Lipogenesis in an adult parasitic wasp

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Introduction

The nature of nutritional resources and the pattern of acquisition and allocation of incoming resources and stored reserves to reproduction and survival has critical consequences for the fitness of organisms and is fundamental to numerous fields of research in behavioural, evolutionary and population ecology (Roff 1992; Godfray 1994; Quicke 1997; Rivero & Casas 1999a; O'Brien *et al.* 2000; Jervis *et al.* 2001; Messina & Fox 2001; Rivero *et al.* 2001; Schliekelman & Ellner 2001).

Considerable amounts of carbohydrate, protein and lipid are needed by most adult insects for survival and reproduction (House 1974; Chapman 1998; Rivero & Casas 1999a). Their nutritional behaviour often reflects their physiological needs but metabolic capabilities - storage or neosynthesis- may frequently compensate for lack of adequate resources in the environment (Simpson & Raubenheimer 1995; Warburg & Yuval 1996). Insects can store large amounts of reserves in their fat body, which is their major energy storage site (Chapman 1998; Canavoso *et al.* 2001). For adults, these reserves are either carried over from the larvae or they are formed from food ingested by adults. Nutrients may also be synthesized *de novo* by the adults following ingestion of the relevant precursors (reviews by Downer & Matthews 1976; McFarlane 1985; Friedman 1985; Waldbauer & Friedman 1991).

Sugars are required by many insect species either to sustain metabolic needs or to serve as precursors. Parasitoid wasps are especially sensitive to sugar deprivation as adults (Hagen 1986; Van Lenteren *et al.* 1987; Heimpel *et al.* 1997; Quicke 1997; Olson & Andow 1998) and several species forage actively for sugar sources in the field such as nectar, pollen, honeydew or plant exudates (Rogers 1985; Hagen 1986; Jervis & Kidd 1986; May 1992; Evans 1993; Jervis *et al.* 1992, 1993; Jervis & Kidd 1996; Sisterson & Averill 2002). These sugar meals supplement carbohydrate resources gained during larval development and can be used immediately to generate energy metabolic purposes or stored for later use by conversion to glycogen (Frideman 1985; Rivero & Casas 1999a).

Lipids appear also to be determinant in the biology of most parasitoids and insects, as they are used in egg provisioning. Indeed, yolk rich eggs contain, in addition to proteins, large amounts of lipids (Troy *et al.* 1975; Kawooya & Law 1988; Giron & Casas in press; Canavoso *et al.* 2001). An important role of the lipids contained in eggs is to supply energy requirements of the developing embryo (Van Handel 1993). In parallel, lipids are generally used to fulfil the various energetic requirements of the insect body as they can be stored anhydrously, have low space requirements and release a lot of energy when burned (Clements

1992; Ellers 1996; Ellers *et al.* 1998; Rivero & Casas 1999a). Lipids used during the adult stage can therefore be available as lipids stored during the larval development, from lipids ingested with food or from lipids synthesized *de novo* by lipogenesis. Synthesising lipids from sugars could indeed permit to replenish the initial lipid stock from larval development in order to sustain the reproductive and potentially metabolic needs during the imaginal stage.

Lipogenesis is reported in a number of insect species including for example mosquitoes, grasshoppers and a great variety of butterflies (Chino & Gilbert 1965; Van Handel 1965; Nayar & Van Handel 1971; Brown & Chippendale 1974; Downer & Matthews 1976; Van Handel 1984; Warburg & Yuval 1996; Naksathit *et al.* 1999). Surprisingly, all parasitoid species studied so far seem unable to synthesize lipids from sugars (Ellers 1996; Olson *et al.* 2000; Rivero & West 2002; J. Casas, unpublished work). All these studies were conducted using feeding experiments followed by biochemical analyses. The aim of the present paper was therefore to determine the extent of lipogenesis of the parasitoid *Eupelmus vuilletti* (Hymenoptera: Eupelmidae) by conducting similar experiments as well as radiotracer studies.

