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Lifespan and patterns of accumulation and mobilization of nutrients in the sugar-fed phorid fly, *Pseudacteon tricuspis*

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Abstract. The effect of sugar feeding on the survival of adult phorid fly *Pseudacteon tricuspis* is investigated. Flies fed 25% sucrose in aqueous solution continuously throughout their lifespan have greater longevity (mean ± SE longevity: female = 7.9 ± 0.8 days, male = 8.9 ± 0.9 days) than completely starved (provided no water and no sugar solution) flies, sugar-starved (provided water only) flies, or flies fed sugar solution only on their first day of adult life. Completely starved flies rarely lived beyond one day. Provision of water increases longevity by 2 days, and one full day of sugar feeding further increases longevity by an additional 1–2 days. Flies fed 50% sucrose have similar survivorship as those fed 25% sucrose. The temporal patterns of nutrient accumulation and utilization are also compared in *P. tricuspis* fed different diets: sugar-starved, sucrose-fed on the first day of adult life only, and sucrose-fed continuously. Adult *P. tricuspis* emerge with no gut sugars, and only minimal amounts of body sugars and glycogen. Although the levels of body sugars and glycogen decline gradually in sugar-starved flies, a single day of sugar feeding results in the accumulation of maximum amounts of gut sugars, body sugars and glycogen. High levels of these nutrients are maintained in female and male phorid flies fed on sucrose continuously over the observation period, whereas nutrient levels decline in flies fed only on the first day of life, beginning 1 day postfeeding. Female and male *P. tricuspis* emerge with an estimated 12.3 ± 2.3 and 7.2 ± 1 g of lipid reserves per fly, respectively. These teneral amounts represent the highest lipid levels detected in adult flies, irrespective of their diet, and are maintained over the life times of sucrose-fed female and male flies, but declined steadily in sugar-starved females. These data suggest that adult *P. tricuspis* are capable of converting dietary sucrose to body sugars and glycogen, but not lipids.

Key words. Anthrone tests, carbohydrate, glycogen, lipid, longevity, metabolism, Phoridae, *Pseudacteon tricuspis*, red imported fire ant, sugar feeding.

Introduction

In addition to energy reserves present at emergence, adults of many insect species depend to a large extent on resources acquired postemergence for survival and reproduction (Chapman, 1982; van Handel, 1984; Nestel *et al.*, 1985; Briegel, 1990; Clements, 1992). Carbohydrates and lipids constitute the main energy resources in insects (Clements, 1992). In insects, sugar-derived energy is potentially available in four forms: (i) as monosaccharides fructose and glucose (gut or crop sugars) ready for immediate absorption in the gut; (ii) as disaccharide trehalose circulating in the haemolymph; (iii) as polysaccharide glycogen stored in the fat body and sometimes in the flight muscle; and (iv) as lipid

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(triglycerides) in the fat body. The synthesis of glycogen and lipid from simple sugars is carried out in the fat body (Downer & Matthews, 1976; Clements, 1992; Rivero & Casas, 1999). By contrast to female mosquitoes, which synthesize lipids from sugar diets (van Handel, 1984; Clements, 1992; Naksathit *et al*., 1999), there is no evidence to date of substantial conversion of dietary sugars to lipids in parasitoids, suggesting that lipid is probably not a major energy reserve in these insects.

Sugar represents the main energy source for adult parasitoids, and several studies have demonstrated the positive effects of sugar feeding on parasitoid survivorship and/or fecundity in the laboratory (Hagen, 1986; Godfray, 1994; Heimpel *et al*., 1997; Olson & Andow, 1998; Olson *et al*., 2000; Fadamiro & Heimpel, 2001; Lee *et al*., 2004), and to a lesser extent in the field (Evans, 1993; Jervis *et al*., 1993). Sugar availability in the field is primarily in form of floral and extrafloral nectar, and homopteran honeydew (Bugg *et al*., 1989; Evans, 1993; Jervis *et al*., 1993, 1996). Nectar and honeydew sugars primarily contain the disaccharide sucrose and its two monosaccharide components, glucose and fructose (van Handel *et al*., 1972; Baker & Baker, 1983; Wäckers, 2001). In addition to these primary components, honeydew also contains several disaccharides, such as maltose and melibiose, and trisaccharides, such as melezitose, raffinose and erlose (Baker & Baker, 1983; Wäckers, 2001). Provision of artificial sugar supplements or nectar sources is increasingly being recommended and utilized in biological control programs (Jacob & Evans, 1998), although little is known about the ability of many species to utilize different types of sugars (Wäckers, 2001). The majority of the studies on effect of sugar feeding on parasitoid biology have focused mainly on parasitioid wasps (Hymenoptera). The effect of sugar feeding on physiology has rarely been demonstrated in dipteran parasitoids, such as phorid flies (Diptera: Phoridae). It is possible that quantitative and qualitative differences exist among different insect groups and species not only in terms of the impact of sugar feeding on their fitness, but also in their sugar preferences.

Phorid flies in the genus *Pseudacteon* (Diptera: Phoridae) are parasitoids of ants, and many species are specific to fire ants, *Solenopis* spp. (Disney, 1994; Morrison, 2000). *Pseudacteon tricuspis* Borgmeier is one of two species of phorid flies that have been introduced in many parts of southern U.S.A. for the biological control of the red imported fire ant, *Solenopsis invicta* Buren (Gilbert, 1996; Porter *et al*., 1999; Graham *et al*., 2003). In Alabama, phorid flies were first released in 1997 (Graham *et al*., 2003) and the release sites are currently being monitored to determine parasitoid establishment and performance. However, little is known about the feeding and foraging behaviour of phorid flies and the impact of sugar feeding on their lifespan. In the present study, the effect of sugar feeding on the longevity of female and male *P. tricuspis* is investigated along with temporal patterns of nutrient accumulation and mobilization in flies provided different diet treatments.

