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Dissecting the variance–covariance structure in insect physiology: The multivariate association between metabolism and morphology in the nymphs of the sand cricket (*Gryllus firmus*)

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Abstract

Energy metabolism in animals has been largely studied in relation to exogenous sources of variation. However, because they give insight into the relationship between whole metabolism and lower organizational levels such as organs and tissues, examination of endogenous determinants of metabolism other than body mass is itself very important. We studied the multivariate association of body parts and several aspects of energy metabolism in an insect, the nymphs of the sand cricket, *Gryllus firmus*. By using a variety of both univariate and multivariate techniques, we explored the resultant variance–covariance matrix to build a path diagram with latent variables. After controlling for body mass, we found a significant canonical correlation between metabolism and morphology. According to the factor loadings and path coefficients, the most important contributions of morphology to the correlation were thorax and abdomen size measures, whereas the most important metabolic contribution was resting metabolism. Activity metabolism was mostly explained by body mass rather than body parts, which could be a result of resting rates being chronic consequences of the functioning of the metabolic machinery that the insect must maintain.

Keywords: Insects; Metabolic rate; Structural equation modelling; Canonical correlation analysis; Morphology

1. Introduction

The most generally used metric of energy use in animals is metabolic rate. From such analyses it has been determined that an animal's metabolism is a response to a complex interaction of exogenous factors such as recent thermal experience (Nespolo et al., 2003), factors correlated with latitude (Rourke, 2000) and food composition (Zanotto et al., 1997). The great majority of eco-physiological studies in insects have studied the exogenous variables that ~~affects~~ metabolism, or the activities of the organism that are indirectly influenced by exogenous factors (e.g., locomotion, Rogowitz and Chappell, 2000; feeding, Zanotto et al., 1997, sexual aggressiveness, Hack, 1997). In addition to exogenous factors, metabolic rate may be significantly affected by endogenous factors.

The most important endogenous variable determining metabolic rate is body mass, which has received a great amount of attention in the form of scaling exponent estimation and comparisons (Hack, 1997; Lehmann et al., 2000; Birchard and Arendse, 2001). However, not many studies have explored the residual effects of body parts (i.e., an endogenous factor) on metabolism (e.g., Lighton and Fielden, 1995; Crnokrak and Roff, 2002; Tartes et al., 2002), although different body parts could affect different aspects of metabolism (e.g., resting, activity, average). In vertebrates, for example, it is known that some organs and systems are more linked to

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certain metabolic rates such as BMR (digestive and metabolically active tissues, Konarzewski and Diamond, 1995) and MMR (skeletal muscles, Chappell et al., 1999; Hammond et al., 2001). There are concrete physiological reasons to expect such specific associations: for example, in mammals different tissues, organs and systems have different specific rates of oxygen consumption/CO_2 production during aerobic metabolism (Konarzewski and Diamond, 1995).

In insects, the proportional contributions of different tissues to the metabolism of the whole animal have been rarely studied (e.g., Djawdan et al., 1997; Crnokrak and Roff, 2002; Tartes et al., 2002). During periods of inactivity, insects are ectotherms and differ considerably from endotherms, both in anatomy and physiology. Because of their size and specific integration of the systems with the rest of the insect body, they do not have clearly demarked organs that can be extracted and measured easily. Instead, in nymphs of hemimetabolous insects the body is clearly divided into head, thorax and abdomen. In vertebrates, activity metabolism is mostly affected by muscular activity, whereas resting metabolism is mainly a consequence of the minimum costs of maintenance (Konarzewski and Diamond, 1995) which is greatly affected by ingestive/digestive activity (Bradley et al., 2003). Hence, if this holds for insects in absence of digestive activity we would predict a strong link between thorax and activity metabolism, and another strong association between abdomen and resting metabolism. In contrast, if body mass is the most important determinant of metabolism in insects, a complete absence of associations would be expected between morphology and physiology after removing body mass.

One of the simplest approaches to the study of such endogenous relationships in insects is to measure linear dimensions and metabolic rate and to explore the resulting covariance structure (Quinn and Keough, 2003). However, animal researchers continue to rely heavily on restrictive ANOVA models (Henderson, 2005). Although this approach gives insight into the response of physiology to fixed treatments, aspects of the complex relationship among endogenous variables cannot be addressed in a single experiment. Combinations of random factors, factorial, repeated-measures and nested designs give some flexibility, but often at the cost of great replication and difficult interpretations when complex interactions appear (Quinn and Keough, 2003). In contrast, the full exploration of the variance–covariance structure in a set of different types of variables measured in the same sampling units (i.e., insects) could be a powerful approach to determine the precise underlying (i.e., biological) associations among variables (Quinn and Keough, 2003).

In this paper, we addressed this issue by a statistical decomposition of the covariance matrix by a combination of univaried and multivariate correlational techniques, to finally show in a path diagram the associations among morphology and physiology in the nymphs of the sand cricket, *Gryllus firmus*.

2. Material and methods

2.1. Insects

The number of individuals needed for this study was obtained from a culture of inbred lines. These inbred lines were derived from a stock culture that originated from approximately 20 males and 20 females collected in northern Florida in 1981. The stock culture is maintained with a standing adult population of several hundred individuals (generally 100–500 adults, with occasional bottlenecks in which the population may have declined to about 50 adults). To prevent diapause the temperature is maintained in excess of 25 °C. Nymphs and adults in both the stock and the experiment were fed Purina rabbit chow.

The inbred lines were created from the stock population by 14 generations of brother–sister mating, full details of which are given in Roff (2002). After generation 14 the seven extant inbred lines were maintained separately in the same manner as the stock culture. As with the original stock, the inbred lines are maintained at population levels of several hundred per cage, with two cages per inbred line. A total of 120 nymphs were drawn haphazardly from the stock cages of the four inbred lines (30 per line), with approximately equal numbers coming from each of two cages per line.

2.2. Morphometric measurements

In all individuals, in addition to body mass, we measured head width (HW), head length (HL), thorax width (PW), thorax length (PL), abdomen width (AW) and abdomen length (AL) by using digital photography. Whereas body mass changes with adult age, all the other measures remain constant after eclosion into the adult morph.

2.3. Respirometry

Our respirometry system was similar to Lighton and Turner (2004) and Rogowitz and Chappell (2000). In brief, carbon dioxide production was measured continuously with an infrared CO_2 analyzer (LI-COR LI6251) capable of resolving differences of 0.2 part per million (ppm) of CO_2 in air. The analyser was calibrated periodically against a precision gas mixture; there were almost no drift between calibrations. Flow rates of dry, CO_2-free air were maintained at $\pm 1\%$ by a Sierra mass flow controller. Air was drawn from the ambient, CO_2 and vapour water was scrubbed with a Drierite–Ascarite