

# Lipogenesis in an adult parasitic wasp

D. Giron \*, J. Casas

Université de Tours, Institut de Recherche sur la Biologie de l'Insecte (CNRS UMR 6035), 37200 Tours, France

Received 9 August 2002; received in revised form 31 October 2002; accepted 31 October 2002

## Abstract

The goal of this study was to determine the extent of lipogenesis in the parasitoid *Eupelmus vuilletti* (Hymenoptera: Eupelmidae). Carbohydrate and lipid metabolism were followed in glucose-fed and starved females over 3 days. Fed females increased their glycogen level, while maintaining their lipid level. Starved females used most of their glycogen, while maintaining their lipid level too. Thus, females either use exclusively sugars to preserve their lipid reserves, or maintained a steady renewal of lipids through lipogenesis. The incorporation of radioactively marked glucose into lipids showed however that lipogenesis did not occur at a sufficient level to increase lipid reserves and to compensate for lipid use. This result has important implications for understanding nutrients allocation strategy in this species as the amount of lipids is almost totally fixed upon the emergence. From an evolutionary perspective, we call for detailed physiological studies of lipogenesis in a wide range of adult hymenopterans, as the absence of lipogenesis could be common to all of Aculeata.

© 2003 Elsevier Science Ltd. All rights reserved.

**Keywords:** Carbohydrate metabolism; Lipid metabolism; Nutrients acquisition and allocation; Parasitoid; *Eupelmus vuilletti*

## 1. 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 and Casas 1999; O'Brien et al., 2000; Jervis et al., 2001; Messina and Fox, 2001; Rivero et al., 2001; Schliekelman and Ellner, 2001).

Considerable amounts of carbohydrate, protein and lipid are needed by most adult insects for survival and reproduction (House, 1974; Chapman, 1998; Rivero and Casas, 1999). 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 and Raubenheimer, 1995; Warburg and 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 and Matthews, 1976; McFarlane, 1985; Friedman, 1985; Waldbauer and 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 and 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 and Kidd, 1986; May, 1992; Evans, 1993; Jervis et al., 1992, 1993; Jervis and Kidd, 1996; Sisterson and 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 (Friedman, 1985; Rivero and Casas, 1999).

Lipids appear also to be determinant in the biology

\* Corresponding author. Tel.: +33-247-366-966; fax: +33-247-366-911.

E-mail address: giron@univ-tours.fr (D. Giron).

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; D. Giron and J. Casas unpublished work; 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 (Clement, 1992; Ellers, 1996; Ellers et al., 1998; Rivero and Casas, 1999). 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 and Gilbert 1965; van Handel 1965; Nayar and van Handel 1971; Brown and Chippendale 1974; Downer and Matthews 1976; van Handel, 1984; Warburg and 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 and 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., in press). 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 and Casas 1999, Rivero et al. 2001; Giron et al., in press). However, host-feeding provides only a small amount of lipids to the female (Giron et al., in press). 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.

## 2. Materials and methods

### 2.1. Parasitoid biology

*Eupelmus vuilletti* (CRW) (Hymenoptera: Eupelmidae) is a tropical solitary host-feeding ectoparasitoid of third- to fourth-instar 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., in press). Females lay an average of some 28 eggs over a period of some 11 days, depending of experimental conditions. The time span of 72 h used in our experiment (see below) corresponds therefore to about one third of the total lifetime of females.

### 2.2. 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 33°C (light): 23°C (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 microcapillary 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 h after which they were killed and stored by deep freezing them at  $-80^{\circ}\text{C}$  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)  $^{14}\text{C}$ -uniformly marked glucose solution (999 GBq  $\text{mmole}^{-1}$ , 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 h after which they were killed and stored by deep freezing them at  $-80^{\circ}\text{C}$  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.

### 2.3. 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; van Handel 1985b; van Handel and Day, 1988) as modified by Giron et al. (in press).

### 2.4. 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  $\mu$ l of 2% sodium sulphate. Samples were then mixed 2 min with 200  $\mu$ l of chloroform and 100  $\mu$ l of methanol. Then each sample was mixed 30 seconds with 80  $\mu$ l of ultrapure milliQ® water and then centrifuged 3 min 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.

One hundred and fifty  $\mu$ l 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 min each and the number of disintegrations per min (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.

### 2.5. 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 and Tishirbani, 1990; Bowman and Azzalani, 1997). The influence of

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

## 3. Results

### 3.1. Nutrient analyses

#### 3.1.1. Body sugars

The mean amount of body sugars present in female *E. vuilletti* upon emergence was  $10.69 + 1.01\mu\text{g}$  ( $n = 15$ ) (Fig. 1). Body sugar level then showed a very weak but significant positive increase over the first 76 h 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 h after emergence (Fig. 1).