Materials and methods

Insects

Pseudacteon tricuspis flies used in this study were reared on workers of red imported fire ants, *S. invicta,* at the fire ant rearing facility of the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, U.S.A., as described by Porter *et al*. (1997). Larvae of *Pseudacteon* phorid flies have the habit of decapitating fire ant workers and pupating inside the empty head capsule (Porter *et al*., 1995). Parasitized fire ant worker heads were received in batches and maintained in a plastic jar with a lid (25-cm diameter × 13-cm high) at 26 ± 1 °C, under an LD 14 : 10 h photoperiod and 65 ± 5% RH, until emergence. The jar was checked at least five times a day for fly emergence. Emerging flies did not have access to food or water in the jar. Flies were removed with an aspirator immediately upon emergence and the sex determined by the presence or absence of the distinct female ovipositor.

Longevity

Adult female and male *P. tricuspis* were placed in four diet treatments: (i) completely starved (provided no water and no sugar); (ii) sugar-starved (provided water only); (iii) sugar-fed only during the first day of emergence and starved thereafter until death; and (iv) sugar-fed continuously from emergence throughout life. Sugar was provided as a 25% sucrose solution in water and flies were fed *ad libitum* for 24 h during the day of feeding. With the exception of the complete starvation treatment, water was provided in all treatments by filling a 0.5 mL microcentrifuge tube with distilled water and threading a cotton string through a hole in the cap of the tube. Water tubes were refilled as needed. Pairs of newly-emerged flies of the same sex were placed in a 6-cm diameter plastic Petri dish. For the treatments involving sugar feeding, 25% sucrose solution was smeared on the inside of the Petri dish cover with a cotton-tipped applicator. Flies that were fed only during the first day of emergence and then starved until death were transferred to new dishes without sucrose (but with water tubes) after exposure in sucrose-smeared dishes for 24 h. Petri dishes were checked once daily for dead flies, which were promptly removed from the dishes. Female and male *P. tricuspis* emerging on the same day from the same batch of attacked imported fire ants were distributed evenly across the four treatments. At least 30 flies of each sex were tested for each treatment. All experiments were conducted at 28 ± 1 °C, under an LD 14 : 10 h photoperiod and 65 ± 5% RH.

In another experiment, the longevity of female and male phorid flies was compared at two sugar levels: 25% sucrose and 50% sucrose. The experiment was conducted using the protocols described above. Sugar solution was provided continuously from emergence throughout the lifespan and water was provided in both treatments, as described above.

At least 15 flies were tested for each diet and sex combination. Data from both longevity experiments were analysed by using proportional hazard modelling (SAS Institute, 1998) to determine the effect of diet, sex, and interaction terms on survivorship. In addition, mean longevity was calculated for each diet treatment. Data for each sex were analysed separately using analysis of variance followed by the LSD test for multiple comparisons of means (SAS Institute, 1998).

Nutrient metabolism

Temporal patterns of accumulation and utilization of energy resources were followed in female and male *P. tricuspis* provided different diet treatments. Daily changes in the amounts of fructose, total sugars, glycogen and lipid were quantified for three groups of phorid flies: (i) sugar-starved (provided water only) until frozen; (ii) fed only during the first day of emergence and starved thereafter until frozen; and sugar-fed continuously (*ad libitum*) until frozen. (iii) Female and male *P. tricuspis* emerging on the same day from the same batch of attacked imported fire ants were distributed evenly across the three treatments. Flies were placed in pairs of the same sex within 6-cm diameter plastic Petri dishes. The dishes holding the flies were randomly assigned to the treatments. Treatments were prepared as described in the longevity experiment. Flies from each treatment were frozen and assayed daily from ages 1–5 days in the two sucrose-fed treatments (treatments ii and iii). Because a significant proportion of starved flies do not live beyond 4 days under the test conditions (see Results), nutrient assays were conducted only for 1–3-day-old flies in the starvation treatment. The amount of each nutrient obtained from newly emerged unfed (day 0) flies was regarded as the teneral amount present in female and male flies at emergence. Forewing length was used to estimate fly size. One forewing was pulled from each fly, slide mounted and measured to the nearest 0.05 mm. Measurements were taken from the outer edge of the anal cell to the outer edge of the tip of the wing. At least 10 individuals of each sex per age were sampled for each treatment, with the exception that only seven males were bioassayed from 3-day-old, sugar-starved treatment because of the difficulty of keeping sugar starved flies beyond 3 days. Due to limited supply of phorid flies for laboratory research, nutrient data collected for 1-day-old female and male flies fed only during the first day of emergence (treatment ii) were also used to represent data for 1-day-old flies fed continuously (treatment iii) because both treatments are essentially the same for 1-day-old flies.

