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Feeding activity pattern in a parasitic wasp when foraging in the field

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Abstract Individual decision-making and behavioral plasticity, and hence reproductive success, depend on nutritional state. Despite the importance of food for life-history functions, when and how often parasitoids encounter and consume food in natural settings remain largely unknown. In this study, we aimed at determining the food intake history of the *Venturia canescens* parasitoids in field conditions. To fulfill this objective, we compared the nutritional state of lab individuals of known feeding history and of field-released individuals allowed to freely forage under natural conditions. Nutritional state and feeding history were determined by using a combination of physiological parameters based on sugar assays. Field-released wasps were caught during periods starting either 5 or 24 h after release. Our results show that in the field, the wasps search actively for carbohydrate food sources. Our work also indicates that feeding activity of wasps in the field can be better understood by using the combination of key physiological parameters. The adaptive value of the feeding pattern and the relevance of the approach used are discussed.

Keywords Nutritional ecology · Carbohydrates · Release-recapture experiment · *Venturia canescens*

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Introduction

Nutrient acquisition and allocation strongly influence internal state (e.g., energetic reserves) and fitness of all organisms, which in turn influences decision-making processes (Mangel and Clark 1988; Clark and Mangel 2000). Indeed, in parasitoid insects for instance, along with the ecological characteristics of their foraging environment, the internal state (egg load, metabolic reserves) of parasitoids can influence their motivation to search for metabolic resources. This consequently affects their life-history traits (Siekmann et al. 2001; Lee et al. 2004) and especially their host-attack efficiency (see Lewis et al. 1998 for a review, Takasu and Hirose 1991; Stapel et al. 1997; Lucchetta et al. 2007; Pelosse et al. 2007). Additionally, knowledge of the feeding strategies of parasitoids can also be of great importance in understanding how they share their available time between the competing tasks of hosts and food searching (Sirot and Bernstein 1996; Desouhant et al. 2005; Tenhumberg et al. 2006). Surprisingly, however, much of our understanding of how parasitoids acquire and manage nutritional resources comes from laboratory and field cage studies and little is known about nutrient acquisition and energy dynamics in insects when they are free to forage in the field. A way to fill this gap is to compare parasitoid behavior in the field and laboratory and/or to quantify energy and nutrient dynamics in wild and/or field-released animals and to compare results with lab-controlled individuals of known feeding history (Strand and Casas 2008). This approach enables us to assess which resources are used and how frequently animals should search for food to cover their energetic needs (Strand and Casas 2008).

Most of the detailed physiological information on nutrient acquisition and allocation strategies comes from experiments conducted in laboratory conditions. Usually, the proteins obtained from host-feeding have been shown to serve to meet the high amino acid demands associated with egg production (Rivero and

Casas 1999; Rivero et al. 2001), while sugars acquired by host-feeding significantly improve parasitoid longevity (e.g., Giron et al. 2002). Additionally, common sugars, floral nectar, or honeydew allow female parasitoids to meet energetic requirements for maintenance metabolism (Jervis and Kidd 1986; Rivero and Casas 1999; Bernstein and Jervis 2008; Jervis et al. 2008) improving parasitoid longevity and, either directly or indirectly, fecundity (see Lee and Heimpel 2008 for potential mechanisms).

In natural conditions, many parasitoid species feed during the adult phase (Jervis and Kidd 1986; Bernstein and Jervis 2008; Hogervorst et al. 2007a). Some parasitoids feed on the hosts themselves (Jervis and Kidd 1986; Heimpel and Collier 1996; Gilbert and Jervis 1998). Other, the non-host feeding parasitoids, feed on plant-derived sugar sources such as extrafloral and floral nectar, fruits, pollen and sap (Eijs et al. 1998; Leius 1960; Jervis et al. 1993; Jervis and Kidd 1996; Lee and Heimpel 2002; Wäckers and Steppuhn 2003) or on the honeydew excreted by some Homoptera (Jervis et al. 1993; Hogervorst et al. 2007b). Some combine both host and non-host feeding (Heimpel et al. 1997) and potentially various non-host food sources successively. Casas et al. (2003) were the first to assess dynamics of metabolic resources in a parasitoid under field conditions, through release-recapture experiments with the non-host-feeder *Venturia canescens*. This pioneer study presented, however, two shortcomings: experiments were carried out in somewhat exotic conditions in a botanic garden with few endemic plants that could provide sugar rich resources, and this study did not reveal the pattern of parasitoid feeding history. Recently, field-conducted experiments in agroecosystems investigated the influence of floral resources on sugar feeding and nutrient dynamics and their link with parasitoid life-history traits and parasitism efficiency (e.g., Steppuhn and Wäckers 2004; Lee et al. 2006; Lee and Heimpel 2008).

