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HPLC sugar analysis reveals the nutritional state and the feeding history of parasitoids

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Summary

1. Adult parasitoids depend on sugar-rich foods such as nectar and honeydew to meet their energy requirements. Many laboratory studies have established fitness benefits of sugar feeding for parasitoids.
2. Nevertheless, we know little about the nutritional ecology of parasitoids in the field, chiefly because of the limited specificity of methods applicable for field studies.
3. Here we use high-performance liquid chromatography (HPLC) analysis to study the sugar profile of *Cotesia glomerata* L. and *Microplitis mediator* Haliday relative to feeding-treatment. We identify the overall sugar level and the ratio of glucose to fructose as two parameters that in combination unambiguously characterize an individual's nutritional state and feeding history.
4. Unfed parasitoids contained low total sugar levels and glucose levels that typically exceeded fructose levels more than five-fold. Parasitoids with constant access to sucrose had high overall sugar levels with a balanced glucose–fructose ratio.
5. The marked shift of the glucose–fructose ratio after feeding was still evident in *C. glomerata* individuals whose total sugar content had decreased to unfed levels after 3 days of starvation. This makes it possible to distinguish between unfed individuals and those that did feed several days ago.
6. In addition to revealing past feeding events, HPLC analysis also enables the identification and quantification of a wide spectrum of sugars present in each specimen. When food sources contain specific sugars this can provide additional information about the type of sugar source consumed.
7. HPLC analyses revealed that trehalose, considered to be the 'insect haemolymph sugar', is not present in the two species investigated here.
8. Overall, HPLC sugar analysis provides a powerful tool to investigate energy status, feeding history and nutritional physiology of field-collected parasitoids.

Key-words: carbohydrate, *Cotesia glomerata*, fructose, glucose, haemolymph sugar, honeydew, *Microplitis mediator*, nectar, nutrient state, signature sugar, trehalose

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Introduction

Owing to their ability to regulate herbivore populations, parasitoids play an important role both as keystone species in natural ecosystems and as biological control agents. While parasitoid larvae are carnivorous, developing in or on their arthropod host, the majority of adult parasitoids depend on carbohydrates as an energy source. Those parasitoids that engage in host-feeding may obtain some carbohydrates this way (Jervis & Kidd 1986). However, host haemolymph is probably a relatively poor source of carbohydrates. Not only are haemolymph carbohydrate levels generally low (Kimura *et al*. 1992), the main haemolymph sugar (trehalose) is rather poorly metabolized by parasitoids (Wäckers 2001). The majority of parasitoids are believed to use plant-derived sugar sources such as extrafloral and floral nectar or honeydew to cover their energetic needs (Jervis *et al*. 1993). The fitness of parasitoids can be strongly affected by the availability of suitable sugar sources. Both longevity and fecundity are often enhanced through sugar feeding (e.g. Olson & Andow 1998; Wäckers 2001). Empirical as well as model studies showed that the availability of sugar

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sources can be a key factor in parasitoid–host dynamics (Krivan & Sirot 1997; Wäckers 2003).

The majority of studies addressing insect nutrition have been conducted in the laboratory. While these studies are undoubtedly instrumental in revealing nutritional benefits and physiological mechanisms, they do not necessarily provide insight into insect food use under field conditions. To better understand the food ecology of parasitoids, we need tools to study and quantify parasitoid feeding and nutritional state in the field. Most of the available field studies have investigated the effect of food sources only indirectly by correlating the presence of nectar-providing plants or other sugar sources with parasitoid abundance or parasitization rate (e.g. van Emden 1962; Leius 1967; Lingren & Lukefahr 1977). Increased parasitism in the presence of nectar-providing plants is not necessarily attributable to nectar feeding. The nectar plants may be visited for shelter, alternative hosts or more favourable climatic conditions (Landis, Wratten & Gurr 2000). Moreover, flowering plants may attract additional individuals from adjacent areas even if they do not provide accessible nectar (Wäckers 1994; Patt, Hamilton & Lashomb 1999; Wäckers 2004a). These confounding factors could be excluded by directly determining the nutritional state and feeding history of field-collected specimens.

