Journal of Insect Physiology 79 (2015) 27–35

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Increasing metabolic rate despite declining body weight in an adult parasitoid wasp

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ARTICLE INFO

Article history:
Received 2 April 2015
Received in revised form 22 May 2015
Accepted 25 May 2015
Available online 27 May 2015

Keywords:
Respirometry
Metabolic ecology
Ecological energetics
Structural mass
Reserves
Starvation
Parasitoids
Host-feeding
Respiratory Quotient (RQ)
Dynamic Energy Budget (DEB)

ABSTRACT

Metabolic rate is a positive function of body weight, a rule valid for most organisms and the basis of several theories of metabolic ecology. For adult insects, however, the diversity of relationships between body mass and respiration remains unexplained. The aim of this study is to relate the respiratory metabolism of a parasitoid with body weight and foraging activity.

We compared the metabolic rate of groups of starving and host-fed females of the parasitoid *Eupelmus vuilleti* recorded with respirometry for 7 days, corresponding to the mean lifetime of starving females and over half of the lifetime of foraging females. The dynamics of carbohydrate, lipid and protein in the body of foraging females were quantified with biochemical techniques. Body mass and all body nutrients declined sharply from the first day onwards. By contrast, the CO_2 produced and the O_2 consumed increased steadily. Starving females showed the opposite trend, identifying foraging as the reason for the respiration increase of feeding females.

Two complementary physiological processes explain the unexpected relationship between increasing metabolic rate and declining body weight. First, host hemolymph is a highly unbalanced food, and the excess nutrients (protein and carbohydrate) need to be voided, partially through excretion and partially through respiration. Second, a foraging young female produces eggs at an increasing rate during the first half of its lifetime, a process that also increases respiration. We posit that the time-varying metabolic rate contributions of the feeding and reproductive processes supplements the contribution of the structural mass and lead to the observed trend. We extend our explanations to other insect groups and discuss the potential for unification using Dynamic Energy Budget theory.

1. Introduction

Metabolic rate has been studied across many organisms, during ontogeny within a species or over short periods of time related to behavioral activities of individuals (Nisbet et al., 2012; Maino and Kearney, 2014). Metabolic rate has been found to increase with body mass, a phenomenon named metabolic scaling that is widespread in nature (West et al., 1997; Ehnes et al., 2011). Metabolic rate is defined as the energy turnover of an organism and is usually quantified using either heat production with calorimetry or CO_2 production and O_2 consumption with respirometry (Sibly et al., 2012). The simplest mechanistic explanations for metabolic scaling do however not apply to growth of individuals for many insect species (Maino and Kearney, 2014). For example, respiration rate increases while body mass decreases through time during the embryonic stage (see Kooijman, 1986; Kooijman, 2010). Another case is the pupal stage of holometabolous insects, which shows a U-shaped respiration curve through time while the body mass of the animal decreases (Maino and Kearney, 2014). Thus, the general rule of increased metabolic rate with increased body weight requires refinements for these cases. The Dynamic Energy Budget (DEB) theory of Kooijman (Kooijman, 2010; Llandres et al., in press), in which biomass is the sum of contributions of the reserve and structure compartments, can explain the patterns of respiration in these cases. Reserve is the sum of intermediary materials between the uptake of food and their use for organism's functioning. This includes the growth and maintenance of structure and

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http://dx.doi.org/10.1016/j.jinsphys.2015.05.005

reproduction (if any). The remaining tissue (e.g. cuticle, muscles, structural lipids, etc.) make up the structure compartment. Reserve biomass does not need any maintenance, only structural mass does. Thus, if one follows the development of an insect, one observes that a recently laid egg is composed almost entirely of reserve, with very little structure, so it hardly respires. Structural mass and respiration both increase with egg development (Woods, 2010), even though the total biomass declines, due to the costs associated with the growing structure (Kooijman, 1986). In the larval stage, vigorous growth implies a marked increase in structural mass, implying in turn an increase in respiration. The U-curve of respiration of pupae of holometabolous insects is explained by the histolysis of reserves first, followed by the buildup of the adult structure. Thus, a positive relationship between *structural* mass and metabolic rate eventually holds for all those cases, from embryos to pupae (Maino and Kearney, 2014).

For adults, the picture is blurred, because metabolic rate and changes in mass, and more importantly mass distribution between structure and reserves, are usually not reported together and rarely on long time spans. Three schemes can be however identified. First, adults are observed losing weight while their metabolic rates is declining over time. This scheme is common, see Woods et al. (2010) and Niitepold et al. (2014; for Speyeria sp., once the mass-corrected RMR is multiplied by the mass), for example. Second, the reverse is also observed: feeding adults increasing weight and CO_2 outputs (see Calabrese and Stoffolano, 1974 for example). Third, a constant metabolic rate despite increasing body weight has also been observed (e.g., Gray and Bradley, 2003). In the discussion, we interpret these three schemes in the light of our results, and add a fourth pattern. In conclusion, adult insects show several patterns of association between total body weight and metabolic rate, and their dynamics over time. Detailed nutrient and energy budgets are thus required to explain this diversity of dynamic relationships, as successfully done for immature stages.