Assessing nutritional state and feeding history of parasitoid free to forage in the field is however not an easy task. One way to fulfill this objective relies on the definition of appropriate physiological parameters that can be used to estimate whether insects fed or not, to estimate the time elapsed since their last meal, and to identify the nature of this meal. While sugars, as the main nutrients obtained by non-host feeding parasitoids, are mainly used in all studies, the physiological parameters estimated vary from total sugars, to glucose/(fructose + glucose) or fructose/total sugars ratios (Casas et al. 2003; Heimpel et al. 2004; Steppuhn and Wäckers 2004; Lee et al. 2006). For example, Steppuhn and Wäckers (2004) identified two parameters that together describe the nutritional state and the feeding history of female parasitoids the glucose/(fructose + glucose) ratio and the total sugar amount. The combination of both parameters enabled to distinguish between starved, recently fed, and earlier-fed wasps in *Cotesia gomerata* and *Microplitis mediator*, the total sugar amount providing information on the time since the last meal and the glucose/(fructose + glucose) ratio being an index of nutritional state. The total sugar amount is higher in individuals that fed recently than in earlier-fed or in starved individuals. Additionally, the ratio was found to be higher in unfed females than in fed ones, independently of the time since the last meal. After feeding sucrose (glucose-fructose dimer), which is the most common nutrient acquired from sugary food, fructose level increases, leading the ratio to decrease. Following this approach, Lee et al. (2006) successfully used biochemical analyses to determine whether sugar feeding occurred in field-collected *Diadegma insulare* (using total sugar amounts) and whether parasitoids fed on nectar or honeydew. In this system, the ratio of fructose to total sugars was found to be higher in nectar- than in honeydew-fed females. Such approaches using physiological parameters to identify potential food sources and to examine the influence of food consumption on parasitoid metabolism appears to be promising to unravel insect foraging strategies in the field but important differences in how these parameters can be used and interpreted exist according to the species under study (Olson et al. 2000; Casas et al. 2003; Steppuhn and Wäckers 2004 for parasitoids; Hogervorst et al. 2007b for predator; Lee and Heimpel 2008). In this context, more empirical data are required on feeding activity pattern of parasitic wasp foraging in the field using physiological parameters that can be quickly and easily obtained and are appropriate for the species at play.

This study aims to investigate the feeding history and nutrients dynamics of the parasitoid *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae) foraging in the field. We first assessed two physiological parameters based on carbohydrate amounts (fructose and all the remaining sugars (RS), see below) in lab individuals of known feeding history, to test whether we could discriminate individuals of different nutritional state and feeding history. From this experiment, we obtained reference values for feeding history and carbohydrate dynamics that were used to determine the feeding activity pattern and resource metabolic dynamics of individuals released in natural conditions. These individuals were allowed to forage freely for hosts and/or food and recaptured after different periods of time.

Materials and methods

Biological details and cultures

V. canescens is a solitary (at most one parasitoid emergence per parasitized host), koinobiont (allows host development after oviposition), larval endoparasitoid of pyralid moths (Salt 1976) known to be pests in granaries, mils, and orchards. We used a thelytokous strain founded with individuals collected in 2004 from a wild population in Valence (44.93°N; 4.90°E), France. The wasps were reared on the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) in wheat semolina (the host food medium), maintained in a constant environment of 25 (± 1) °C and 75 (± 5) % relative humidity (12 h L: 12 h D). The hosts originated from a mass rearing facility located in Antibes (Biotop), France.

Host foraging *V. canescens* females are attracted by the odor of a host mandibular gland secretion (kairomone) (Thorpe and Jones 1937; Castelo et al. 2003). The females mature most of their eggs when adult and therefore are confronted with the decision of whether to allocate reserves to egg production, maintenance, or both. This wasp species does not feed on host hemolymph, but often other food sources in the field (nectar and exudates of fruits or leaves are potential sources, Casas et al. 2003). Fruits harboring hosts can also provide food (Desouhant et al. 2005). The food foraging wasps use volatile cues to detect and locate food sources (Desouhant et al. 2005). Adult survival and fecundity are dependent on nutrition. Food can extend an individual's lifespan up to six times (i.e., around 3 weeks), in laboratory conditions (Desouhant et al. 2005). Fecundity is enhanced by a factor 2 when food is providing (Ozkan 2007).

Biochemical analyses and choice of physiological parameters

We used a colorimetric analysis technique to assess levels of different carbohydrate for individual insects (van Handel 1985a, 1985b; van Handel and Day 1988). This method, easy to implement, was already successfully used for parasitoid, including *V. canescens,* to assess different nutrients in the same individual (Casas et al. 2003; Pelosse et al. 2007). We determined the concentration of fructose with the cold anthrone test. Because fructose originates from sucrose, which is a specifically acquired sugar from nectars and honeydew (Petanidiou 2007), this technique allows assessment if feeding occurred. With the hot anthrone test, we assayed the total sugar content (fructose and all the others sugars) for each female. Sugar concentrations were assessed from absorbance curves of known concentrations of glucose and fructose. We then used the difference between results of the cold and hot anthrone tests to assess the concentration of the others sugars except fructose (van Handel and Day, 1990), which we called "remaining sugars" (RS).

At least two main methods are available to assay the nutrient content in insects. The first one relies on nutrient assays via HPLC techniques. This method is accurate but also expensive and less convenient to implement than the second, the colorimetric method. The latter does not allow the assessment of the quantity of glucose independently of other sugars. However, the colorimetric method has the advantage of being efficient to compare nutrient contents between treatments allowing for building the global energy budget of an organism (Casas et al. 2003; Pelosse et al. 2007). Indeed, this technique provides total lipid, sugar, glycogen, and protein contents simultaneously on a single animal. Results obtained can also allow us to easily link physiology and foraging strategy of insects foraging freely in the field. Finally, by using similar biochemical techniques, our results can be confronted to our pre-existing data obtained on field collected *V. canescens*, the first example of dynamics of metabolic resources in a parasitoid under field conditions (Casas et al. 2003).