Insects may store their energy reserves as carbohydrates (sugar, glycogen) and/or fat. The latter seems unreliable as an indicator of parasitoid nutritional state. In previous studies (Ellers 1996; Olson *et al*. 2000; Fadamiro & Heimpel 2001), the lipid levels of parasitoids decreased over their lifetime, even if food was available, indicating that these species are unable to synthesize lipids from sugars. Giron & Casas (2003) came to the same conclusion, even though they found no decrease in lipid levels over a 3-day period. Carbohydrate levels (glycogen and sugars) increase when parasitoids are provided with sucrose and are therefore a suitable indicator of the nutritional state of parasitoids (Fadamiro & Heimpel 2001; Giron & Casas 2003). The seminal study by Olson *et al*. (2000) used biochemical assays to quantify total body sugars, as well as fructose and glycogen levels in sucrose-fed *Macrocentrus grandii* parasitoids (Hymenoptera: Braconidae) and provided the first evidence that quantitative changes in sugar levels can be used as a general indicator of previous feeding. Casas *et al*. (2003) and Wäckers & Steppuhn (2003) demonstrated that field-collected parasitoids showed raised carbohydrate levels compared with freshly emerged individuals, indicating that parasitoids obtained sugars in the field. The biochemical assays used in most studies give only limited information on overall sugar composition. Since total carbohydrate content of parasitoids declines when feeding is followed by food deprivation (Fadamiro *et al*. 2001), it can be difficult to differentiate between parasitoids that consumed sugars several days ago, and those that have not fed. Since both categories are likely prevalent in the field, this differentiation can be crucial. Moreover, as the common biochemical methods do not identify food-source specific sugars, they do not provide information on the food source consumed.

The ultimate goal of this study is to use high-performance liquid chromatography (HPLC) to establish a differentiated and conclusive method to demonstrate and quantify food consumption in field-collected hymenopteran parasitoids. We conducted a series of experiments to analyse quantitative and qualitative changes in the overall sugar spectrum of two braconoid parasitoids *Cotesia glomerata* L. and *Microplitis mediator* Haliday relative to their feeding history, sex and age. In addition to being highly sensitive, the use of HPLC has the advantage that it identifies and quantifies a range of sugars in one procedure. Thus, it provides detailed information on the feeding history, the nutritional status, as well as the type of food sources consumed for each of the specimen examined.

Materials and methods

INSECTS

For this study we chose the braconid parasitoid *C. glomerata*, based on the fact that the feeding ecology of this species has been thoroughly studied (e.g. McGovran *et al*. 1969; Wäckers 2001). *C. glomerata* had been reared on larvae of the Large Cabbage White (*Pieris brassicae*) feeding on *Brassica oleracea*. To examine possible differences between the laboratory colony and a native population, we also used parasitoids emerging from field-collected *C. glomerata* cocoons from the same plant–host complex. To assess possible variation in the physiological sugar dynamics between species, we included *M. mediator* as a second parasitoid species. *M. mediator* had been reared on *Mamestra configurata*.

All cocoons were held at room temperature. Newly emerged parasitoids were collected daily and after brief anaesthetisation with CO_2 their weight at eclosion was determined using a microbalance, Mettler MT5 ± 2 µg (Mettler-Toledo B.V., 4000 HA, TIEL).

SUGAR LEVELS UNDER STANDARDIZED CONDITIONS RELATIVE TO WASP FEEDING HISTORY, AGE AND SEX

To determine the initial sugar levels at the time of eclosion, a group of parasitoids was analysed directly after weighing (newly emerged parasitoids). In addition, changes in sugar levels over time in unfed and *ad libitum* sucrose-fed parasitoids were examined. Sucrose was chosen as food substrate because it commonly occurs in floral and extrafloral nectars, as well as in honeydew. Parasitoids were kept individually in plastic Petri-dishes (Ø 5·5 cm) and either provided with water plus six 1-µl drops of a 2 M sucrose solution (fed parasitoids), or with water only (unfed controls). Petri dishes were placed in a climate chamber at standardized

conditions (15 °C; 100% r.h.; 16L:8D) and sucrose solution was renewed every second day. Parasitoids of both treatments were analysed from the first to the fifth day after eclosion. Sucrose-fed individuals were also analysed at days 8, 11 and 14. The number of replicates for each age and sex category ranged from 7 to 20 individuals in the case of *C. glomerata* and from 2 to 9 in the case of *M. mediator*.

In a further experiment, 16 *C. glomerata* females and 11 males were provided with sucrose (2 M) for the first 2 days after eclosion and subsequently kept with water only for 3 additional days before HPLC analysis (fed parasitoids after 3 days of starvation).

