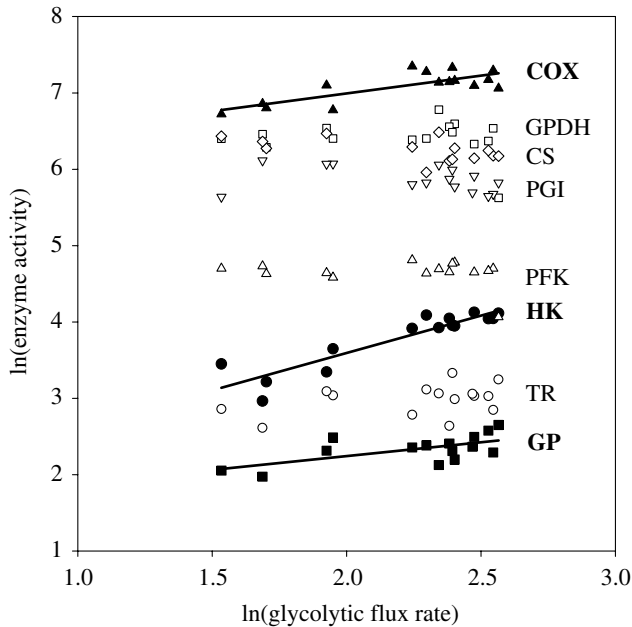


Fig. 1.  $\ln(\text{enzyme activity in } \mu\text{mol g}^{-1} \text{min}^{-1})$  vs  $\ln(\text{flux rate in } \mu\text{mol g}^{-1} \text{min}^{-1})$  across orchid bee species. Slopes of regressions represent hierarchical regulation coefficients,  $\rho_h$ . These values are equal to 0.46 (COX, cytochrome oxidase), 0.98 (HK, hexokinase), and 0.36 (GP, glycogen phosphorylase), and significant for these three enzymes ( $P$  values are indicated in Table 1). Slopes are not significantly different from zero in the case of GPDH (glycerol 3-phosphate dehydrogenase), CS, citrate synthase, PGI (phosphoglucoisomerase), PFK (phosphofructokinase) and TR (trehalase).

observed at the hexokinase (HK) step, where  $\rho_h=0.98$ , while steady-state flux rates at cytochrome *c* oxidase (COX) and glycogen phosphorylase (GP) are the result of control shared by hierarchical regulation ( $\rho_h=0.46$  and  $0.36$ , respectively) and metabolic regulation ( $\rho_m=0.54$  and  $0.64$ , respectively). The finding that hierarchical regulation accounts for interspecific variation in HK flux remains robust after PIC analysis (Table 1). However, the statistical relationships for COX and GP are no longer significant after controlling for phylogeny. All other steps examined yield  $\rho_h=0$ ; this means  $\rho_m=1$  and that metabolic regulation completely accounts for interspecific variation in flux at these steps in energy metabolism.

Our results are similar to those obtained by ter Kuile and Westerhoff (2001) in the sense that most of the variation in glycolytic flux in their intraspecific study of protozoa was due to metabolic regulation, and complete or partial hierarchical control contributed to the regulation of only a few steps in energy metabolism. It is important to point out that  $\rho_h$  and  $\rho_m$  values are not flux control coefficients; i.e. they are not measures of the contributions of individual enzyme-catalyzed steps to the control of flux relative to other steps in a pathway. Rather, they allow quantitative determination of the relative contributions of two alternative mechanisms (i.e. hierarchical or metabolic regulation) in explaining pathway flux changes at individual steps.

Although allometric variation in biochemical flux capacities is widespread among animals (Suarez et al., 2004), the regulatory mechanisms underlying allometry in [E] remain largely unexplored. Two quite noteworthy exceptions to this are intraspecific studies on fish by Yang and Somero (1996) and Burness et al. (1999). In both studies are found examples wherein [E] does not correlate directly with the tissue content of the corresponding mRNA. This indicates that translation rates and/or rates of enzyme degradation may play important roles in the allometric variation in [E]. It would be worthwhile to determine whether HK mRNA content is size-dependent in orchid bees. However, given the preponderance of metabolic regulation revealed by our results, how such mechanisms lead to the allometric scaling of metabolism in orchid bees also merits further consideration.