The two physiological parameters we used are: total sugars and a ratio called ρ. Total sugar amount corresponds to the sum of all sugar quantities (RS and fructose). The ρ ratio is defined by RS/(RS + fructose). These RS (expressed as equivalent glucose) include glucose but also other sugars stored during larval development (such as trehalose), obtained by adult nutrition (such as melezitose) and/or synthesized de novo (i.e., synthesized from simple sugars). As a consequence, RS could represent an over-estimation of real glucose amount. However, as non-glucose sugars, concentrations are usually low in parasitoids (Wäckers et al. 2006) our results can be confronted to existing data on total sugars amount and glucose/(glucose + fructose) ratio measured in semi-field conditions (Steppuhn and Wäckers 2004). We predict that total sugar amount should provide information on the time since the last meal and the ρ ratio should be higher in unfed females than in fed ones, independently of the time since the last meal.

We focus our analyses on carbohydrates because (i) they reflect and allow quantifying the acquisition of food in non host-feeder parasitoids, (ii) they are the main compartment affected by environmental variables such as food availability in thelytokous *Venturia canescens*, while lipids and proteins show monotonous decrease during lifetime (Pelosse 2008).

As female size varies, all measurements on sugar contents were corrected for hind tibia length and are given in terms of µg per 0.02 mm tibia length (as in Casas et al. 2003). Given (i) the positive correlation between dry body mass and hind tibia length in thelytokous *V. canescens* (Harvey and Vet 1997), and (ii) positive correlations between hind tibia length and numerous life-history traits in parasitoids, such as teneral reserves (e.g., Ellers et al. 1998), egg load and longevity (Harvey et al. 1994), we considered the left hind tibia length as a good index of adult body size.

Laboratory experiment: Nutrient dynamics and feeding history

First, we aimed at assessing the dynamics of nutrients (RS and fructose) in females kept in the laboratory. This experiment allowed us to create a range of references for nutrient values that were used to assess the nutritional state of field-released wasps at the time of capture (see next section). Second, this experiment aimed at testing whether the ρ ratio and total sugar amount could be used to discriminate individuals with different nutritional status (fed and starved), and to determine the feeding history of *V. canescens*.

Dynamics of nutrients in the laboratory

To test the effect of feeding status on total sugars amount, two groups of individuals were compared: females that fed once (fed group) and females kept unfed (starved group). The nutrients contents of wasps were estimated by biochemical assays. Wasps used were collected just after their emergence between 08:00 am and 10:00 am and randomly assigned to the fed or the starved group. They were kept in the rearing room.

The fed group comprised 64 individuals that 24 h post-emergence were individually placed in tubes (7.5 × 2 cm) and offered a single meal of 5 µl of 50% water-diluted honey for 10 min. Honey composition (mainly fructose and glucose in equal proportion) closely resembles nectar and honeydew in being sugar-rich but both nitrogen and lipid-poor (Crane and Walker 1984; Foldhazi et al. 1996). Next, groups of 16 individuals were frozen to halt metabolic processes at 2, 12, 18, and 24 h after the meal (i.e., 26, 36, 42, and 48 h after emergence) (Fig 1).

The starved group comprised 80 females that were used to assess the dynamics of teneral metabolic reserves. Groups of 16 individuals were frozen immediately after emergence or 12, 24, 36, and 48 h after emergence (Fig 1). The experiment was ended after 48 h, the usual life-span of food deprived females (Desouhant et al. 2005).

Total sugar amount and ρ ratio as classification parameters

In order to assess whether ρ and total sugar amount are efficient to discriminate between individuals of different nutritional state (fed versus starved) and feeding history (short versus long time since last meal) we created four categories of individuals from previous fed and starved groups (see above, Fig 1). Two categories comprised "just emerged" and "unfed" (2 days without food) wasps, both of which never had access to food. The two other categories comprised "just fed" wasps, which had access to food only once 2 h before, and "fed then starved" wasps, which had access to food only once 1 day before.

Field experiment: Feeding pattern of wasps and nutrients dynamics

By calculating the ρ ratio and the amount of total sugars in natural conditions and comparing them to values obtained in controlled conditions (laboratory experiment), we aimed to determine the nutrient dynamics and pattern of feeding activities of free-foraging wasps in the field.

To achieve this objective, food-deprived wasps from our laboratory cultures were released and recaptured after different periods in a mixed landscape combining an orchard, grass, and a small natural wood, near Valence (France). On the day of emergence (between 08:00 am and 10:00 am), each wasp was color-marked with a dot of water-soluble acrylic paint on the upper side of the thorax. The animals were marked with different colors for each release experiment. The wasps were then isolated in individual plastic tubes (7.5 × 2 cm), and kept in the rearing room at 25°C and water was supplied. On the second day, at 08:00 am, they were transferred into release tubes, each containing a single female (see Desouhant et al. 2003) and then transported to the experimental field (travel of 1 h). Release tubes were kept closed during 30 min at the release point to allow wasps to settle in the new conditions. To match the laboratory experiment, release started 24 h post-emergence (at 10:00 am) by opening the tubes (as in Desouhant et al. 2003). Some wasps, randomly chosen, were not released and were kept as controls (CTRL group comprising 5% of the released number of individuals). They were frozen at the time of release.