The aim of our work is to examine the relationship between metabolic rate and body mass in insect adulthood by understanding the lifelong management of nutrient and energy. We already benefit from a large experimental basis on the nutrient and energetic metabolism for the adult parasitoid *Eupelmus vuilleti* (Hymenoptera: Eupelmidae) (Casas et al., 2005). The state of knowledge on this system is summarized below. Here, we specifically compared CO_2 production, O_2 consumption and the Respiratory Quotient (RQ) between starving and foraging females, understood both as food intake through host-feeding and egg laying (Godfray, 1994). We relate the findings to the dynamics of body contents, assessed using colorimetric techniques, and to feeding and reproduction. The respirometry measurements lasted 7 days, corresponding to the mean lifetime of starving females and over half of the lifetime of foraging females (Casas et al., 2005). Any divergence between the two groups should be apparent by then.

2. Current state of the overall program

E. vuilleti (Crawford) (Hymenoptera: Eupelmidae) is a tropical solitary wasp. This species is an ectoparasitoid of third- to fourth-instar larvae of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae), infesting the cowpea *Vigna unguiculata* (Fabaceae) seeds and pods. It also feeds from some of its hosts. Females obtain nutrients by extracting hemolymph during host-feeding, in particular substantial amounts of carbohydrate and protein, but little lipid (Giron et al., 2002). In an earlier paper (Casas et al., 2005), we quantified carbohydrate, protein, and lipid reserves of females at birth and death and their daily maintenance needs in the absence of hosts and food, i.e. under starving conditions. In another set of experiments, each host-feeding and oviposition event was recorded over the entire lifetime of another group of females in the presence of hosts, and the amounts acquired and invested in each egg was quantified (Giron and Casas, 2003a; Giron et al., 2004). Model simulations with hosts showed that the nutrient budget originally developed for starving females had to be drastically altered to match the observed data of foraging females (Casas et al., 2005). Energy gains through host feeding, and losses from egg laying, are large and had to be taken into account. More unexpectedly, nutrient-specific allocation rules had to be altered. For the budget to be balanced, the protein gained through host-feeding would have to be excreted or respired, possibly replacing some of the lipid and carbohydrate used during starvation. However, there are no data confirming such a shift in fuel type. These previous studies did not directly measure the metabolic rate of the adult, nor its dynamics, and thus relied on indirect measurement and logical inference. As the discussion below shows, however, there are multiple pathways for managing excess nutrients, making the existence of multiple hypotheses built on logical grounds possible and measurements even more necessary.

3. Material and methods

3.1. Biology of species and rearing

Rearing and experiments were carried out in a controlled-environment room with a 13:11 light:dark photoperiod, a light:dark temperature cycle of 33 °C:23 °C, and a mean of 75% relative humidity.

For both the respirometry experiments and the biochemical essays, females were isolated at emergence and kept with water *ad libitum* on cotton balls in individual Petri dishes of 8.5 cm diameter. Each female was put in presence of six new cowpea seeds harboring beetle larvae of 3rd or 4th instar every day for six hours, from 08:30 to 14:30 over the duration of the experiments (7 days for the respiration experiment) or their entire lifetime (for the nutrient and energy budget experiment). Females had no other dietary source than hosts. This treatment is thereafter called "*feeding*". A single feeding event enables a female to gather up to 20% of its own weight (Giron et al., 2004) so that a handful of host-feeding events is sufficient to attain full satiation.

3.2. Respirometry

Five groups of five foraging females were isolated at emergence and processed as above. Five other groups of 5 newly emerged females were deprived of hosts during the experimental duration of 7 days. These starving females acted as control group. The metabolic rate of each group of 5 *E. vuilleti* females was measured each day in a longitudinal fashion (i.e. the same females in each pooled were used over the entire duration of the experiment) using a flow-through respirometry system (Sable Systems International, SSI; Las Vegas, Nevada, USA). The large number of possible configurations of the instrument implies that our results cannot be replicated or interpreted without a detailed explanation of the set-up (Fig. 1) and the ensuing calculations (see Lighton, 2008).

Immediately before data collection, the carbon dioxide analyzer was calibrated with two known gas samples, CO_2-free nitrogen gas (zero gas) and 0.5% CO_2 in nitrogen gas (span gas) (CAN-Gas, Messer France S.A.). The oxygen analyzer was daily spanned using the air taken from outside and assumed to be 20.95% O_2. Outside air was pumped through two columns containing water vapor and CO_2 scrubbers, Drierite ($CaSO_4$; W.A. Hammond Drierite Co. LTD, OHIO, USA) and Soda Lime (>75% $CaOH_2$, <3.5% NaOH) respectively, to obtain a stable baseline. Dry and CO_2-free air passed through SS3 subsamplers, followed by the mass flow controller