The amounts of fructose, total sugars, glycogen and lipid in individual flies in the different treatments were quantified using a series of biochemical tests originally developed by van Handel (1965, 1967, 1985a, b; van Handel & Day, 1988) for mosquitoes, and recently adapted for parasitoids (Olson *et al.*, 2000; Fadamiro & Heimpel, 2001; Lee *et al.*, 2004). Briefly, an individual fly was crushed with a plastic pestle in a 1.5 mL microcentrifuge tube containing 50 μL of 2% sodium sulphate solution and placed on ice. The dissolved nutrients were then extracted with 450 μL of chloroform-methanol (1 : 2), after which the tube was vortexed. The tube was then centrifuged at 14 000 ***g*** for 2 min and 200 μL of the resulting supernatant was transferred to a glass tube (12-mm diameter × 75-mm long) for the sugar assays. Another 200 μL was transferred to a similar glass tube for the lipid assay. The precipitate was left in the microcentrifuge tube for the glycogen assay. All tubes were heated at 90 °C until approximately 50 μL of solution was left in the sugar tube and all solution was evaporated from the lipid and glycogen tubes.

Fructose. To estimate the amount of fructose, 950 μL anthrone reagent was added to the sugar tube, mixed thoroughly and left to react at room temperature for 1.5 h (cold anthrone reading). After the reaction time elapsed, the solution was poured into a 1.5 mL methacrylate cuvette and the optical density (absorbance) measured at 625 nm using a spectrophotometer. To convert absorbance readings to absolute fructose amounts (μg), standard curves were generated by determining the cold anthrone absorbance (at 625 nm) of different amounts (1–50 μg; three replicates per dose) of pure fructose (Fisher, Fairlawn, New Jersey). A linear regression was the best fit and highly significant ($F = 403$, d.f. $= 16$, $P < 0.0001$, $r^2 = 0.96$), and generated the linear equation: absorbance $= 0.208 + 0.036$ (μg fructose). The total amount of *gut sugars* (amount of sugars present in the insect crop) in each fly was estimated by multiplying the fructose amount by five. This was carried out because sucrose (which was fed to the flies) consists of equal parts of fructose and glucose, and the glucose does not react at room temperature (van Handel, 1967; Fadamiro & Heimpel, 2001). Therefore, the cold anthrone reading must be multiplied by two to give an estimate of total gut sugars. Furthermore, because only 200 μL of the original 500 μL was used for the fructose (cold anthrone) assay, it was necessary to multiply this amount further by 2.5 to estimate the total amount of gut sugars.

Body sugars. The amount of total sugars in each fly was estimated by using the hot anthrone test. Total sugars refer to the total amount of sugars present in an insect and consisted of two components, one (body or blood sugars) present in the haemolymph and body tissues, and one (gut sugars) contained in the crop (van Handel & Day, 1988). The same solution used for the cold anthrone test was poured back into a glass tube, heated at 90 °C for 10 min and cooled on ice. The absorbance was again read at 625 nm to give an estimate of total sugars. To convert absorbance readings into absolute amounts (μg), standard curves were generated by determining the hot anthrone absorbance (at 625 nm) of different amounts (1–50 μg, three replicates per dose) of pure sucrose (Fisher). The resulting linear regression was highly significant ($F = 461$, d.f. $= 16$, $P < 0.0001$, $r^2 = 0.97$), and generated the linear equation: absorbance $= 0.218 + 0.033$ (μg sucrose). The total amount of total sugars present in each fly was estimated by multiplying the amount

of sugars from the hot anthrone test by 2.5 because only μ200 μL of the original 500 μL was used for the hot anthrone assay. To estimate the amount of body sugars (nonglycogen sugars) present within the haemolymph and tissues of phorid flies (i.e. body sugars), the estimated total amount of gut sugars was subtracted from the estimated total amount of total sugars. Presumably, body sugars of *P. tricuspis* are comprised mainly of trehalose, the dominant sugar in insect haemolymph and other tissues (Wyatt, 1967; van Handel, 1969).

Glycogen. One mL of anthrone reagent was added to the microcentrifuge tube containing the precipitate. After centrifugation, the tube was heated at 90 °C for 10 min and then cooled on ice and the absorbance read at 625 nm. Glycogen standard (calibration) curves were generated by determining the absorbance of oyster glycogen (ICN Biomedicals, Aurora, Ohio) at a range of 1–50 μg (three replicates per dose). Regression analyses showed that a quadratic fit (second-order polynomial) was the best fit and highly significant ($F = 226$, d.f. = 17, $P < 0.0001$, $r^2 = 0.97$), and generated the quadratic equation: absorbance = 0.085 + 0.034 (μg glycogen) − 0.00028 (μg glycogen − 20.1667)^2. The equation was thus used to convert absorbance readings to absolute glycogen amount (g). The amount of glycogen estimated above was considered to be representative of the whole fly because all glycogen in the sample is presumed to precipitate to the bottom of the tube.

Lipids. The amount of lipids in each fly was determined by adding 40 μL of sulphuric acid to the tube containing the lipid precipitate. The tube was then heated at 90 °C for 2 min, cooled on ice, and 960 μL of a vanillin phosphoric acid reagent was added. The solution in the tube was left to react at room temperature for 30 min, mixed, and the absorbance read at 525 nm. To convert absorbance values to absolute lipid amounts (μg), lipid standard curves were generated by determining the absorbance of pure vegetable oil at a range of 1–50 μg (three replicates per dose). The resulting quadratic equation (second-order polynomial) was highly significant ($F = 336$, d.f. = 20, $P < 0.0001$, $r^2 = 0.97$), and generated the quadratic equation: absorbance = 0.05 + 0.027 (μg lipid) − 0.00033 (μg lipid − 14.667)^2. The equation was thus used to convert absorbance readings to absolute lipid amount. To estimate the total amount of lipids present in each fly, the lipid amount was multiplied by 2.5 because 200 μL of the original 500 μL was used for the assay. All reagents were prepared as described by van Handel (1985a).