SUGAR LEVELS IN *C. GLOMERATA* UNDER SEMI-FIELD CONDITIONS

As we ultimately intend to study the nutritional state of parasitoids in the field, we conducted a second test series to investigate the dynamics of parasitoid sugar levels under semi-field conditions. Upon eclosion, groups of 30–40 *C. glomerata* were kept according to sex in outdoor gauze cages ($35 \times 35 \times 62$ cm^3). Each cage contained two cabbage plants in pots and a Petri dish with water. The size of the cages allowed parasitoids to fly freely. Every day an aerosol of either water (unfed) or a 2 M sucrose solution (fed) was sprayed into the cages. Five to six fed individuals per sex were analysed from the first to the fourth day after eclosion and five to eight unfed individuals on the first 2 days after eclosion. At the third day only two unfed females and three males were available. No individual survived more than 3 days.

HPLC SUGAR ANALYSIS

At the allocated age, individual insects were collected in an Eppendorf tube containing 1 ml 70% ethanol and subsequently stored at room temperature until analysis. To prepare the samples for HPLC analysis, the specimens were homogenized in the ethanol solution using a pestle. Subsequently samples were centrifuged at 13 000 r.p.m. for 10 min. We collected 500 µl of the supernatant and diluted it 10-fold with Milli-Q water (Millipore, Amsterdam). Of each sample 10 µl was injected into a Dionex DX 500 HPLC-system (Dionex Corp., Sunnyvale, CA). The system was equipped with a GP 40 gradient pump, a Carbopac PA1 guard (4×50 mm^2), and analytical column (4×250 mm), as well as an ED 40 electrochemical detector for pulsed amperimetric detection (PAD) (Dionex, Breda, The Netherlands). The column was eluted with 1 M NaOH and Milli-Q water (10:90%, 1 ml min^{-1}) and kept at 20 °C during analysis. Daily reference curves were obtained for sorbitol, mannitol, trehalose, glucose, fructose and sucrose by injecting calibration standards with concentrations of 2·5 p.p.m., 5 p.p.m., 7·5 p.p.m. and 10 p.p.m. of these sugars. The concentrations of the individual sugars were analysed using the program PEAKNET Software Release 5·1 (DX-LAN module) (Dionex, Breda, The Netherlands). The overall sugar content of a wasp was obtained from the sum of the concentrations of the individual sugars. To adjust the measured concentrations for insect size, sugar amounts were expressed relative to the wasp weight at time of eclosion. The glucose–fructose ratio was calculated as the glucose fraction of the sum of both monosaccharides.

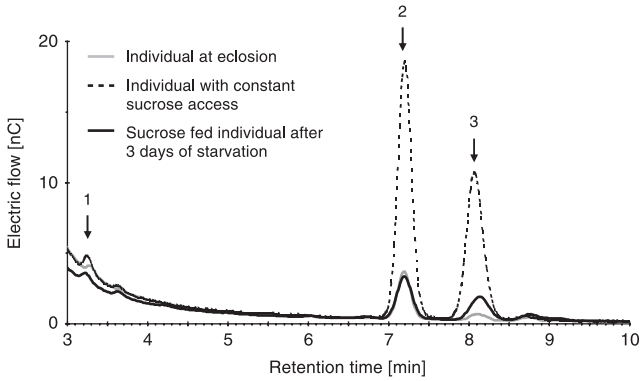

Fig. 1. HPLC chromatograms of ethanol extracts of *Cotesia glomerata* individuals of different feeding treatments: (a) unfed at time of eclosion, (b) constant sucrose access, (c) 2 days sucrose provided and subsequently 3 days of starvation. The peaks represent sorbitol (1), glucose (2) and fructose (3).

STATISTICAL ANALYSIS

For statistical analysis we used the program STATISTICA 5·0 (SURF diensten, Houten, The Netherlands). As the data were not normally distributed and the variance heterogeneity was high, non-parametric statistics were used. Parasitoids of different treatments, and sexes, were compared pair-wise using the Mann–Whitney *U*-test with Bonferoni corrections. When testing for differences between ages, Kruskal–Wallis ANOVA was used.