#### 3.1.2. Body glycogen

The glycogen level of emerging females was  $53.59 + 4.14\mu\text{g}$  ( $n = 15$ ) (Fig. 2). It then dropped rapidly over the first 76 h 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

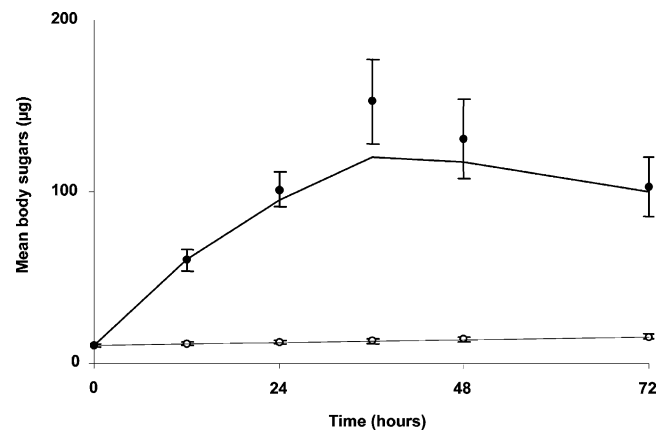


Fig. 1. Mean body sugars (mean+s.e.) measured in unfed (empty dots) and glucose-fed females (full dots) at different time intervals. For unfed females, solid lines represent linear regression of body sugars measured against time. For glucose-fed females, solid lines represent spline regression of body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

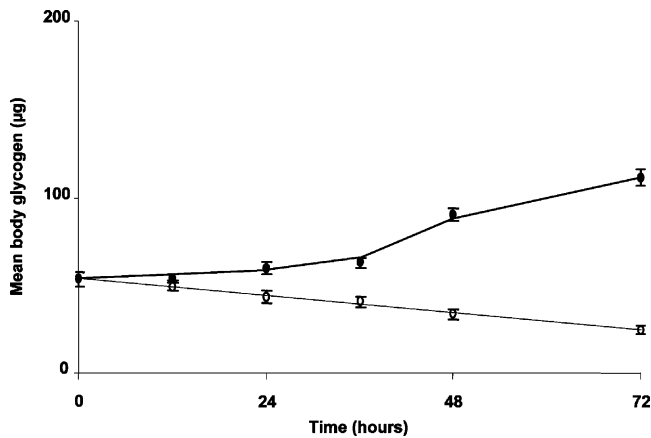


Fig. 2. Mean body glycogen (mean+s.e.) measured in unfed (empty dots) and glucose-fed females (full dots) at different time intervals. For unfed females, solid lines represent linear regression of body glycogen measured against time. For glucose-fed females, solid lines represent spline regression of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

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 h after emergence.

### 3.1.3. Body lipids

The mean estimated amount of lipids present in emerging females was  $191.3 \pm 9.73 \mu\text{g}$  ( $n = 15$ ) (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).

### 3.2. 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 +

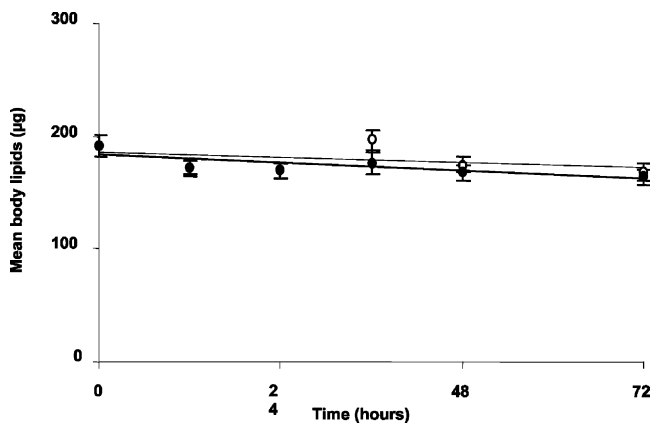


Fig. 3. Mean body lipids (mean+s.e.) measured in unfed (empty dots) and glucose-fed females (full dots) at different time intervals. Solid lines represent linear regression of body lipids against time.

s.e.  $0.0048 \pm 0.0003$  Bq). The level of radioactivity found in the control group was  $0.28 \pm 0.04$  Bq.

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  $6.71 \pm 0.76\%$  of the total radioactivity obtained and the radioactivity measured in this fraction never exceed 324 Bq.