Nutrient data were analysed by using multiple regression analysis to test the effects of diet (fed sucrose for 1 day vs. fed sucrose continuously), age, diet–age interaction and wing length on nutrient levels. Each sex was analysed separately because male flies were smaller ($F = 96.5$, d.f. = 1, $P < 0.001$) and had generally lower nutrient contents than females. Statistical analyses were run on absolute nutrient amounts rather than on absorbance values. Nutrient values were checked for unequal variances and, if necessary, transformed using Box-Cox transformation (SAS Institute, 1998) to equalize variances. Gut sugar, body sugar and glycogen levels from the starvation treatment (treatment i) were clearly different from those obtained from the two sugar-feeding treatments (treatments ii and iii), and thus were excluded from multiple regression analyses. However, all three treatments were compared in the analyses of lipids because lipid values were not remarkably different among the three treatments. Newly emerged flies were not included in the statistical analyses because they were not part of any of the treatments.

Results

Longevity

Proportional hazard analysis revealed a significant effect of diet ($\chi^2 = 168.1$, d.f. = 3, $P < 0.001$) on lifespan of adult *P. tricuspis*. Survivorship curves for both sexes are shown in Figure 1. Table 1 shows the average longevity of *P. tricuspis* at the different treatments. Sucrose-fed female and male phorid flies lived significantly longer than flies that were provided with water only (sugar-starved flies), or completely starved flies (no water, no sugar) (Table 1). Female *P. tricuspis* fed 25% sucrose throughout their lifespan lived longer (7.9 ± 0.8 days) than females provided 25% sucrose only on the first day of emergence and starved thereafter (4.2 ± 0.3 days). Females provided water had a greater longevity (3 ± 0.2 days) than completely starved females (1.2 ± 0.1 days). Similar results were obtained for male *P. tricuspis:* males fed sucrose throughout their lifespan lived longer than males in the remaining three treatments (Table 1). Water increased average longevity by approximately 2 days for males and by approximately 1.8 days for females. Although males provided sucrose for 1 day only had a higher survivorship than males provided water only, longevity of females provided sucrose for 1 day was not significantly higher than that of females provided with water only. The effect of sex was significant ($\chi^2 = 4.35$, d.f. = 1, $P = 0.04$) with sugar-fed males living longer than females. No significant diet–sex interaction was recorded ($\chi^2 = 0.5$, d.f. = 3, $P = 0.92$). Further data analysis shows that approximately 25% of female and male phorid flies fed sucrose continuously lived for 12 days or more, with some individuals surviving for over 15 days (Fig. 1).

The 25% and 50% sucrose concentrations showed no significant difference in extending the longevity of female and male *P. tricuspis* ($\chi^2 = 2.27$, d.f. = 1, $P = 0.99$). Sex also did not affect longevity ($\chi^2 = 0.06$, d.f. = 1, $P = 0.97$), and no significant concentration × sex effect was recorded ($\chi^2 = 0.16$, d.f. = 2, $P = 0.92$). Female and male phorid flies fed 25% sucrose *ad libitum* had similar survivorship to their counterparts fed 50% sucrose (Table 2).