Results

INITIAL SUGAR LEVELS

The total sugar level of *C. glomerata* parasitoids upon eclosion was 7·2 ± 0·09 µg (mean ± SE; $n = 38$) per mg wasp. Newly emerged individuals of *M. mediator* contained an average of 2·9 ± 0·14 µg overall sugars per mg wasp ($N = 10$). The HPLC sugar spectrum of newly emerged parasitoids of both species was clearly dominated by glucose (Fig. 1a), representing on average 70% of the total sugar concentration. In addition, low levels of fructose and sucrose as well as the sugar alcohols sorbitol and mannitol occurred. The common insect haemolymph sugar trehalose was never detected.

TIME SERIES

Unfed parasitoids

The low initial sugar level decreased rapidly with age in unfed parasitoids (Fig. 2). Within 3 days of eclosion

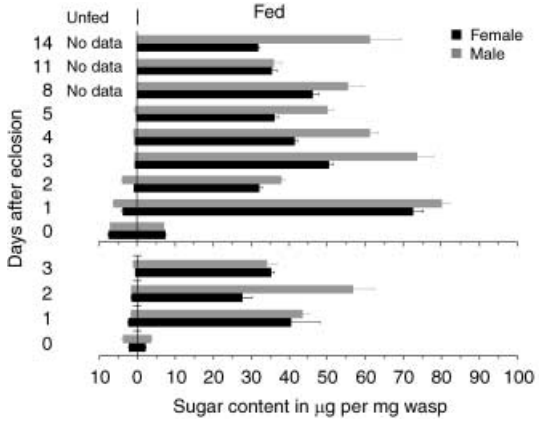

Fig. 2. Sugar content (mean + SE; in μg mg^{-1} wasp mass) of (a) *Cotesia glomerata* and (b) *Microplitis mediator* of different ages and both sexes. Parasitoids were either unfed (left) or provided with a 2 M sucrose solution (right) and data for the day of eclosion are presented twice to enable a comparison with both. Differences between fed and unfed parasitoids are significant for each age category (Mann–Whitney *U*-test: $P < 0{\cdot}01$). Sugar content of fed individuals differ at each age and that of unfed individuals at least after 2 days from newly eclosed parasitoids. The data of *M. mediator* males and females were combined prior to analysis.

the sugar content of *C. glomerata* had declined ($P < 0{\cdot}05$) to 0·49 μg (females) and 0·63 μg per mg wasp (males), i.e. less than 10% of the initial sugar level. In *M. mediator*, sugar levels decreased ($P < 0{\cdot}05$) to 0·43 μg (females) and 1·02 μg per mg wasp (males), i.e. 15 and 35% of the initial level. *C. glomerata* females showed a faster decline in sugar levels than males ($P < 0{\cdot}05$). On the first day after eclosion the total sugar level of females had already been reduced by half while males still retained about 90% of their initial level. Females had almost exhausted their body sugars on the second day, while males still retained 50% of their initial sugar level. Unfed parasitoids retained the glucose dominance throughout their life (Fig. 3).

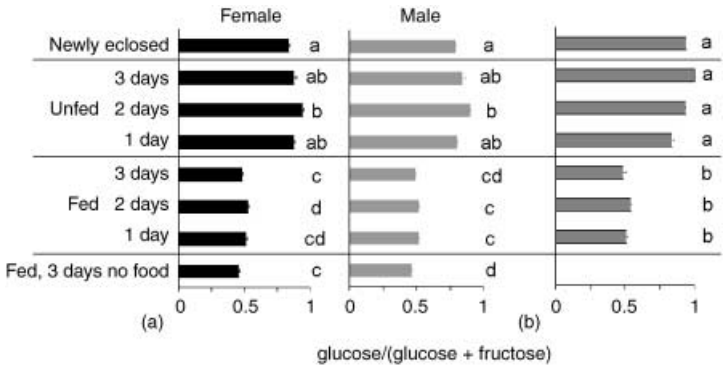

Fig. 3. Ratio between glucose and fructose (mean + SE) of (a) *Cotesia glomerata* males and females and (b) *Microplitis mediator* parasitoids with different feeding histories and of different ages. Significant differences are indicated by variables on the end of the bars (Mann–Whitney *U*-test: $P < 0{\cdot}5$).

Fed parasitoids

Feeding resulted in a significant increase in sugar levels ($P < 0{\cdot}01$; Fig. 2). Sucrose-fed parasitoids of both species, irrespective of age or sex, had overall sugar levels that were five- to ten-fold higher than the maximum levels of unfed parasitoids. Furthermore, feeding caused a significant change in sugar composition (Fig. 3). Whereas glucose was the main sugar in unfed parasitoids, sucrose-fed parasitoids contained approximately equal levels of fructose and glucose. Sucrose peaks were usually small or absent, indicating a rapid breakdown of sucrose into its hexose components. As in unfed individuals, the common insect haemolymph sugar trehalose was never detected.