## 4. Discussion

*E. vuilletti* females emerged with relatively high reserves of body sugars and glycogen. The initial level

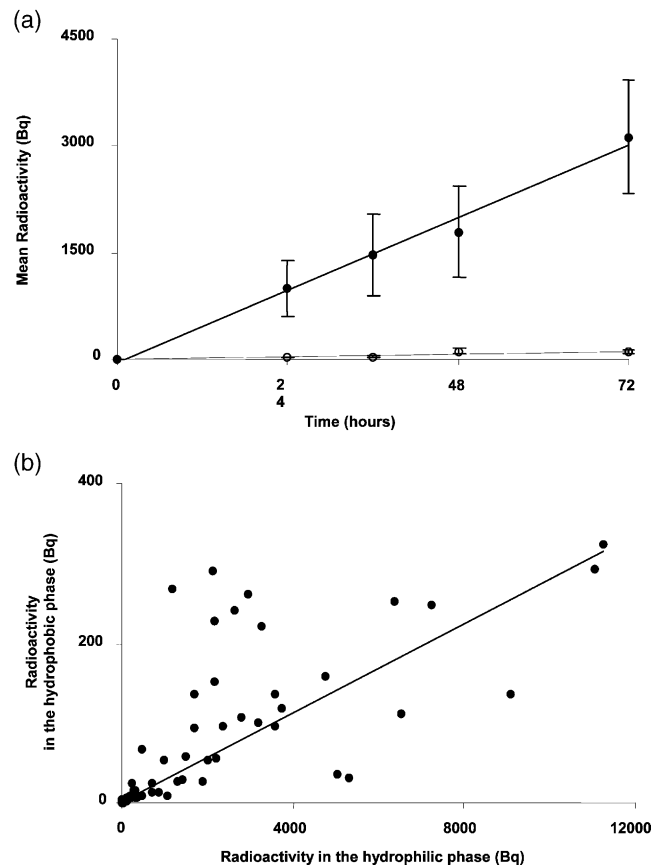


Fig. 4. (a) Mean radioactivity measured in the hydrophobic (empty dots) and hydrophilic phases (full dots) at different time intervals. Solid lines represent linear regression of radioactivity against time. (b) Linear regression of radioactivity measured in the hydrophobic phase against radioactivity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

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 h 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 h after emergence.

Concentration of haemolymph sugars is assumed to control the mobilization and the storage of glycogen (Ziegler and 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 and Schultz, 1986; Rivero and Casas, 1999). 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; van Handel, 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 exceeded 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 and 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 and Casas, 1999) 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 and 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., in press; D. Giron and J. Casas unpublished work).

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 and 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 Aculeata 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 and 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.

## Acknowledgements

We would like to thank G. Boileau for his help with the laboratory work. We would also like to thank K. Crailsheim and M. Wells for their useful comments on the manuscript. This study was financed by a PhD scholarship from the French Ministry for education and Research to D.G. Further support was provided by the National Centre of Scientific Research (CNRS).