E. vuilletti is a synovigenic solitary parasitoid producing yolk rich eggs during its imaginal stage. *E. vuilletti* female consumes host haemolymph during host-feeding and despite intensive surveys in the habitat, *E. vuilletti* was never observed attending others nutritional sources (J.P. Monge personal communication). Thus, host haemolymph constitutes the only source of food available for the female as adult (Giron *et al.* 2002). For *E. vuilletti,* host-feeding enables the female to obtain a large amount of proteins and sugars used to maintain the highly proteinic demand associated to egg production and the energetic costs associated to maintenance (Rivero & Casas 1999a; Rivero *et al.* 2001; Giron *et al.* 2002). However, host-feeding provides only a small amount of lipids to the female (Giron *et al.* 2002). Therefore, as lipid reserves cannot be fully replaced through feeding, females are assumed to depend either on lipids available at emergence or lipids synthesized *de novo* by lipogenesis to sustain their lipid requirements, or on both sources.

Materials and methods

Parasitoid biology

Eupelmus vuilletti (CRW) (Hymenoptera: Eupelmidae) is a tropical solitary host-

feeding ectoparasitoid of third- to fourth-instars larvae of *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) infecting *Vigna unguiculata* (Fabaceae) pods and seeds. Females are synovigenic, i.e. they are born with a very limited number of mature eggs (between 2 and 4 eggs ready to oviposit) and need to feed from the host in order to sustain egg production and maturation. Females, however, rarely use the same host for egg laying and for feeding (personal observation). In this species, females feed from the host by puncturing its cuticle and creating a feeding tube with secretions from their ovipositor (Fulton 1933). The females then turn and use the feeding tube to extract the host haemolymph with their mouthparts (Giron *et al.* 2002). Females lay an average of some 28 eggs over a period of some 11 days, depending of experimental conditions. The time span of 72 hours used in our experiment (see below) corresponds therefore to about one third of the total lifetime of females.

Experimental set-up

Culturing and all experimental procedures were carried out in a controlled temperature room with a 13:11 light:dark photoperiod, a temperature cycle of 33ºC(light): 23ºC(dark), and a constant 75% humidity. Experimental females were individually placed inside a gelatine capsule (length 2 cm and diameter 0.6 cm) the cap of which had been finely pierced to allow the introduction of one of the extremes of a micro-capillary tube (length 10 cm and internal diameter 0.6 mm) containing one of the nutrient solutions.

In a first experiment, females were allowed to feed *ad libitum* from the tube for 12, 24, 36, 48 or 72 hours after which they were killed and stored by deep freezing them at –80°C for subsequent biochemical analyses. The control diet consisted of water and a second batch of females were allowed to feed on a 10% (w:v) glucose solution. A total of 15 females were allocated to each of the control and glucose treatment for each of the specified experimental period.

In a second experiment, females were allocated to one of the two following feeding treatments. The radioactively marked diet consisted of a 10% (w:v) ^{14}C-uniformly marked glucose solution (999 GBq mmole^{-1}, ICN Pharmaceuticals). The control diet simply consisted of water. Females were allowed to feed *ad libitum* from the tube for 24, 36, 48 or 72 hours after which they were killed and stored by deep freezing them at –80°C for subsequent radioactivity quantification. A total of 15 females were allocated to each of the control and glucose marked treatment for each of the specified experimental period.

Biochemical analyses

Quantification of the amount of the body lipids, sugars and glycogen of the females were carried out using the colorimetric techniques developed for mosquito analysis (Van Handel 1985a, 1985b; Van Handel & Day 1988) as modified by Giron *et al.* (2002).