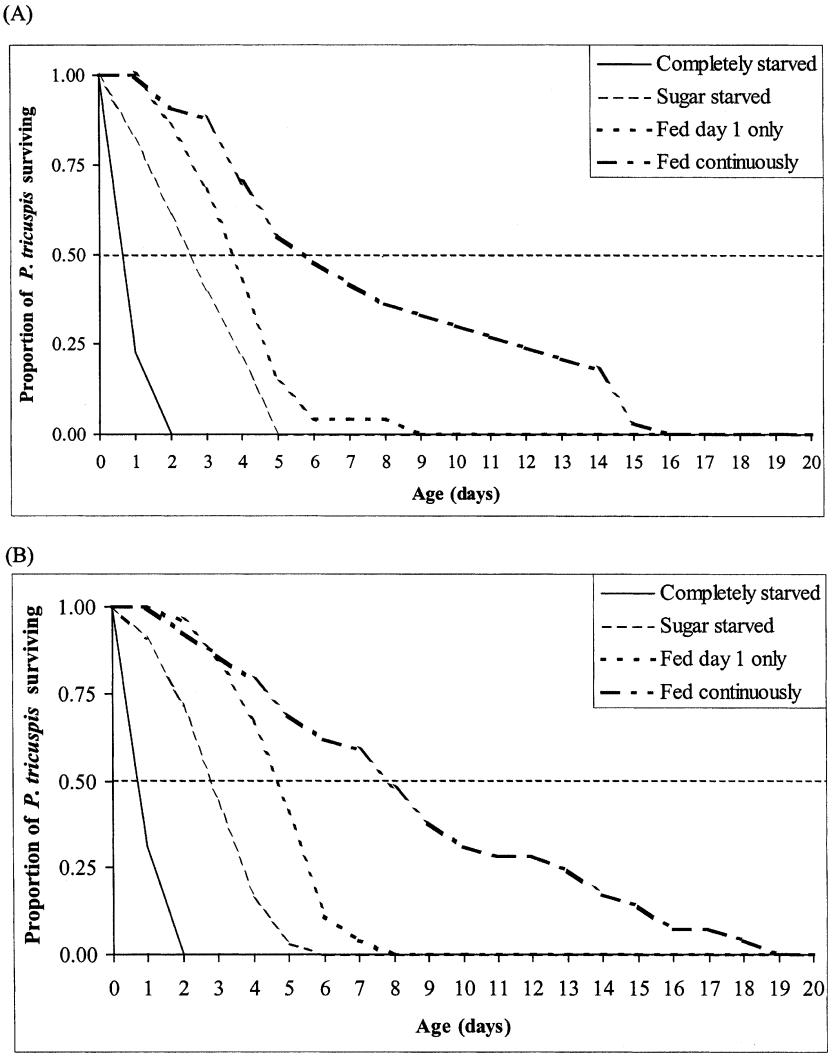

Fig. 1. Survivorship curves for (A) female and (B) male *Pseudacteon tricuspis* fed different diet treatments. The dashed line at 0.5 survivorship indicates median longevity for each treatment.

Nutrient metabolism

Gut sugars. Female and male *P. tricuspis* emerged with virtually no gut sugars (sugar fraction contained in the crop), which also were not detected in sugar-starved flies. However, high levels of gut sugars were detected in female and male flies fed sucrose on the first day of emergence and flies fed sucrose continuously (Fig. 2). The amount of gut sugars accumulated by female and male *P. tricuspis* fed sucrose continuously reached a peak on the first day of feeding and did not significantly increase during subsequent days of feeding, indicating that adult flies reached their limits of gut sugars accumulation quickly during the first day of feeding (Fig. 2). However, gut sugars declined gradually over time in female and male flies fed on day 1 only, reaching lowest levels in 5-day-old flies, leading to a significant effect of age and a significant interaction

Table 1. Mean longevity (days ± SE) of female and male *Pseudacteon tricuspis* subjected to different feeding treatments.

Diet	Females	Males
Completely starved[1]	1.2 ± 0.1^{c}	1.3 ± 0.1^{d}
Sugar-starved	3.0 ± 0.2^{b}	3.3 ± 0.2^{c}
Sugar-fed day 1 only	4.2 ± 0.3^{b}	5.0 ± 0.3^{b}
Sugar-fed throughout lifespan	7.9 ± 0.8^{a}	8.9 ± 0.9^{a}
F	37.7	46.7
P	<0.001	<0.001

Means within the same column having different superscript letters are significant ($P < 0.05$). [1]Completely starved = flies provided with no water and no sugar solution.

Table 2. Mean longevity (days ± SE) of female and male *Pseudacteon tricuspis* fed two concentrations of sucrose *ad libitum*.

% Sucrose	Females	Males
25	7.5 ± 1.2	8.7 ± 1.5
50	8.5 ± 1.3	8.0 ± 1.1
F	0.28	0.13
P	0.60	0.72

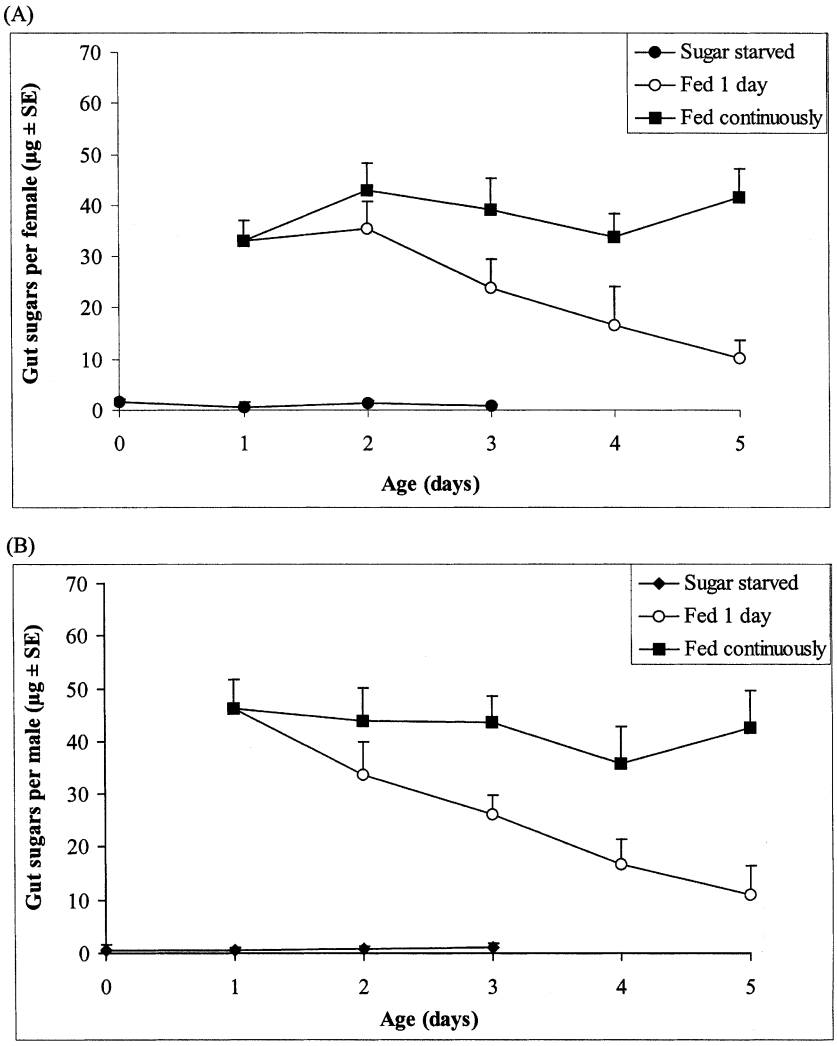

Fig. 2. Amounts (μg ± SE) of gut sugars in (A) female and (B) male *Pseudacteon tricuspis* that were either sugar-starved (provided water only), fed 25% sucrose on the first day of life only, or fed sucrose continuously throughout their lifespan.

between effects of age and diet treatment (Table 3). Gut sugar amounts in sugar-fed males were higher than in females.