## References

- Bligh, E.G., Dyer, W.J., 1959. Method of lipid extraction. *Canadian Journal of Biochemical Physiology* 37, 911–917.
- Bowman, A.W., Azzalini, A., 1997. *Applied Smoothing Techniques for Data Analysis*. Oxford Science, Oxford.
- Brown, J.J., Chippendale, G.M., 1974. Migration of the monarch butterfly, *Danaus plexippus*: energy sources. *Journal of Insect Physiology* 20, 1117–1130.
- Canavoso, L.E., Jouni, Z.E., Karnas, K.J., Pennington, J.E., Wells, M.A., 2001. Fat metabolism in insects. *Annual Review of Nutrition* 21, 23–46.
- Chapman, R.F., 1998. *The Insects: Structure and Function*. Cambridge University Press, Cambridge.
- Chino, H., Gilbert, L.I., 1965. Studies on the interconversion of carbohydrate and fatty acid in *Hyalophora cecropia*. *Journal of Insect Physiology* 11, 287–295.
- Clement, A.N., 1992. *The Biology of Mosquitoes*. Chapman and Hall, London.
- Downer, R.G.H., 1981. Physiological and environmental considerations in insect bioenergetics. In: Downer, R.G.H. (Ed.), *Energy Metabolism in Insects*. Plenum Press, New York, pp. 1–17.
- Downer, R.G.H., Matthews, J.R., 1976. Patterns of lipid distribution in insects. *American Zoologist* 16, 733–745.
- Ellers, J., 1996. Fat and eggs: an alternative method to measure the trade-off between survival and reproduction in insect parasitoids. *Netherlands Journal of Zoology* 46, 227–235.
- Ellers, J., van Alphen, J.J.M., Sevenster, J.G., 1998. A field study of size-fitness relationships in the parasitoid *Asobara tabida*. *Journal of Animal Ecology* 67, 318–324.
- Evans, E.W., 1993. Indirect interactions among phytophagous insects: aphids, honeydew and natural enemies. In: Watt, A.D., Leather, S.R., Walters, K.E.F., Mills, N.J. (Eds.), *Individuals, Populations and Patterns in Ecology*. Intercept, Andover, pp. 63–80.
- Friedman, S., 1985. Intermediary metabolism. In: Blum, M.S. (Ed.), *Fundamentals of Insect Physiology*. John Wiley and Sons, New York, pp. 467–506.
- Fulton, B.B., 1933. Notes on *Habrocytus cerealellae*, a parasite of Angoumois grain moth. *Annals of the Entomological Society of America* 26, 536–553.
- Giron, D., Rivero, A., Mandon, N., Darrouzet, E., Casas, J. The physiology of host-feeding: implications for survival. *Functional Ecology* 16 (in press).
- Godfray, H.C., 1994. *Parasitoids: Behavioural and Evolutionary Ecology*. Princeton University Press, Princeton, New Jersey.
- Hagen, K.S., 1986. Ecosystem analysis: plant cultivars (HPR), entomophagous species and food supplements. In: Boethel, D.J., Eikenbary, R.D. (Eds.), *Interactions of Plant Resistance and Parasitoids and Predators of Insects*. Ellis Horwood Limited Press, West Sussex, pp. 151–197.
- Hastie, T.J., Tishirbani, R.J., 1990. *Generalized Additive Models*. Chapman and Hall, London.
- Heimpel, G.E., Rosenheim, J.A., Kattari, D., 1997. Adult feeding and lifetime reproductive success in the parasitoid *Aphytis melinus*. *Entomologia Experimentalis et Applicata* 83, 305–315.
- House, H.L., 1974. Nutrition. In: Rockstein, M. (Ed.), *The Physiology of Insecta*. Academic Press, New York, pp. 1–62.
- Jervis, M.A., Kidd, N.A.C., 1986. Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews* 61, 395–434.
- Jervis, M.A., Kidd, N.A.C., Walton, M., 1992. A review of methods for determining dietary range in adult parasitoids. *Entomophaga* 4, 565–574.
- Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T., Dawah, H.A., 1993. Flower-visiting by hymenopteran parasitoids. *Journal of Natural History* 27, 67–105.
- Jervis, M.A., Kidd, N.A.C., 1996. Phytophagy. In: Jervis, M.A., Kidd, N.A.C. (Eds.), *Insect Natural Enemies. Practical Approaches to their Study and Evaluation*. Chapman and Hall, London, pp. 375–394.
- Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A., Kidd, N.A., 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. *Journal of Animal Ecology* 70, 442–458.
- Kawooya, J.K., Law, J.H., 1988. Uptake of the major hemolymph lipoprotein and its transformation in the insect egg. *Journal of Biological Chemistry* 263, 8740–8747.
- Kiernan, J.A., 1990. *Histological and Histochemical Methods: Theory and Practice*. Pergamon Press, Oxford.
- May, P.G., 1992. Flower selection and the dynamics of lipid reserves in two nectarivorous butterflies. *Ecology* 73, 2181–2191.
- McFarlane, J.E., 1985. Nutrition and digestive organs. In: Blum, M.S. (Ed.), *Fundamentals of Insect Physiology*. John Wiley and Sons, New York, pp. 59–89.
- Messina, F.J., Fox, C.W., 2001. Offspring size and number. In: Fox, C.W., Roff, D.E., Fairbairn, D.J. (Eds.), *Evolutionary Ecology: Concepts and Case Studies*. Oxford University Press, Oxford, pp. 113–127.
- Naksathit, A.T., Edman, J.D., Scott, T.W., 1999. Amounts of glycogen, lipid, and sugar in adult female *Aedes aegypti* (Diptera: Culicidae) fed sucrose. *Journal of Medical Entomology* 36, 8–12.
- Nayar, J.K., van Handel, E., 1971. The fuel for sustained mosquito flight. *Journal of Insect Physiology* 17, 471–481.
- O'Brien, D.M., Schrag, D.P., Martinez del Rio, C., 2000. Allocation to reproduction in a hawkmoth: a quantitative analysis using stable carbon isotopes. *Ecology* 81, 2822–2831.
- Olson, D.M., Andow, D.A., 1998. Larval crowding and adult nutrition effects on longevity and fecundity of female *Trichogramma nubilale* (Hymenoptera: Trichogrammatidae). *Environmental Entomology* 27, 508–514.
- Olson, D.M., Fadamiro, H., Lundgren, J.G., Heimpel, G.E., 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiological Entomology* 25, 17–26.
- Quicke, D.L.J., 1997. *Parasitic Wasps*. Chapman and Hall, London.
- Rivero, A., Casas, J., 1999. Incorporating physiology into parasitoid behavioural ecology: the allocation of nutritional resources. *Researches in Population Ecology* 41, 39–45.
- Rivero, A., Giron, D., Casas, J., 2001. Lifetime allocation of juvenile and adult nutritional resources to egg production in a holometabolous insect. *Proceedings of the Royal Society of London. B* 268, 1231–1237.