Radioactivity quantification

A simple method to extract and separate lipids and sugars was used. Female parasitoids from both the control and marked diet treatments, for each experimental period, were individually prepared by crushing them in an Eppendorf tube with a glass rod in 40 µl of 2% sodium sulphate. Samples were then mixed 2 minutes with 200 µl of chloroform and 100 µl of methanol. Then each sample was mixed 30 seconds with 80 µl of ultrapure milliQ® water and then centrifuged 3 minutes at 3000 g. This technique adapted from Bligh and Dyer (1959) allowed us to obtain two layers. The lower, hydrophobic, phase is composed of chloroform and contains lipids, while the upper phase, hydrophilic phase consists of methanol and water, and contains sugars.

150 µl of each phase was recovered and placed in a liquid scintillation tube. Then, 5 ml of the liquid scintillation cocktail (Hionic-Fluor™, Packard) was added in each tube. Quantification of the amount of isotope in each phase for each female was carried out using a liquid scintillator analyser (LSA, TriCarb1900, Packard Instruments). After 1 h, all tubes were read in the LSA for 10 minutes each and the number of disintegrations per minute (DPM) calculated (using the transformed spectral index of external spectrum as a quenching indicating parameter). Increasing quantities of radioactively marked glucose were added to extraction mixture in order to measure the possible contamination of the hydrophobic phase with radioactive sugars. Females fed only with water were used in order to control for the presence of natural radiation. The mean radioactivity in these control females was considered as the background noise and was then subtracted to the measures from marked females.

Statistical analyses

The effect of time on body lipid, sugar and glycogen contents (in both starved and *ad libitum* fed females) were analysed with generalized linear modelling techniques available in the SPSS statistical package (SPSS Inc., USA). For glucose-fed females, data were also analysed using spline regression (Hastie & Tishirbani 1990; Bowman & Azzlani 1997). The

influence of radioactively marked diet on sugar and lipid contents was explored using the same generalized linear modelling technique.

Results

Nutrient analyses

Body sugars. The mean amount of body sugars present in female *E. vuilletti* upon emergence was 10.69 ± 1.01µg (n=15) (fig. 1). Body sugar level then showed a very weak but significant positive increase over the first 76 hours of the lifespan of **unfed** females (Linear regression: slope=0.07, intercept=10.68, r^2=0.12, $F_{1,88}$=12.52, p<0.05). For **glucose-fed** females, body sugar level increased rapidly over the same time interval (Linear regression, slope=1.33, intercept=50.53, r^2=0.16, $F_{1,88}$=17.94, p<0.05). A spline regression analysis showed that the pattern of body sugars in glucose-fed female can be however divided in broadly two phases. In a first phase, the body sugar level showed a positive increase, while in a second phase it levelled off, about 36 hours after emergence (fig. 1).

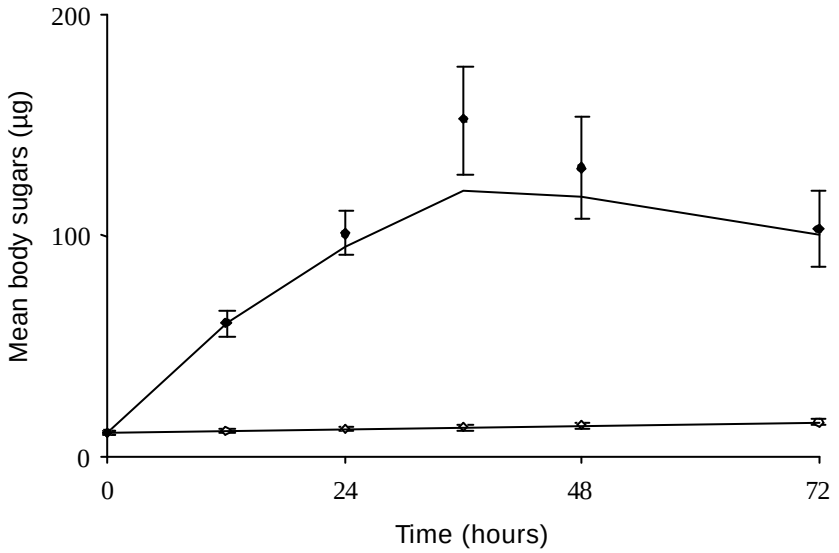

Figure 1. Mean body sugars (mean ± s.e.) measured in unfed (empty dots) and glucose-fed females (full dots) at different time intervals. For unfed females, solid lines represent linear regression of body sugars measured against time. For glucose-fed females, solid lines represent spline regression of body glycogen measured against time (Lowess algorithm, tension=0.055).