#### Metabolic regulation at the PGI step

In previous work using honeybees *Apis mellifera*, we combined empirical and modeling approaches to try to understand the relations between flux capacities and flux rates at the PGI step, a near-equilibrium, reversible, glycolytic reaction at which glucose 6-phosphate, G6P, is converted to fructose 6-phosphate, F6P (Staples and Suarez, 1997). At such a near-equilibrium step, the enzyme catalyzes reactions in both directions, and net glycolytic flux equals the forward rate minus the reverse rate. Using Haldane's equations (Haldane, 1930) in a similar manner as Veech et al. (1969), we showed that the high  $V_{\max}$  at this step could be rationalized on the bases of the enzyme's dual roles, i.e. to maintain a net forward flux, equal to the overall rate of glycolysis, and to maintain the reaction

Table 1. Relationship between glycolytic flux rate ( $J$ ) and the activities of hexokinase (HK), cytochrome oxidase (COX) and glycogen phosphorylase (GP) in orchid bee flight muscle, analysed using conventional statistics and phylogenetically independent contrasts (PIC)

Enzyme	Conventional			PIC gradual			PIC speciational		
	Equation	$r^2$	$P$	$b$	$r^2$	$P$	$b$	$r^2$	$P$
HK	$5.11J^{0.98} (0.72, 1.25)$	0.83	<0.001	0.83 (0.34, 1.31)	0.59	<0.005	0.84 (0.25, 1.43)	0.50	<0.01
COX	$429.7J^{0.47} (0.24, 0.69)$	0.61	<0.001		0.12	NS		0.19	NS
GP	$4.56J^{0.36} (0.07, 0.65)$	0.38	<0.05		0.28	NS	0.66 (0.14, 1.18)	0.44	<0.05

$N=14$  species for conventional analysis and 11 contrasts for PIC analysis.

For gradual evolution, branch lengths from fig. 1 in Darveau et al. (2005a) were used; for speciational model, branch lengths were set to 1. NS, not significant.

close to equilibrium. We now consider how substrate and product concentrations *in vivo* would have to change to account for the range of net flux rates observed interspecifically among orchid bees. The Haldane (1930) equation applied to PGI is:

$$J = \frac{V_f \times \frac{[G6P]}{K_f} - V_r \times \frac{[F6P]}{K_r}}{1 + \frac{[G6P]}{K_f} + \frac{[F6P]}{K_r}}, \quad (5)$$

where  $J$  is the net flux, [G6P] and [F6P] are glucose 6-phosphate and fructose 6-phosphate concentrations, respectively,  $K_f$  and  $K_r$  are Michaelis constants for the forward (G6P is substrate) and reverse (F6P is substrate) reactions, respectively. We used  $K_f=1.07 \text{ mmol l}^{-1}$  and  $K_r=0.117 \text{ mmol l}^{-1}$ , obtained under simulated intracellular conditions using purified honeybee PGI (Staples and Suarez, 1997). It is assumed that both  $K_f$  and  $K_r$  are mass-independent and conserved across orchid bee species.  $K_{eq}$  is the equilibrium constant obtained *in vitro*, equal to 0.3 (Staples and Suarez, 1997).  $V_r$  represents the  $V_{max}$  value in the direction of F6P conversion to G6P. These scale isometrically in orchid bees and average  $345.6 \mu\text{mol g}^{-1} \text{ min}^{-1}$  (Darveau et al., 2005b).  $V_f$  is the  $V_{max}$  in the glycolytic direction, estimated from the Haldane (1930) equation for  $K_{eq}$ ,