Body sugars. Female and male flies emerged with approximately 4 μg of body sugars (Fig. 3). Body sugar (sugar fraction in the haemolymph and body tissues) levels declined gradually in sugar-starved females and males, but increased to high levels on the first day of feeding in sucrose-fed flies of both sexes. One day of sucrose feeding resulted in a net accumulation of approximately 0.4 and 1.8 μg of body sugars in female and male flies, respectively. Body sugars accumulated by female flies fed sucrose continuously did not significantly increase after the first day of feeding, but remained stable in adult flies of both sexes throughout the experiment. There was no significant effect of diet treatment on body sugar levels in flies from the two sucrose treatments (Table 3). Although male flies fed sucrose only on their first day of life had the highest body sugar level on day 1, female flies from the same treatment had the highest body sugar level on day 2. Moderate body sugar levels were maintained for 3 days in female and male flies fed sucrose only on their first day of life, after which body sugar levels began to decline, leading to a significant diet × age interaction for females and a significant age effect for male flies (Table 3).

Glycogen. Individual adult *P. tricuspis* of both sexes emerged with approximately 8.5 μg of glycogen, and this teneral level was maintained over time in starved female and male flies (Fig. 4). Glycogen levels increased significantly on day 1 in adults of both sexes fed sucrose, and remained stable in flies fed sucrose continuously. One day of sucrose feeding resulted in net accumulation of approximately 8.6 and 5.6 μg of glycogen in female and male flies, respectively. Glycogen levels in males fed on day one only remained high over the entire observation period, but were slightly less than levels in males fed continuously, leading to significant effects of diet and age on glycogen (Table 3). However, glycogen levels in females fed on day 1 only began to decline

Table 3. Multiple regression analyses testing for effects of diet, age, the interaction between diet and age, and wing length on nutrient levels of female and male *Pseudacteon tricuspis*.

		Females			Males		
	d.f.	MS	*F*	*P*	MS	*F*	*P*
Gut sugars							
Diet	1	4702	15.0	0.001	7030	23.2	<0.001
Age	1	2009	6.4	0.013	7107	23.4	<0.001
Diet × Age	1	2474	7.9	0.006	5858	19.3	<0.001
Wing length	1	4689	14.9	0.001	2745	9.1	0.003
Body sugars							
Diet	1	89.6	1.2	0.28	104	3.7	0.06
Age	1	38.2	0.49	0.48	118	4.2	0.04
Diet × Age	1	349	4.5	0.03	38.6	1.4	0.24
Wing length	1	583	7.5	0.007	177	6.3	0.01
Glycogen							
Diet	1	308	5.8	0.02	64.4	9.8	0.002
Age	1	366	6.8	0.01	105.5	15.9	0.001
Diet × Age	1	111	2.1	0.15	19.8	3.0	0.09
Wing length	1	56.9	1.1	0.30	73.8	11.2	0.001
Lipids							
Diet	2	29.6	2.5	0.09	4.5	1.2	0.30
Age	1	105	8.8	0.004	3.2	0.9	0.35
Diet × Age	2	7.3	0.31	0.74	0.54	0.14	0.87
Wing length	1	4.0	0.34	0.56	0.01	0.002	0.96

Two diet treatments (fed only on day 1 vs. fed continuously) were compared for gut sugars, body sugars and glycogen, whereas all three diet treatments were compared for lipids.

gradually on day 2, leading to a significant effect of age, although levels were always maintained above the teneral amount throughout the observation period.

Lipids. The mean estimated amount of lipids present in newly emerged female and male *P. tricuspis* was $12.3 \pm 2.3\,\mu g$ ($n = 16$) and $7.2 \pm 1.0\,\mu g$ ($n = 12$), respectively (Fig. 5). This teneral amount represented the highest lipid levels detected in female and male flies in the different treatments. There was no significant effect of diet treatments on lipid levels of female and male flies (Table 3), and sucrose feeding did not significantly increase lipid levels (Fig. 5). There was a slight decrease over time in lipid levels of starved females, resulting in a significant effect of age on female lipid levels (Table 3). However, lipid levels remained relatively stable in sucrose-fed flies throughout their lifetime. No significant diet–age interaction was recorded for adult flies of both sexes.