Body glycogen. The glycogen level of emerging females was 53.59 ± 4.14µg (n=15) (fig. 2). It then dropped rapidly over the first 76 hours of the lifespan of **unfed** females (Linear regression, slope=-0.40, intercept=53.63, r^2=0.40, $F_{1,88}$=59.74, p<0.05). For **glucose-fed** females, body glycogen level then increased rapidly over the same time interval (Linear regression, slope=0.85, intercept=44.74, r^2=0.64, $F_{1,88}$=158.37, p<0.05). By using spline regression analysis, the pattern of body glycogen in glucose-fed female could be however divided in broadly two phases. In a first phase, the body glycogen level remained stable, while it increased about 36 hours after emergence.

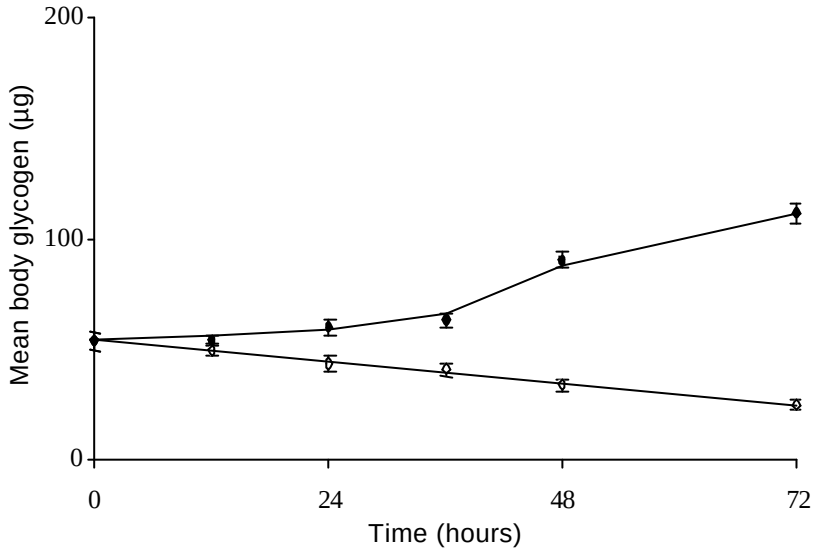

Figure 2. Mean body glycogen (mean ± s.e.) measured in unfed (empty dots) and glucose-fed females (full dots) at different time intervals. For unfed females, solid lines represent linear regression of body glycogen measured against time. For glucose-fed females, solid lines represent spline regression of body glycogen measured against time (Lowess algorithm, tension=0.050).

Body lipids. The mean estimated amount of lipids present in emerging females was 191.3 ± 9.73µg (n=15) (fig. 3). The body lipid level did not change with time for **unfed** female (Linear regression: $F_{1,88}$=1.70, *NS*), as well as for **glucose-fed** females (Linear regression: $F_{1,88}$=4.45, *NS*).