The released wasps were free to forage for two different periods of time: the captures started either 5 h (henceforth called 'group A'), or 24 h after release

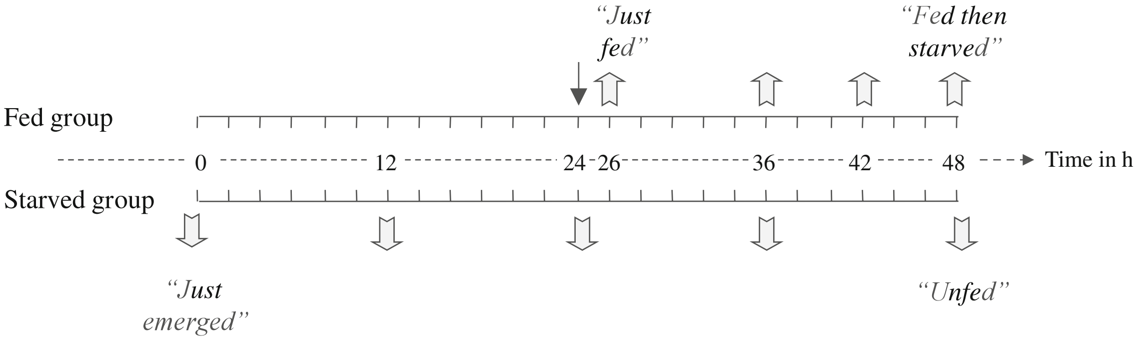

Fig. 1 Summary of the laboratory experiment design. *Black arrow* indicates the meal the wasps of the fed group received 24 h after their emergence. *Grey arrows* indicate each time a sub sample of wasps was used for sugar assessments. "just emerged": sub sample of starved wasps at emergence; "unfed": starved wasps since 48 h; "fed then starved": wasps of 2 days old that have received a meal 24 h before; "just fed": wasps of 26 h old that have fed once 2 h before

('group B'). The wasps were released in two different groups to avoid recapturing all the released wasps after 5 h of foraging in the field. Captures started by suspending 40 traps in the surrounding vegetation in a 20-m-radius circle centered in the release point. Traps consisted of 2 × 5 × 5 cm honeycomb-like structured pieces of cardboard containing fifth-instar host larvae settled in their food medium. Each trap was checked for wasps every 15 min on average (see Desouhant et al. 2003). In both cases (groups A and B), trapping was prolonged until no wasp was caught within 1 h. Each marked wasp on a trap was caught and frozen with carbo-ice within 10 min. The time of each capture was recorded. Back in the laboratory, the wasps were stored at –20°C for future biochemical analyses.

Three experiments were conducted: two short-term experiments in which recaptures started 5 h after release (group A), and one long-term experiment lasting at least 24 h (group B). The long-term experiment was not repeated because of bad weather conditions. The short-term recapture experiments were carried out on July 27 and September 9, 2005. We released 60 and 39 wasps, and caught 38 (63.3%) and 16 (41.0%) marked females, respectively. Wasps were caught between 3:00 pm (that is, 5 h after release) and 8:30 pm (10.5 h after release) for one of the experiments of group A. The long-term experiment was carried out on September 14, 2005, where 60 wasps were released and 37 (61.6%) marked individuals were caught, between 10:00 am (24 h after release) and 2:00 pm (28 h after release) the following day (group B). Weather conditions were quite similar during the 3 days of experiment: sunny with little wind, and with temperatures ranging from 20 to 26°C.

Data analyses

Dynamics of nutrients in the laboratory and in the field

The amounts of sugars were analyzed by the means of a generalized linear model (gamma error and inverse link). In the "laboratory experiment", the explanatory variables were *time since emergence* (a quantitative variable) and *nutritional status* (a factor with two modalities fed and starved). In the "field experiment", *time since beginning of captures* (a quantitative variable) and *group of release* (factor with two modalities A and B) were used as explanatory variables. The CTRL group was not included in this analysis since the wasps belonging to it were "captured" at the same time. The potential explanatory factor, *day of experiment* (i.e., 2 days of experiments for groups A and 1 day for B) was not included in the model because the total sugar amount was not significantly different between the two days of experiment for group A ($t = -1.58$, 1df, $p = 0.12$). To compare the nutrient contents for wasps of groups A and B with those of CTRL group, Kruskal-Wallis rank sum tests were performed.

Total sugar amount and ρ ratio as classification parameters

A MANOVA, with a Pillai test to take into account of potential heteroscedasticity, was used to test whether (i) the four "feeding" categories of females ("just emerged", "unfed", "just fed" and "fed then starved") from the laboratory experiment, and (ii) the individuals from the field belonging to A, B, and CTRL groups were distinct according to their relationship between the ρ ratios and total sugars. MANOVA allows the response variable (ρ ratio and totals sugars amount) to be multidimensional (Tomassone et al. 1988). Since MANOVA does not provide contrast analysis, we assessed differences in total sugar amount and ratio values between groups (field) and "feeding categories" (lab) by changing the matrix of contrasts in generalized linear models (gamma error and inverse link).