$$K_{eq} = \frac{V_f \times K_r}{V_r \times K_f}, \quad (6)$$

as  $948.2 \mu\text{mol g}^{-1} \text{ min}^{-1}$ . This is close to the  $V_f$  value measured empirically in honeybee flight muscles (Staples and Suarez, 1997). Eq. 5 is used to solve for the mass action ratios, i.e. the ratios of [F6P]/[G6P] in the flight muscles, required to account for the range of estimated flux rates. The decline in mass action ratio (Fig. 2A) is extremely small, a result that can also be demonstrated by holding [F6P] constant to  $0.1 \text{ mmol l}^{-1}$ , a value within the range measured *in vivo* (Staples and Suarez, 1997), and solving for the range in [G6P] required to obtain the range of observed net forward flux rates (Fig. 2B). The model predicts that a change of about  $0.02 \text{ mmol l}^{-1}$  in [G6P] is enough to account for the entire range of variation in flux across orchid bee species. A highly amplified response to a small change in substrate concentration, as seen here, is a characteristic of near-equilibrium reactions (Newsholme and Crabtree, 1976). Given this result, it seems unlikely that the expected range of variation in [F6P]/[G6P] in orchid bee flight muscles would be detectable in a comparative, interspecific study. At many other steps in metabolism, this problem is exacerbated by even lower steady-state concentrations of substrate and product (e.g. Kashiwaya et al., 1994). Thus, a modeling approach such as the one described here may be the only way to address the issue of metabolic regulation at such steps.

#### A broader view of metabolic regulation

Metabolic control analysis using skinned muscle fibers from mammals has revealed that as much as half of the control of

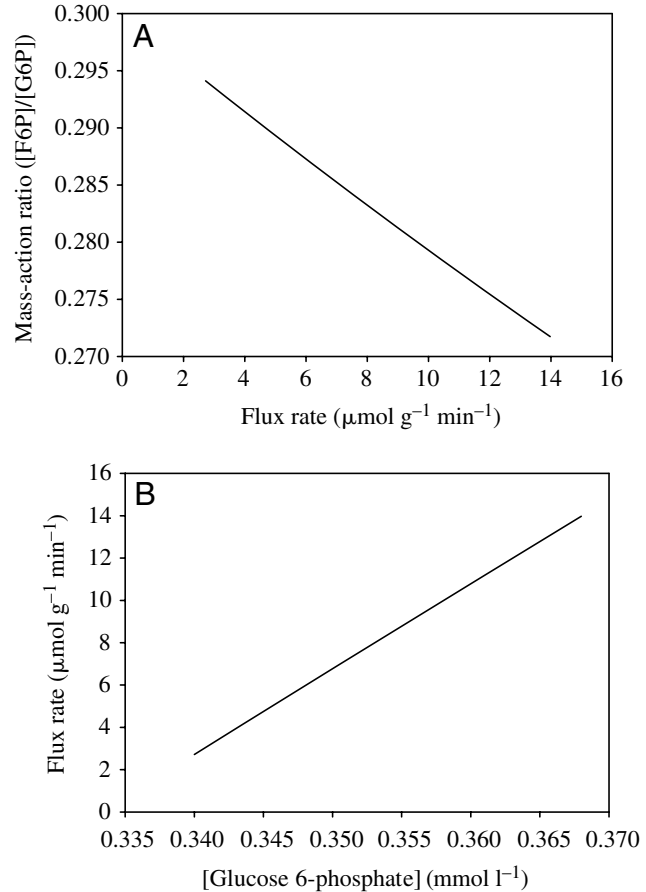


Fig. 2. (A) Mass action ratio vs glycolytic flux rate (i.e. net forward flux at the phosphoglucosomerase reaction). The Haldane relationship predicts that extremely small variation in the mass action ratio is enough to account for the range of glycolytic flux rates of  $4.64\text{--}13.0 \mu\text{mol g}^{-1} \text{ min}^{-1}$  observed across species. (B) Glycolytic flux rate (i.e. net forward flux at the PGI reaction) vs glucose 6-phosphate concentration. Holding fructose 6-phosphate concentration at  $0.1 \text{ mmol l}^{-1}$ , the Haldane relationship predicts that the range of glycolytic flux rates of  $4.64\text{--}13.0 \mu\text{mol g}^{-1} \text{ min}^{-1}$  observed across species is achieved by a change in glucose 6-phosphate concentration of about  $0.02 \text{ mmol l}^{-1}$ .

oxidative metabolism in muscles is exerted by actomyosin ATPase (Wisniewski et al., 1995). In the context of metabolic scaling in orchid bees, a possible interpretation of our results is that, as muscle operating frequencies decline with increasing body mass (Darveau et al., 2005a), muscle ATP hydrolysis rates decline (Darveau et al., 2005b) and this drives the allometric scaling of metabolism. Such an interpretation follows from the similarity in allometric exponents of wingbeat frequency and hovering metabolic rate and is consistent with the predominant role played by metabolic regulation seen in the results reported here.