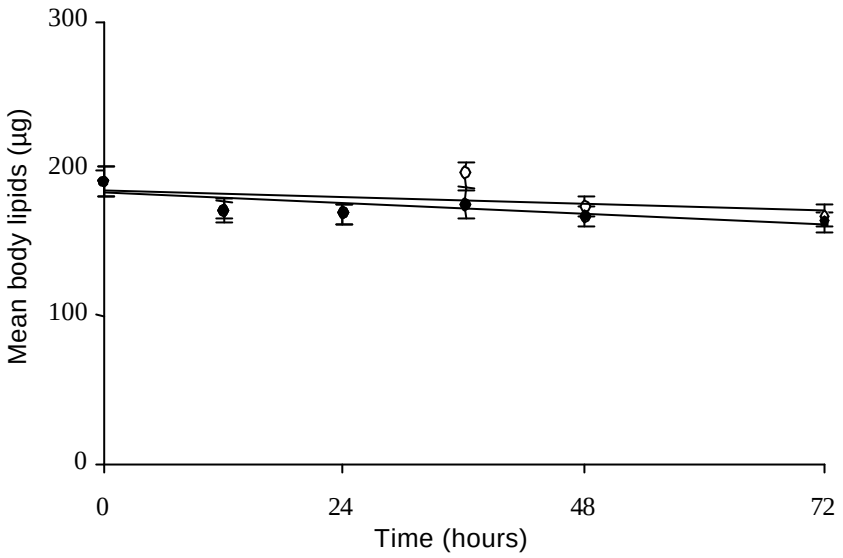

Figure 3. Mean body lipids (mean ± s.e.) measured in unfed (empty dots) and glucose-fed females (full dots) at different time intervals. Solid lines represent linear regression of body lipids against time.

Radioactivity analyses

For all tests realised, only a very weak, constant and therefore insignificant percentage of radioactive glucose was carried over into the hydrophobic phase (mean±s.e. 0.0048 ± 0.0003Bq). The level of radioactivity found in the control group was 0.28 ± 0.04Bq.

The amount of radioactivity measured in the hydrophilic fraction increased significantly with time (Linear regression: slope=42.30, intercept=-46.50, r^2=0.10, $F_{1,68}$=7.30, p<0.05) (fig. 4a). The radioactivity level measured in the hydrophobic fraction showed a very weak but significant positive increase with time (Linear regression: slope=1.72, intercept=-6.98, r^2=0.12, $F_{1,68}$=9.16, p<0.05). The increase of the amount of radioactivity measured in the hydrophobic fraction is positively correlated to the amount of radioactivity measured in the hydrophilic fraction (Linear regression: slope=0.03, $F_{1,68}$=76.45, r^2=0.50, p<0.05) (fig. 4b). The mean amount of radioactivity measured in the 'hydrophobic' fraction corresponds to 6.71 ± 0.76% of the total radioactivity obtained and the radioactivity measured in this fraction never exceed 324 Bq.