Wing length. Wing length data obtained from female and male flies of all ages in the different treatments (in the nutrient experiment) were pooled and analysed by sex. *Pseudacteon tricuspis* females (mean ± SE: 1.16 ± 0.007 mm; $n = 194$; range = 0.85–1.4 mm) had a significantly longer wing length than males (1.06 ± 0.007 mm; $n = 164$; range = 0.85–1.3 mm; $t_{358} = 9.9$; $P < 0.001$). Wing length was positively correlated with the amounts of gut and body sugars in female and male flies, and also with glycogen levels in male flies. However, no significant relationship between wing length and lipid amounts was detected in female or male flies, suggesting that the amount of lipid reserves at emergence is not a function of size (Table 3). To test this hypothesis, a further analysis was conducted by placing newly emerged adult phorid flies of each sex into two groups based on wing length and comparing teneral lipid levels between the two groups. The results show no significant trend in the mean ± SE teneral lipid levels of small (wing length = 0.85–1 mm) and large (wing length ≥ 1 mm) females (small = $14.8 \pm 5.3\,\mu g$, $n = 6$; large = $10.9 \pm 2.0\,\mu g$, $n = 10$; $P = 0.43$) and males (small = $6.4 \pm 0.6\,\mu g$, $n = 5$; large = $7.8 \pm 1.7\,\mu g$, $n = 7$; $P = 0.54$).

Wing length data were further analysed by pooling data for all ages for each diet treatment and running linear regression analysis to determine the relationship between wing length and nutrient types (i.e. gut sugars, body sugars and glycogen). Generally, wing length was positively correlated with gut sugars and body sugars only for the treatments in which sucrose was offered, but not for starved flies (Table 4). Wing length was also related to glycogen for starved males, and males fed sucrose continuously, but the relationship between wing length and lipids was not significant for female and male phorid flies (Table 3).

Patterns of nutrient mobilization. A single day meal of sugar resulted in the net accumulation of approximately 32 μg of gut sugars, 1 μg of body sugars and 9 μg of glycogen in female *P. tricuspis*. The amounts of sugars accumulated by the male were generally higher (approximately 46 μg of gut sugars, 4 μg of body sugars). However, male flies accumulated lower glycogen amounts (6 μg) than females. After a single day of starvation, female and male flies that were previously treated to a full day of sucrose meal began to mobilize accumulated gut sugars and, to a reduced extent, body sugars. Glycogen was mobilized at a minimal rate by starved female and male flies. Similarly, lipids were mobilized at a low rate by females, but rarely by male flies.

Discussion

One major objective of this study is to demonstrate sugar feeding and its impact on longevity of phorid flies. The results show that female and male *P. tricuspis* are capable of feeding on sucrose solution and that continuous sugar feeding increases their longevity by at least a factor of 2. Under the conditions of the present study, completely starved phorid flies survive on average for only 1 day. Provision of water increases fly longevity by approximately 2 days (compared with completely starved flies) and one full day of sugar feeding further increases longevity by an additional 1–2 days, suggesting that even limited sugar availability can significantly increase adult lifespan of *P. tricuspis*. The data also show that one in four adult

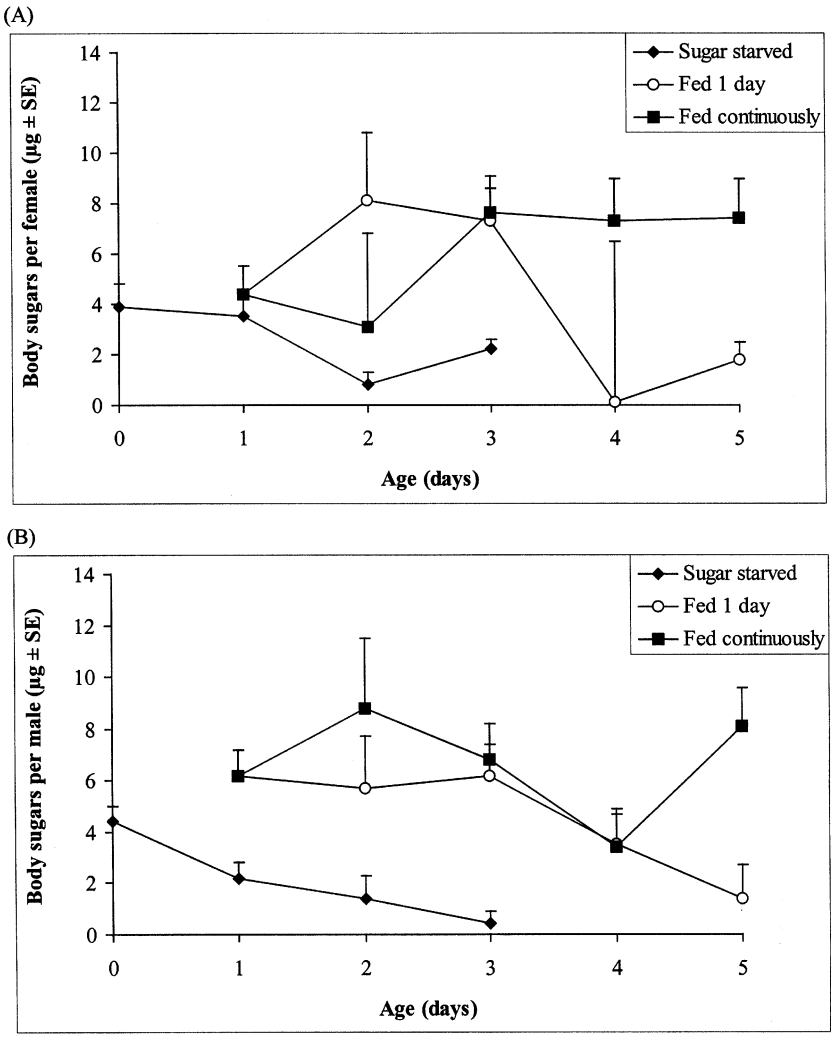

Fig. 3. Amounts (μg ± SE) of body sugars in (A) female and (B) male *Pseudacteon tricuspis* that were either sugar-starved (provided water only), fed 25% sucrose on the first day of life only, or fed sucrose continuously throughout their lifespan.