Fed parasitoids after 3 days of starvation

Cotesia glomerata females that had been provided with sucrose-provided for 2 days and subsequently starved for 3 days contained sugar levels similar to those found in 1-day-old unfed parasitoids. However, they could be clearly distinguished from unfed individuals by their balanced glucose–fructose ratio, typical for fed wasps (Fig. 3).

Parasitoids of field-collected cocoons

The sugar levels of these *C. glomerata* parasitoids strongly resembled the patterns found for the laboratory colony, both with respect to the initial sugar level and its composition (glucose dominance). Unfed parasitoids from the field population also showed a strong decrease in sugar levels over the first 2 days after eclosion, which again was faster for females than for males ($N = 6$–15; $P < 0{\cdot}05$). Similar to laboratory-reared individuals, sucrose provision resulted in a strong increase in the overall sugar level ($N = 7$–15; $P < 0{\cdot}01$) and a change from glucose domination to a balanced glucose–fructose ratio.

Parasitoids in field cages

Under semi-field conditions, the decline in overall sugar levels of unfed parasitoids seemed to be sharper than under laboratory conditions. One-day-old females and males retained a mere 25% and 50% of the initial sugar levels and almost all unfed individuals in outdoor cages died within 3 days. Sugar levels of fed parasitoids significantly exceeded the levels at eclosion ($N = 4$–19; $P < 0{\cdot}05$). Similar to the climate chamber studies, fed parasitoids in the outdoor cages showed a balanced glucose–fructose ratio, whereas the sugar spectrum of unfed individuals was again glucose-dominated.

DISTINGUISHING FED FROM UNFED INDIVIDUALS

Both the overall sugar content as well as the ratio between glucose and fructose are suitable parameters

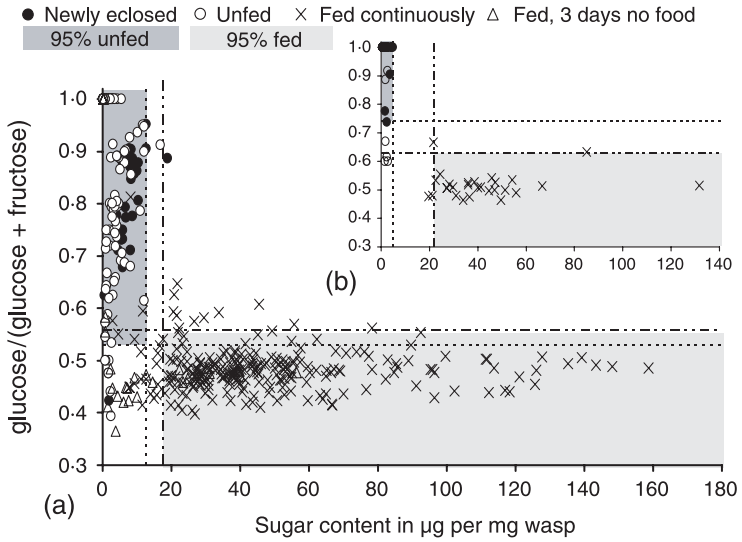

Fig. 4. Plot of the overall sugar content (µg mg^{-1} wasp mass) *vs* the glucose–fructose ratio of every individual (a) *Cotesia glomerata* and (b) *Microplitis mediator* parasitoid (laboratory-reared). Dashed lines indicate the thresholds including 95% of the fed or unfed parasitoids, respectively.

to distinguish between continuously sucrose-provided and starved *C. glomerata* and *M. mediator* parasitoids. The overall sugar level is useful as a parameter indicating the nutritional state of the individual but is not absolutely reliable as indicator for its feeding history. This is exemplified by the fact that parasitoids that fed once and were subsequently food deprived had sugar levels similar to unfed wasps. The glucose–fructose ratio, on the other hand, does not provide explicit information about the nutritional state, but is reliable in separating individuals that have fed earlier from those that never fed.