Further insights into the role played by metabolic regulation can be derived from work concerning bioenergetic scaling in mammalian hearts. Top-down control analysis has revealed that work rate (therefore ATP hydrolysis rate) dominates the

control of oxidative metabolism in the heart (Diolez et al., 2002). Using  $^{31}\text{P}$ -NMR spectroscopy, Dobson and Headrick (1995) and Dobson and Himmelreich (2002) found that cytosolic free [ADP] declines as body mass increases in hearts, such that  $1/[\text{ADP}]$  and the cytosolic phosphorylation potential  $[\text{ATP}]/[\text{ADP}][\text{P}_i]$  both scale allometrically with exponents close to  $-0.25$ . Dobson and colleagues propose that these result in higher 'kinetic gain' such that small changes in [ADP] result in greater fractional changes in metabolic rate in the hearts of small mammals compared with larger ones. This scheme provides a plausible control mechanism by which allometry in work rate drives allometry in metabolic rates. That this applies to orchid bee flight muscles is a testable hypothesis.

### Conclusion

The relative importance of the roles played by hierarchical regulation, resulting from variation in [E], and metabolic regulation in accounting for interspecific variation in metabolic rates is not well understood. Among orchid bees, the allometry in metabolic rates during hovering flight involves the hierarchical regulation of flux at the HK step and metabolic regulation at all other enzyme-catalyzed reactions that we examined. Thus, although techniques for the study of gene expression can be brought to bear on the problem of metabolic scaling, the predominance of metabolic regulation in accounting for interspecific variation in flux rates also warrants the application of a systems approach to the control of flux (Fell, 1997) and the manner in which it scales (Darveau et al., 2002; Suarez et al., 2004).