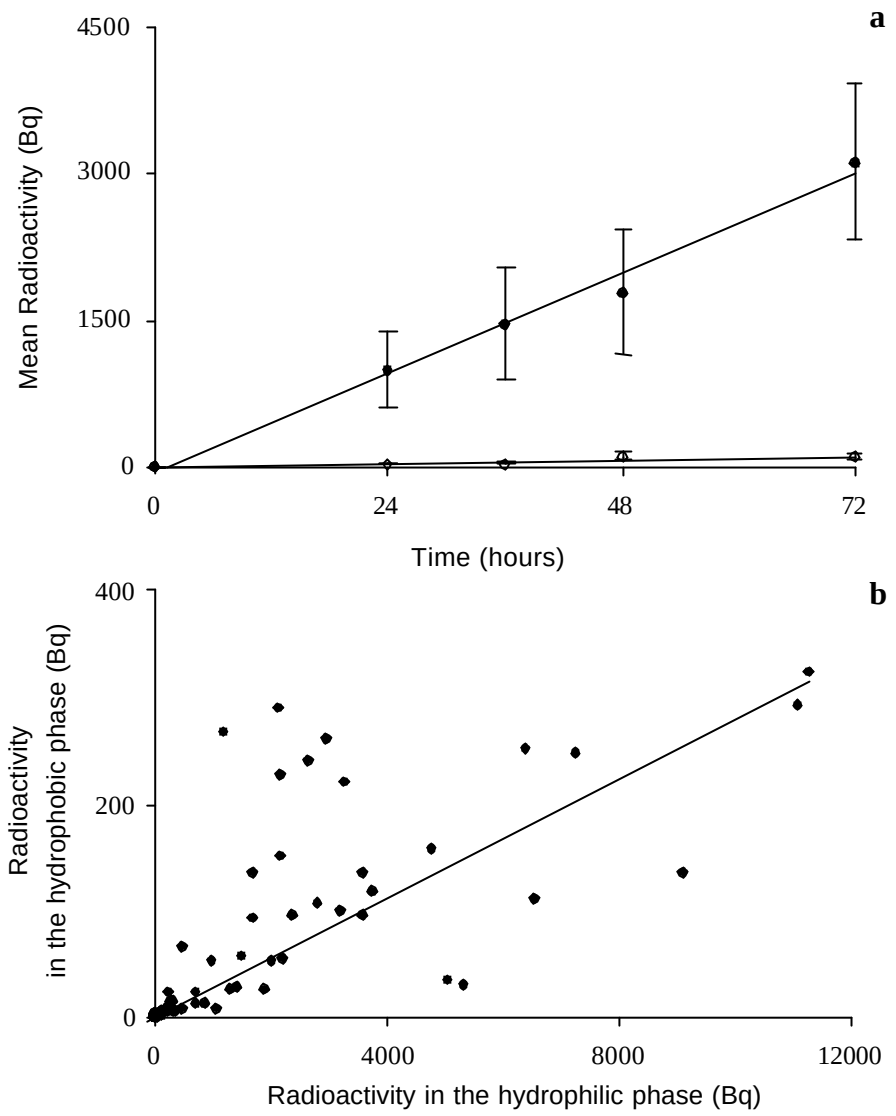

Figure 4. (a) Mean radioactivity measured in the hydrophobic (empty dots) and hydrophilic phases (full dots) at different time intervals. Solid lines represent linear regression of radioactivity against time. **(b)** Linear regression of radioactivity measured in the hydrophobic phase against radioactivity measured in the hydrophilic phase.

Discussion

E. vuilletti females emerged with relatively high reserves of body sugars and glycogen. The initial level of sugars (teneral reserves) of unfed females did not decrease after emergence. On the contrary, their level of body sugars increased weakly during the first 76 hours following the emergence, in spite of the absence of a nutritional supply. This pattern is associated with a concomitant decrease in glycogen reserves. In conclusion, females in the absence of food seem to supply their metabolic needs by glycogen. Feeding on glucose, on

the other hand, increased the level of body sugars. Moreover, glycogen synthesis and levelling off of sugars occurred simultaneously, about 36 hours after emergence.

Concentration of haemolymph sugars is assumed to control the mobilization and the storage of glycogen (Ziegler & Schultz 1986). Indeed, glycogen is known to be stored only when sugar concentration in haemolymph reaches a certain threshold. On the contrary, glycogen reserves are mobilized when the sugars concentration of the haemolymph has declined below a certain value (Van der Horst *et al.* 1980; Ziegler & Schultz 1986; Rivero & Casas 1999a). The increase of body sugars observed in unfed females is in accordance with such a glycogen regulatory mechanism in *E. vuilletti.* The mobilization of the stored glycogen reserves in unfed females and the consumption of sugars in fed females indicates that carbohydrates are the fuels used by females to sustain their metabolic needs. Using sugars enables females to maintain their lipids reserves at a constant level.