A linear discriminant analysis (LDA) was performed to estimate how ρ and total sugar amount contribute to the correct classification of individuals into the four "feeding categories". A predictive model for the category membership using the laboratory animals was first used to determine the percentage of laboratory wasps, belonging to a feeding category, that were classified into this category. Then it was used to classify the field wasps.

To assess whether all recaptured individuals of groups A and B have had similar feeding behavior, the distributions of the amount of total sugars of groups A and B were compared using the Kolmogorov-Smirnov test. We also tested whether in group A and B some of the recaptured individuals had no access to food by testing unimodality Hartigan's dip test (Hartigan and Hartigan 1985). If some individuals were found not to have fed, the null hypothesis of unimodality was rejected.

All the statistical analyses were performed using R software (R Development Core Team 2008).

Results

Sugar dynamics

Dynamics of sugars in laboratory conditions

Both explanatory variables *time since emergence* and *nutritional status* significantly affected the quantity of total sugars. The decrease with *time since emergence* differed between fed and starved individuals (Fig. 2; interaction: $F = 7.03$, $df = 1$ and 140, $p = 0.008$). Fed individuals showed a steeper decline of total sugars with time than starved ones (difference between slopes: $t = 2.68$, $df = 140$, $p = 0.009$). This means that sugar amounts differ between the two groups at different times after emergence. Fed wasps had in average higher sugars contents than starved ones (1.10 μg/0.02 mm tibia ± 0.74 (SE) and 0.15 μg/0.02 mm tibia ± 0.07 (SE), respectively).

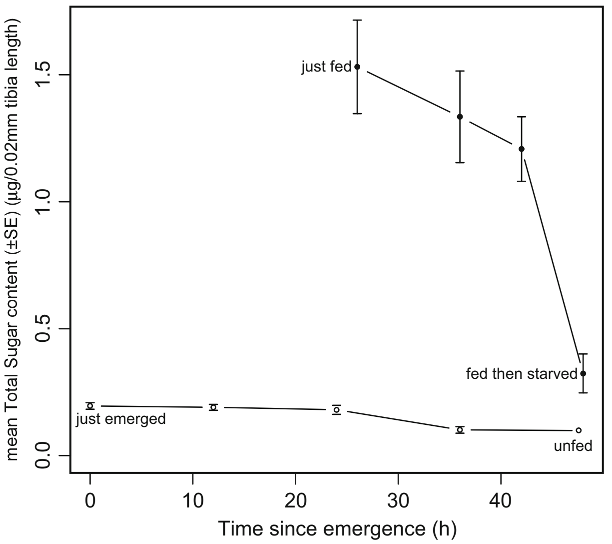

Fig. 2 Total sugar dynamics in *V. canescens* females. *Black circles*: average nutrients for 26, 36, 42, and 48-h-old wasps after a meal taken 24 h after emergence (fed group in Fig 1); *white circles*: average nutrients for wasp that never have access to food (starved group in Fig 1). "just emerged", "unfed", "just fed", "fed then starved" refer to groups of wasps described in Fig. 1 legend. Amounts of nutrients are corrected for the wasp size (as in Casas et al. 2003)

Dynamics of sugars in the field

Total sugar amount was significantly affected by the interaction between the *time since beginning of recaptures* and the *groups of release* (total sugar amount: $F = 8.34$, $df = 1$ and 41, $p = 0.006$; Fig. 3).

The significantly larger reserves of total sugars of recaptured wasps than the control ones (comparison of groups CTRL, A and B, Kruskal-Wallis, $\chi^2 = 26.52$, $df = 2$, $p < 0.0001$, Fig. 3) indicated that, independently of the time spent free in the field, the recaptured wasps had fed in the wild. Individuals of group B had significantly more sugars than those of group A (U-Mann-Whitney test with Bonferroni correction, $W = 123$, $p = 0.004$). Group A had significantly more sugars than the CTRL individuals ($W = 227$, $p < 0.0001$). The mean values per 0.02 mm of tibia length were 0.11 µg ± 0.02 (± SE) for group CTRL, 0.74 µg ± 0.10 for group A, and 1.17 µg ± 0.09 for group B.

Reliability of total sugar amount and ρ ratio

Pattern of feeding history in laboratory conditions

The four "feeding categories" of wasps ("just emerged", "unfed", "just fed", and "fed then starved") were significantly discriminated by the combination of ρ ratio value and total sugar amount (MANOVA, $F = 30.67$, $df = 6$ and 120, $p < 0.0001$; Fig. 4a and b).

The wasps that had never fed ("just-emerged" and "unfed") had a lower total sugar amount than the wasps that had access to food ("just fed" and "fed then starved"; contrasts: $t = 6.79$, $df = 30$, $p < 0.0001$; Figs. 2; 4b). Among the wasps that had fed, "just fed" individuals had a higher total sugar content than wasps that fed 24 h before ("fed then starved") (contrasts: $t = 3.08$, $df = 30$, $p = 0.003$). Total sugar content did not allow, however, to discriminate between, on one hand, "just-emerged" and "unfed" groups and on the other hand, "just emerged" and "fed then starved" (Fig. 4b).