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### References

- Burness, G. P., Leary, S. C., Hochachka, P. W. and Moyes, C. D. (1999). Allometric scaling of RNA, DNA, and enzyme levels: an intraspecific study. *Am. J. Physiol.* **277**, R1164-R1170.
- Casey, T. M. and Ellington, C. P. (1990). Energetics of insect flight. In *Energy Transformations in Cells and Organisms* (ed. W. Wieser and E. Gnaiger), pp. 200-210. Stuttgart: Georg Thieme Verlag.
- Casey, T. M., May, M. L. and Morgan, K. R. (1985). Flight energetics of euglossine bees in relation to morphology and wing stroke frequency. *J. Exp. Biol.* **116**, 271-289.
- Casey, T. M., Ellington, C. P. and Gabriel, J. M. (1992). Allometric scaling of muscle performance and metabolism: insects. In *Hypoxia and Mountain Medicine* (ed. J. R. Sutton G. Coates and C. S. Houston), pp. 152-162. Oxford: Pergamon Press.
- Darveau, C.-A., Suarez, R. K., Andrews, R. D. and Hochachka, P. W. (2002). Allometric cascade as a unifying principle of body mass effects on metabolism. *Nature* **417**, 166-170.
- Darveau, C.-A., Hochachka, P. W., Welch, K. C., Jr., Roubik, D. W. and Suarez, R. K. (2005a). Allometric scaling of flight energetics in Panamanian orchid bees: a comparative phylogenetic approach. *J. Exp. Biol.* **208**, 3581-3591.
- Darveau, C.-A., Hochachka, P. W., Roubik, D. W. and Suarez, R. K. (2005b). Allometric scaling of flight energetics in orchid bees: evolution of flux capacities and flux rates. *J. Exp. Biol.* **208**, 3593-3602.
- Diolez, P., Raffard, G., Simon, C., Leducq, N., Dos Santos, P. and Canioni, P. (2002). Mitochondria do not control heart bioenergetics. *Mol. Biol. Rep.* **29**, 193-196.
- Dobson, G. P. and Headrick, J. P. (1995). Bioenergetic scaling: metabolic design and body-size constraints in mammals. *Proc. Natl. Acad. Sci. USA* **92**, 7317-7321.
- Dobson, G. P. and Himmelreich, U. (2002). Heart design: free ADP scales with absolute mitochondrial and myofibrillar volumes from mouse to human. *Biochim. Biophys. Acta* **1553**, 261-267.
- Fell, D. (1997). *Understanding the Control of Metabolism*. London and Miami: Portland Press.
- Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* **125**, 1-15.
- Garland, T., Jr, Harvey, P. H. and Ives, A. R. (1992). Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* **41**, 18-32.
- Haldane, J. B. S. (1930). *Enzymes*. London: Longmans, Green and Co.
- Hochachka, P. W. and Somero, G. N. (2002). *Biochemical Adaptation. Mechanism and Process in Physiological Evolution*. Oxford: Oxford University Press.
- Kashiwaya, Y., Sato, K., Tsuchiya, N., Thomas, S., Fell, D. A., Veech, R. L. and Passonneau, J. V. (1994). Control of glucose utilization in working perfused rat heart. *J. Biol. Chem.* **269**, 25502-25514.
- Maddison, W. P. and Maddison, D. R. (2004). Mesquite: A modular system for evolutionary analysis. Version 1.02. <http://mesquiteproject.org>.
- Midford, P. E., Garland, T., Jr. and Maddison, W. P. (2003). PDAP Package.
- Newsholme, E. A. and Crabtree, B. (1976). Substrate cycles in metabolic regulation and in heat generation. *Biochem. Soc. Symp.* **41**, 61-109.
- Pennycuik, C. J. and Rezende, M. A. (1984). The specific power output of aerobic muscle, related to the power density of mitochondria. *J. Exp. Biol.* **108**, 377-392.
- Rothe, U. and Nachtigall, W. (1989). Flight of the honeybee. IV. Respiratory quotients and metabolic rates during sitting, walking and flying. *J. Comp. Physiol.* **158**, 739-749.
- Staples, J. F. and Suarez, R. K. (1997). Honeybee flight muscle phosphoglucose isomerase: matching enzyme capacities to flux requirements at a near-equilibrium reaction. *J. Exp. Biol.* **200**, 1247-1254.
- Suarez, R. K. (2000). Energy metabolism during insect flight: biochemical design and physiological performance. *Physiol. Biochem. Zool.* **73**, 765-771.
- Suarez, R. K., Lighton, J. R. B., Joos, B., Roberts, S. P. and Harrison, J. F. (1996). Energy metabolism, enzymatic flux capacities and metabolic flux rates in flying honeybees. *Proc. Natl. Acad. Sci. USA* **93**, 12616-12620.
- Suarez, R. K., Staples, J. F. and Lighton, J. R. B. (1999). Turnover rates of mitochondrial respiratory chain enzymes in flying honeybees (*Apis mellifera*). *J. Exp. Zool.* **283**, 1-6.
- Suarez, R. K., Staples, J. F., Lighton, J. R. B. and Mathieu-Costello, O. (2000). Mitochondrial function in flying honeybees (*Apis mellifera*): respiratory chain enzymes and electron flow from complex III to oxygen. *J. Exp. Biol.* **203**, 905-911.
- Suarez, R. K., Darveau, C.-A. and Childress, J. J. (2004). Metabolic scaling: a many-splendoured thing. *Comp. Biochem. Physiol.* **139**, 531-541.
- Suarez, R. K., Darveau, C.-A., Welch, K. C., Jr., O'Brien, D. M., Roubik, D. W. and Hochachka, P. W. (2005). Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. *J. Exp. Biol.* **208**, 3573-3579.
- ter Kuile, B. H. and Westerhoff, H. V. (2001). Transcriptome meets metabolome: hierarchical and metabolic regulation of the glycolytic pathway. *FEBS Lett.* **500**, 169-171.
- Veech, R. L., Raijman, L., Dalziel, K. and Krebs, H. A. (1969). Disequilibrium in the triosephosphate isomerase system in rat liver. *Biochem. J.* **115**, 837-842.
- Wisniewski, E., Gellerich, F. N. and Kunz, W. S. (1995). Distribution of flux control among the enzymes of mitochondrial oxidative phosphorylation in calcium-activated saponin-skinned rat musculus soleus fibers. *Eur. J. Biochem.* **230**, 549-554.
- Yang, T.-H. and Somero, G. N. (1996). Activity of lactate dehydrogenase but not its concentration of messenger RNA increases with body size in barred sand bass, *Paralabrax nebulifer* (Teleostei). *Biol. Bull.* **191**, 155-158.