The observed pattern of energy acquisition and use is typical of most insect species: under negative energetic balance, insects utilize their energetic reserves (glycogen and/or triglycerides), while under positive energetic balance, they maintain or accumulate their reserves levels (Downer 1981). Insects feeding on sugar usually synthesize glycogen during the first hours but quickly switch to lipid synthesis and eventually accumulate much more lipid than glycogen (Van Handel 1965, 1984; Ziegler 1997). In contrast, in *E. vuilletti* the lipid content of sugar-fed females is not increased significantly. Indeed, the level of lipids never exceeded the teneral level and remained constant even when females consumed glucose for a long time. Two explanations can be put forward. First, the level of lipids never increased because this species is unable to synthesize lipids from sugars. Alternatively, lipid synthesis from sugars was more or less equivalently compensated by lipid use. The simple measurement of the dynamics of nutrients reserves during the life of the female cannot clearly distinguish between these two explanations. Tracing the fate of radioactive sugar enabled us to determine the extent of lipogenesis.

The presence of radioactivity obtained in the hydrophilic phase containing total sugars indicates that females did consume the radioactively marked glucose solution. The concomitant increase of the amount of radioactivity in the hydrophobic phase containing the total lipids could be interpreted as an ability of the female to synthesize lipids from sugars. However, the amount of radioactivity measured in the hydrophobic phase remained very low and never exceed about 7% of the total radioactivity. The radioactivity measured in the hydrophobic phase may come from sugars residuals attached to lipids under the form of glycolipids. Indeed, a large amount of substances are grouped under the common term of

glycolipids and the number and the nature of sugars attached to lipids can greatly influence their hydrophobic or hydrophilic nature (Kiernan 1990; Valet & Richard 1997). If lipogenesis was occurring, the amount of radioactivity in the hydrophilic phase would tend to level off or decline due to a concomitant increase of radioactivity in the hydrophobic phase. These two processes would lead to a variable ratio of radioactivity in the two phases, in contrast to the linear relation observed. Lipids are basically long-term storage molecules (Rivero & Casas 1999a) and lipogenesis from sugars is a costly pathway. Thus, it is difficult to understand why wasps would synthesize lipids from their ingested sugars and burn them immediately afterwards. Indeed the storage and subsequent mobilization of energy reserves is more costly than direct utilization (Wheeler 1989; J. Casas unpublished work). We therefore conclude that lipogenesis either does not occur or occurs at a very low rate in *E. vuilletti*, thus confirming with trace experiments results previously obtained on other parasitoids with feeding experiments (Ellers 1996; Olson *et al.* 2000; Rivero & West 2002; J. Casas, unpublished work). The amount of lipids is therefore almost totally fixed at time of birth and may constrain females to elaborate their eggs mainly from lipids stored during the larval development. The constant level of lipids in both fed and starved females can be explained by the lack of oviposition opportunities. All the results obtained, or previously obtained, in this species seem to indicate that females adopt an accurate management of their lipid reserves with a fine tuning of lipid allocation in their eggs on one hand and a preferential use of a food carbohydrate source to sustain metabolic needs on the other hand (Giron *et al.*2002; Giron & Casas in press).

All parasitoid species in which lipogenesis was explored –*Asobara tabida*, *Macrocentrus grandii*, *Nasonia vitripennis*, *Venturia canescens*– are unable to synthesize lipids from sugars in meaningful quantities despite the fact that these species cover the entire spectrum of life history traits (Ellers 1996; Olson *et al.* 2000; Rivero & West 2002; J. Casas, unpublished work). These traits extend from endoparasitism to ectoparasitism, from moderate to complete synovigeny, and from absence to presence of host-feeding. This surprising result is worth pursuing in depth, as it may well apply to the Aculatea Hymenoptera as a whole group. Indeed, the extent of lipogenesis in ants or bees is at this stage unknown given the paucity of appropriate empirical data (D. Giron & J. Casas, personal observation; M. Wells and K. Crailsheim, personal communication). Detailed physiological studies are now needed to corroborate or infirm the capacity of lipogenesis in all hymenoptera and to identify ecological and physiological conditions making lipogenesis unlikely in this large group of organisms.