The ρ ratio of the wasps that had never fed ("just-emerged" and "unfed"; global mean = 0.73) had a lower ratio than the wasps that had access to food ("just fed" and "fed then starved"; global mean = 0.79; contrasts $t = 3.25$, $df = 30$, $p < 0.001$). Among the wasps that never fed, the ratio of "just emerged" individuals was higher than that of the unfed ones (contrasts: $t = 7.82$, $df = 30$, $p < 0.0001$). The "unfed" wasps had a significantly lower ρ than "fed then starved" (contrast: $t = 4.69$, $df = 30$, $p < 0.001$; Fig. 4a and b). Among the wasps that fed, no difference was observed between "just fed" and "fed then starved" *Venturia canescens* (contrasts: $t = 0.40$, $df = 30$, NS).

The discriminant analysis (LDA) provided, for wasps belonging to each of the four "feeding" categories, percentages of these individuals classified into the right category: 81% for "just emerged" and "unfed", 56% for "fed then starved" and 75% for "just fed". Under a random assignment, the percentage should have been 25% (i.e., prior probability of each group).

Pattern of feeding history in the field

The three groups (CTRL, A, and B) were significantly discriminated by the combination of ρ ratio value and total sugar amount (MANOVA, $F = 10.24$, $df = 4$ and 102, $p < 0.0001$; Fig. 4c and d). The values of ρ were different between the three groups ($F = 4.83$, $df = 2$ and 51, $p = 0.012$). The ratio of the released wasps (groups A and B) did not differ from that of the control group ($t = 1.35$, $df = 52$, $p = 0.18$). On the other hand, ρ of group A was significantly higher than that of B (contrast: $t = 3.01$, $df = 41$, $p = 0.004$; averages: 0.76 ± 0.0007 (SE) and 0.72 ± 0.0004 (SE), for groups A and B, respectively, Fig. 4d). Total sugar amount was different between released wasps and control group (contrast: $t = -2.69$, $df = 52$, $p = 0.009$), and between the groups A and B (contrast: $t = 4.52$, $df = 41$, $p < 0.0001$).

When applying the predictive model of LDA to individuals of group A, 11 (42.3%) individuals were classified into the "just fed" category and 53.8% in "fed then starved". One individual was identified as "just emerged". For recaptured wasps of group B, 14 (73.6%) and five (26.4%) were classified into "just fed" and "fed then starved" categories, respectively. No individual was classified into "unfed".

The distribution of total sugar amount of individuals belonging to group A was significantly different than group B (Kolmogorov-Smirnov test, $D = 0.5385$,

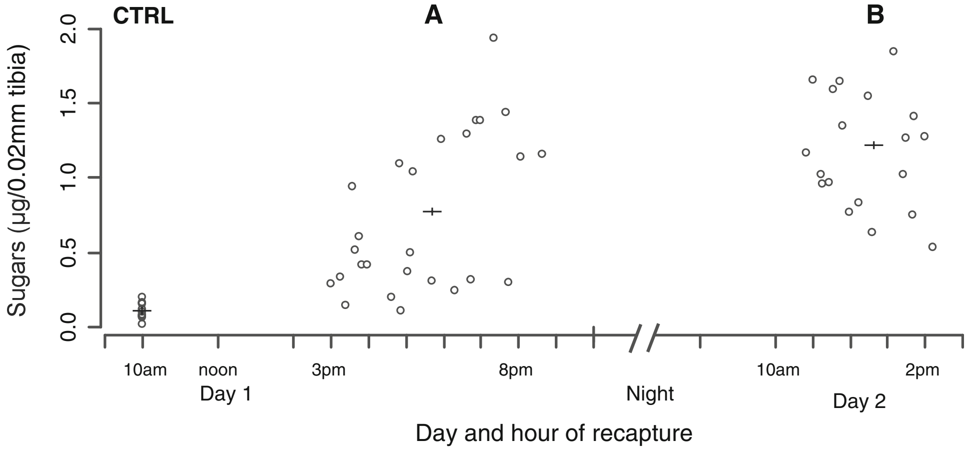

Fig. 3 Individual total sugars for the wasps of the control group (CTRL) and those caught at least 5 h (A) or 24 h (B) after release in the field. Individuals were released at 10:00 am for groups A and B. Each *dot* represents an individual and the means for individuals of group CTRL, A, and B, are represented by a *cross*. Amounts of nutrients are corrected for the wasp size (as in Casas et al. 2003)