A clear differentiation between the feeding treatments arises when the two parameters are used in combination. When the overall sugar content is plotted against the glucose–fructose ratio, the individual parasitoids cluster according to their nutritional state and feeding history (Fig. 4). In the case of *C. glomerata*, three feeding groups could be reliably distinguished by the following thresholds:

1. Unfed individuals are characterized by a low overall sugar content (7·2 ± 0·09 µg per mg wasp at eclosion) and a high glucose–fructose ratio (0·81 ± 7 × 10^{-5}). Out of these 38 parasitoids 95% had an overall sugar content below 12·6 µg per mg wasp and a glucose–fructose ratio above 0·53.
2. Individuals with continuous access to sucrose had a high overall sugar content (51·1 ± 0·16 µg per mg wasp) and a low glucose–fructose ratio (0·48 ± 2 × 10^{-4}). Out of sucrose provided parasitoids (N = 256) 95% had an overall sugar content above 17·6 µg per mg wasp and a glucose–fructose ratio below 0·56.
3. Fed individuals subjected to 3 days of starvation had a low overall sugar content (4·7 ± 0·2 and 25·8 ± 0·6 µg per mg wasp for females and males, respectively), yet retained a low glucose–fructose ratio (0·54 ± 0·01). Ninety-five per cent (N = 27) had a glucose–fructose ratio below 0·56.

Similar patterns could be distinguished in the case of *M. mediator* (Fig. 4b). At eclosion, parasitoids (N = 10) had sugar contents below 4·87 µg and a glucose–fructose ratio above 0·74. The sugar levels of 95% of the fed *M. mediator* individuals (N = 18) exceeded 21·78 µg, with a glucose–fructose ratio below 0·63.

Discussion

In this study, we identified two parameters that together describe the nutritional state and the feeding history of field-collected parasitoids: the overall sugar content and the glucose–fructose ratio. The overall sugar content is an indicator of the current energy reserves. Whereas consumed sugars are also in part transferred to glycogen (Fadamiro & Heimpel 2001; Giron & Casas 2003), a glucose polymer that is not quantified by our analysis, our results show that the overall sugar level is a reliable indicator of parasitoid nutritional state.

The glucose–fructose ratio enables differentiation between unfed individuals and those that metabolized the bulk of their sugar meal. The combination of both parameters makes it possible to distinguish between unfed, recently fed and earlier-fed individuals. Our results correspond with high fructose concentrations found in continuously sucrose-fed and low or no fructose in unfed or starved *M. grandii* individuals (Fadamiro & Heimpel 2001). The fact that the biochemical assays used in the latter study do not reveal levels of other sugars limits the differentiation between individuals with low sugar levels.

A categorization according to the nutritional state and the feeding history of field-collected individuals is particular valuable to study parasitoid feeding ecology in nature. Free-flying parasitoids have high energy requirements (Hoferer, Wäckers & Dorn 2000), resulting in a rapid depletion of sugar reserves. This is confirmed in the present study as all unfed parasitoids in field cages died within 3 days. It has long been assumed that shortage of nectar and other sugar sources in modern agro-ecosystems can limit the efficacy of biological control agents (e.g. Wolcott 1942; Hocking 1966). Recently this has sparked a growing interest in the concept of augmenting beneficials by providing food supplements such as flowering plants (Gurr & Wratten 1999) or sugar sprays (Hagen 1986; Evans & Swallow 1993). Still, very few studies have unambiguously demonstrated a link between sugar supplements and improved biological control (Heimpel & Jervis 2004). Recently, Casas *et al*. (2003) and Wäckers & Steppuhn (2003) reported increased carbohydrates in field-collected individuals. In the latter study we could identify nectar and honeydew feeding in field-collected individuals. Here we show that the glucose–fructose ratio provides additional information on the feeding

history of parasitoids, and thus can be a valuable tool when studying the impact of food sources in biological control.

Two requirements need to be met for the glucose–fructose ratio to be informative. Firstly, the food sources utilized by the parasitoids should contain free fructose or fructose derivatives at concentrations exceeding those in unfed individuals. Secondly, the parasitoid should retain the ratio of glucose–fructose in the absence of additional feeding.