- Rivero, A., West, S.A., 2002. The physiological costs of being small in a parasitic wasp. *Evolutionary Ecology Research* 4, 407–420.
- Roff, D.A., 1992. *The Evolution of Life Histories: Theory and Analyses*. Chapman and Hall, New York.
- Rogers, C.E., 1985. Extrafloral nectar: entomological implications. *Bulletin of the Entomological Society of America* 31, 15–20.
- Schliekelman, P., Ellner, S.P., 2001. Egg size evolution and energetic constraints on population dynamics. *Theoretical Population Biology* 60, 73–92.
- Simpson, S.J., Raubenheimer, D., 1995. The mechanisms of nutritional homeostasis. In: Chapman, R.F., de Boer, G. (Eds.), *Regulatory Mechanisms in Insect Feeding*. Chapman and Hall, London, pp. 251–278.
- Sisterson, M.S., Averill, A.L., 2002. Costs and benefits of food foraging for a Braconid parasitoid. *Journal of Insect Behaviour* 15, 571–588.
- Troy, S., Anderson, W.A., Spielman, A., 1975. Lipid content of maturing ovaries of *Aedes aegypti* mosquitoes. *Comparative Biochemistry and Physiology (B)* 50, 457–461.
- Valet, P., Richard, D., 1997. *Les Lipides et la Cellule Adipeuse*. Editions Nathan, Paris.
- van der Horst, D.J., Houben, N.M.D., Beenackers, A.M.T., 1980. Dynamics of energy substrates in the haemolymph of *Locusta migratoria* during flight. *Journal of Insect Physiology* 26, 441–448.
- van Handel, E., 1965. Microseparation of glycogen, sugars and lipids. *Analytical Biochemistry* 11, 266–271.
- van Handel, E., 1984. Metabolism of nutrients in the adult mosquito. *Mosquito News* 44, 573–579.
- van Handel, E., 1985a. Rapid determination of glycogen and sugars in mosquitoes. *Journal of the American Mosquito Control Association* 1, 299–301.
- van Handel, E., 1985b. Rapid determination of total lipids in mosquitoes. *Journal of the American Mosquito Control Association* 1, 302–304.
- van Handel, E., Day, J.F., 1988. Assay of lipids, glycogen and sugars in individual mosquitoes: correlations with wing length in field-collected *Aedes vexans*. *Journal of the American Mosquito Control Association* 4, 549–550.
- van Handel, E., 1993. Fuel metabolism of the mosquito (*Culex quinquefasciatus*) embryo. *Journal of Insect Physiology* 39, 831–833.
- van Lenteren, J.C., van Vianen, A., Gast, H.F., Kortenhoff, A., 1987. The parasite-host relationship between *Encarsia Formosa* Gahan (hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae). XVI: food effects on oogenesis, life span and fecundity of *Encarsia Formosa* and other hymenopterous parasites. *Zeitschrift für Angewandte Entomologie* 103, 69–84.
- Waldbauer, G.P., Friedman, S., 1991. Self-selection of optimal diets by insects. *Annual Review of Entomology* 36, 43–63.
- Warburg, M.S., Yuval, B., 1996. Effects of diet and activity on lipid levels of adult Mediterranean fruit flies. *Physiological Entomology* 21, 151–158.
- Wheeler, C.H., 1989. Mobilization and transport of fuels to the flight muscles. In: Goldsworthy, G.J., Wheeler, C.H. (Eds.), *Insect Flight*. CRC Press, Boca Raton, Florida, pp. 273–303.
- Ziegler, R., 1997. Lipid synthesis by ovaries and fat body of *Aedes aegypti* (Diptera: Culicidae). *European Journal of Entomology* 94, 385–391.
- Ziegler, R., Schulz, M., 1986. Regulation of carbohydrate metabolism during flight in *Manduca sexta*. *Journal of Insect Physiology* 32, 997–1001.