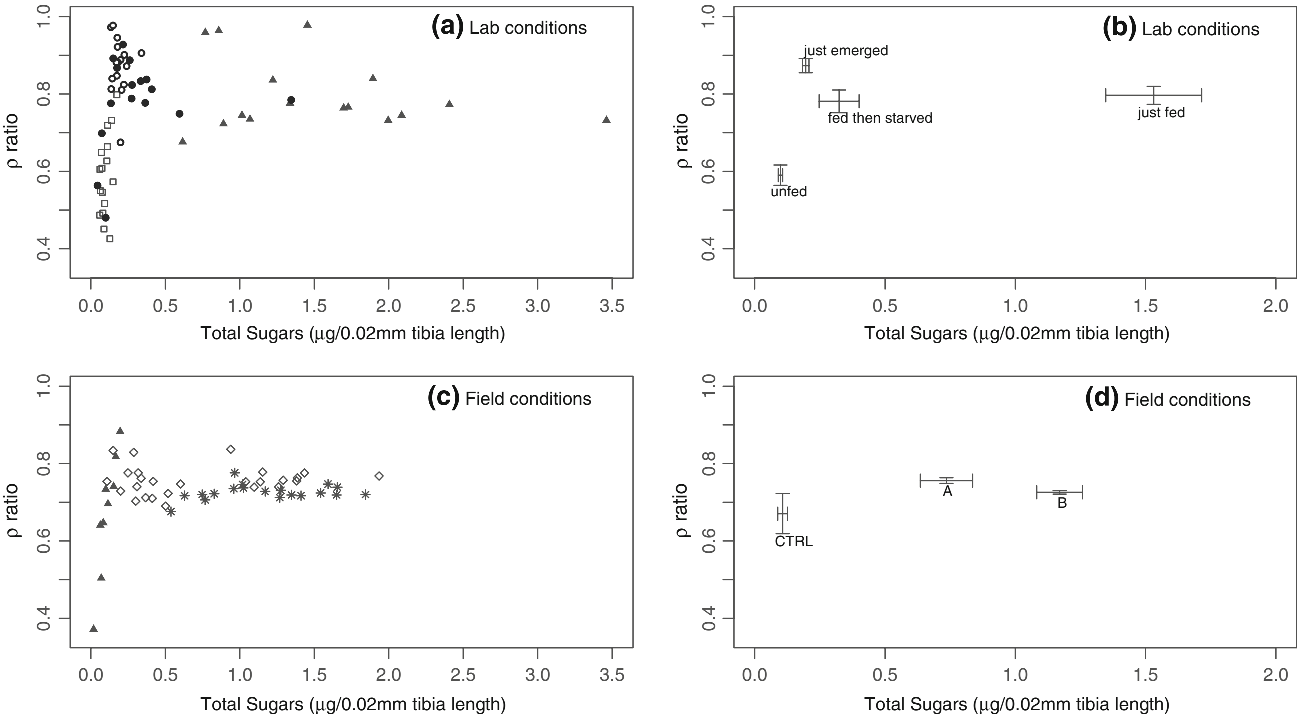

Fig. 4 Total sugar amount vs. ρ ratio of individuals from the laboratory experiment (panels **a** and **b**) and the field experiment (panels **c** and **d**). **a** *Open circles*: "just emerged"; *opened squares*: "unfed"; *filled circles*: "fed then starved"; *filled triangles*: "just fed". "just emerged", "unfed", "just fed", "fed then starved" refer to groups of wasps described in Fig. 1 legend. **b** Individual data of each group are summarized by the mean ± standard error in the two dimensions of the graph. **c** *Black triangles*: control wasps (CTRL); *white diamonds*: group A (wasps caught at least 5 h after release; see Fig. 3); *stars*: group B (wasps caught at least 24 h after release; see Fig. 3). **d** Individual data of each group (A, B, and CTRL) are summarized by the mean ± standard error in the two dimensions of the graph. For each category of wasps, the *arrowhead* indicates the mean for both total sugars and ρ ratio. Amounts of nutrients are corrected for the wasp size (as in Casas et al. 2003)

$p = 0.002$). This is due to the fact that the two groups have different means and that the distribution of total sugars in wasps of group A was not unimodal (Hartigan's dip test, dip statistic = 0.13, $p < 0.05$), while that of group B was unimodal (dip statistic = 0.065, NS).

Discussion

Our results showed that the total sugar amount and ρ ratio were reliable physiological parameters for characterizing *Venturia canescens* females according to their nutritional state and feeding history under laboratory conditions. The combination of both variables enabled us to distinguish between unfed, recently fed, and earlier fed wasps, indicating that these parameters can be used to investigate feeding history in field conditions. Our field results showed (i) that wasps released and caught after different foraging periods easily found carbohydrates food source to feed on, and (ii) that individuals caught in the morning after spending the previous night in the field had fed more recently on sugary sources than those that had spent only the afternoon. Some individuals succeeded in feeding on sugar in the afternoon, particularly early in the evening.

Total sugars amount and ρ ratio as reliable indexes of nutritional state and feeding activity in laboratory conditions

In *V. canescens*, the ρ ratio, in combination with total sugars, are reliable indicators of nutritional state of individuals and allow to define the time since the last meal. Similar to the glucose/(fructose + glucose) ratio (Steppuhn and Wäckers 2004), the ρ ratio enabled us to distinguish between fed and unfed *V. canescens* since the wasps that never fed ("just emerged" and "unfed") had a different ratio than the wasps that fed once ("just fed" and "fed then starved", Fig. 1). The "unfed" wasps also have a lower ratio than the wasps that receive a meal 24 h before. However, as with the glucose/(fructose + glucose) ratio, the ρ ratio is independent of the time since the last meal, "just fed" and "fed then starved" wasps having similar ratios. Consequently, this parameter only prevents us to unravel dynamics of feeding of parasitoid in the field. However, as in *C. glomerata* and *M. mediator* (Steppuhn and Wäckers 2004), the total sugar amount allowed discriminating individuals that fed recently from those that fed earlier. Among the fed animals, those that had fed recently had more sugar reserves than those that acceded to a meal 24 h before. Moreover, both groups had higher sugar contents than the wasps that had never fed ("just emerged" and "unfed").