As far as food source composition is concerned, nectar may vary widely with regard to the ratio between sucrose and its component sugars fructose and glucose, but the overall proportion of glucose and fructose units usually does not deviate much from the 1:1 ratio (Percival 1961; Baker & Baker 1983). Honeydew, the sugar-rich excretion product of sap-feeding insects, represents another important sugar source for parasitoids under field conditions (Wäckers & Steppuhn 2003). Like nectar, honeydew can be balanced in its glucose and fructose units. However, in other instances fructose is selectively assimilated and the glucose moiety is used to synthesize glucose-rich disaccharides (trehalose, bemisiose) or oligosaccharides (melezitose and erlose) excreted in the honeydew (Ashford, Smith & Douglas 2000; Byrne, Hendrix & Williams 2003). Under these conditions honeydew composition can be skewed towards glucose. Even so, the fructose fraction usually still exceeds that of unfed individuals. Other sugar sources available to sugar-feeding insects (Wäckers 2004b) also usually contain substantial amounts of fructose, either in its pure form or as a component of sucrose or other saccharides. Therefore, the first criterion for the use of glucose–fructose ratio as an indicator of sugar feeding is usually met.

In addition, the glucose–fructose ratio of the ingested food should not be shifted towards glucose during physiological processes. This requires that fructose is not transformed to glucose and that glucose is assimilated at a rate equal to, or exceeding the rate of fructose assimilation. In this study, the glucose–fructose ratio in sucrose-fed *C. glomerata* and *M. mediator* is stable over 3 days of starvation (Fig. 3). The fact that the stable glucose–fructose ratio was found both for parasitoids kept in Petri dishes (low activity) and those flying in large field cages (high activity) indicates that the glucose–fructose ratio was not affected by the parasitoid's metabolic requirements. This assimilation appears to be comparable to the pattern reported for honeybees that transport and assimilate fructose and glucose at similar rates (Crailsheim 1988). Overall, this shows that the glucose–fructose ratio is stable and therefore suitable as a parameter for the identification of previous feeding events in the studied parasitoid species, even when the sugar meal has been almost completely metabolized.

Since HPLC analysis provides a broad sugar profile for each individual insect, it not only allows identification of feeding events, but also provides insights into the food sources used. Many food sources are characterized by a specific sugar composition. Detection of such 'signature' sugars in insects can strongly corroborate the consumption of a particular food. Honeydew, for example, often contains specific sugars such as erlose or melezitose and different sugar profiles of honeydew types can even indicate which honeydew has been consumed (Wäckers & Steppuhn 2003). Information on specific sugar composition is especially relevant considering that not all sugars are suitable for parasitoids (Wäckers 2001). If parasitoids feed on unsuitable sugar substrates, e.g. certain honeydew types, the overall sugar content becomes a poor indicator of energy reserves.

Unexpectedly, the specific sugar profiles of *C. glomerata* nor *M. mediator* do not show any trehalose, irrespective of feeding treatment, sex or age. We are confident about this result, given that our HPLC detects trehalose at concentrations of 0·02 p.p.m. and accurately measures trehalose levels in our standards, as well as in other insect species. This lack of trehalose is surprising, as insects in general are thought to metabolize glucose into trehalose, which is often referred to as the 'insect blood sugar' (Blum 1985; Thompson 2003). While this generalization is widely held, it is actually based on a limited number of species. Other insects, in particular dipteran and hymenopteran species, have been reported to have relatively low trehalose levels, at least during part of their development. These include larvae of the fly species *Phormia regina* and *Agria affinis* (Diptera) (Barlow & House 1960; Wimer 1969). Among Hymenoptera, the presence of trehalose has been demonstrated in honeybees (Woodring *et al*. 1993; Blatt & Roces 2001) and ants (Boevé & Wäckers 2003). Nevertheless, these species may feature haemolymph glucose at levels similar to or exceeding trehalose. Trehalose is believed to serve several functions, including energy storage, protection against cold, heat and osmotic stress (Thompson 2003). Furthermore it helps retain a glucose gradient across the gut wall, thus facilitating glucose diffusion (Friedman 1985). The lack of trehalose in *C. glomerata* and *M. mediator* opens up the question how these insects contend with the various functions attributed to trehalose. It should be considered that the absence of trehalose might be more common both among the Braconidae and in other insect families. This study underlines the need to establish trehalose levels rather than assuming the presence of trehalose in systems with undetermined haemolymph metabolites. It also indicates that our knowledge of carbohydrate metabolism, which is largely based on a few model insects, may not be adequate to understand physiological mechanisms in other insect species.

In summary, this study establishes two parameters to reliably distinguish the nutritional state and feeding history of field-collected parasitoids. It furthermore reveals the potential of HPLC sugar analysis for the study of insect feeding ecology in the field. Besides

giving a quantitative indication of energy status, the qualitative sugar profile also provides information on the food sources used and can give insights into the physiology of carbohydrate metabolism.

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