Interestingly, two of our results differ from those obtained when glucose/(fructose + glucose) ratio is used. First, among never fed individuals, Steppuhn and Wäckers (2004) found a constant ratio for "just emerged" and "unfed" wasps. We found that the "just emerged" wasps had a higher ρ ratio than the "unfed" wasps (2 days old). This might be due to a strong decrease in RS quantity after 24 h of food deprivation (from 0.14 to 0.06 µg per 2 mm tibia length), while fructose was almost constant over the 48-h period following emergence (range: 0.025–0.038). Second, females that fed once (either 2 or 24 h before) had ratio values higher than those estimated for the "unfed" animals while the opposite was observed when the glucose/(glucose + fructose) ratio was used. The explanation for this disparity could be that 2 h was enough to metabolize the honey meal decreasing the relative contribution of fructose to total sugars by hexokinase and phosphoglucoisomerase conversion of fructose into glucose (Bailey 1975) hence increasing the ρ ratio.

It is now clear that a general rule describing food consumption from physiological parameters cannot be established. These physiological parameters present different interpretations according to the species under study (Hogervorst et al. 2007b). Indeed, in three species of aphidophagous insects the total sugar amount and the glucose/(fructose + glucose) ratio of insects that were starved for 48 h (for the parasitoid *Aphidius ervi* and the lacewing *Chrysoperla carnea*) or 24 h (for the hoverfly *Episyphus balteatus*) after a previous feeding could not be distinguished from individuals that had never fed before. These interspecific differences highlight the need for more empirical data on various parasitoid species and call a systematic comparison of data from field-caught insects with those from laboratory-controlled conditions.

Pattern of feeding history in the field

Previous study showed that *V. canescens* females released in the field fed on sugary sources (exudates of fruits or leaves, nectar; Desouhant et al. 2005). However, so far, nothing was known of feeding frequency and rhythm. Based on the total sugar amounts and the ρ ratio, our results give some insight into feeding activity pattern of parasitoids in the field.

Almost all released wasps that were captured had fed in the field. Indeed, when compared to lab-collected results, the ρ ratio values of A and individuals B (free to forage during 5 and 24 h, respectively) were comparable to those of "just fed" and "fed then starved" wasps, respectively. The increase of total sugar content between control group individuals (CTRL) and recaptured groups also shows that both groups had fed. Finally, 98% of individual of groups A and B were classified into a category in which animals had fed ("just fed" and "fed then starved"). Our results back up previous findings on other parasitoid species where the majority of individuals captured in agricultural settings were found to have fed upon sugar (from 59.5 to 89% (Wäckers and Steppuhn 2003; Lavandero et al. 2005; Winkler 2005; Lee et al. 2006).

However, pattern of feeding is not the same between individuals of groups A and B. On average, the wasps of the group B (recaptured the day after) had fed more recently than the wasps caught the day of release (group A). Several results lead to this conclusion. First, mean total sugar content of group B was greater than that of group A. Second, the bimodality of the distribution of total sugar contents indicated that part of the wasps in group A did not feed just after release, which was not the case for group B. Third, 42 (group A) versus 74% (group B) of individuals were classified (discriminant analysis) into the category "just fed".

Interestingly, it is worth noticing that despite having fed in the field, released wasps had the same total sugar content after 5 h as individuals that did not feed for 18 or 24 h in the laboratory. This could be explained by intrinsic differences in food sources used in controlled (diluted honey) and natural conditions and/or by the quantity of food consumed in both conditions. To cope with theses issues, laboratory wasps were fed on a diet (honey) that closely resembles natural food sources such as nectar and honeydew (Crane and Walker 1984; Foldhazi et al. 1996). Another explanation relies on differences in energy requirements between free-flying wasps and wasps kept in tubes in the laboratory. Despite having fed more recently (less than 5 h) than corresponding lab-controls (between 18 and 24 h ago), field individuals most likely used more nutritional resources to cover their flying activity. Flight is a highly energy demanding behavior in insects (Harrison and Roberts 2000) and parasitoids could fuel their flight with glucose as shown for honeybee and orchid bee (Suarez et al. 2005).

In this paper, we show that investigating nutrients dynamics and feeding-activity patterns in the field provide interesting results to link physiology and foraging strategy of insects free to forage in the field) and should help to better understand the effects of nutritional state on female parasitoid's parasitization rate (Fisher et al. 2004; Casas et al. 2005; Min et al. 2006; Lee and Heimpel 2008). Our study, combined with the work of Casas and collaborators (2003), provides a more complete picture of feeding pattern of *Venturia canescens* foraging in the field. From the methodological point of view, we show that the colorimetric method provides a quick, cheap, and easy way to access key physiological parameters.

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