phorid flies fed sucrose lived for 12 days or more, with certain individuals surviving for over 15 days, suggesting the presence of relatively long-lived individuals in the population. Similar results have been obtained for several hymenopteran parasitoids, although the increase in lifespan was usually more substantial, with longevity typically ranging from 1 to 5 days for starved parasitoids and 2–8 weeks for sugar-fed parasitoids (Hagley & Barber, 1992; Fletcher *et al*., 1994; Heimpel *et al*., 1997; Thompson, 1999; Olson *et al*., 2000; Fadamiro & Heimpel, 2001; Wäckers, 2001). For example, adult *Macrocentrus grandii* have an average longevity of 3 days when provided with only water, 5–7 days when provided with a sugar meal only on the first day of life, and 14–21 days when provided with sugar continuously throughout their lifetime (Fadamiro & Heimpel, 2001). Little is known about the effect of sugar feeding on longevity of dipteran parasitoids. Leeper (1974) investigated the feeding behaviour of the tachinid parasitoid, *Lixophaga sphenophori*, and Topham & Beardsley (1975) reported that adults of this species may feed on nectar in the field. Preliminary results from an ongoing study also suggest that another tachinid parasitoid, *Voria ruralis*, may benefit from nectar in the field (Lee & Heimpel, 2004). In general, adult phorids are presumed to be generalist feeders on plant nectar, sap or honeydew (Morrison, 2000), and there are a few anecdotal reports of adult feeding by phorid flies in general (Disney, 1994; Pesquero *et al*., 1995; Porter, 1998). Not much is known about the lifespan of adult phorids in nature, but adults have been observed to live a week or less in the laboratory (Porter *et al*., 1995, 1997). Pesquero *et al*. (1995) recorded a longevity of up to 5 days for adult phorid flies held in glass vials containing sugar water. Apart from the above studies, the authors are unaware of any previous studies systematically conducted to demonstrate sugar feeding and its impact on lifespan and accumulation of nutrients in dipteran parasitoids in general, let alone phorid flies. The significant effect of sugar feeding on lifespan of *P. tricuspis* recorded in the present study may indicate the possibility of a synovigenic (i.e. emergence with some immature eggs) life-history strategy because species that are strictly pro-ovigenic (i.e. emerge with their lifetime egg complement mature) have characteristically short lifespans and may have little need

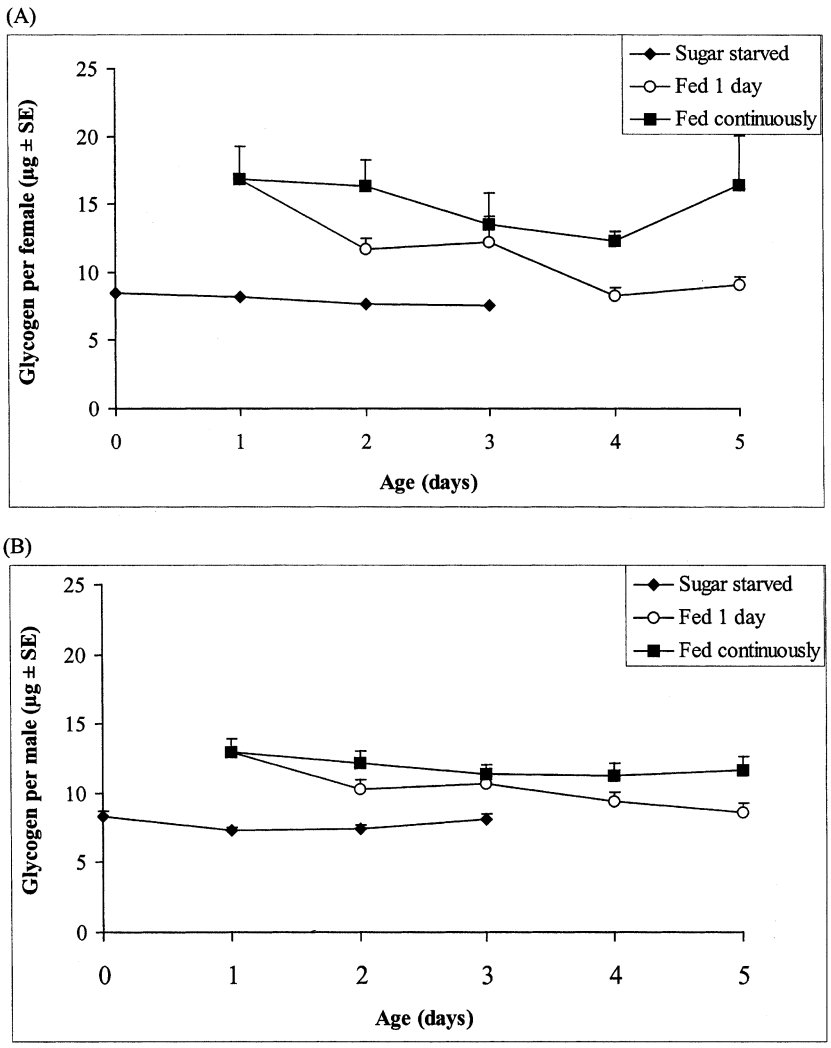

Fig. 4. Amounts (µg ± SE) of glycogen in (A) female and (B) male *Pseudacteon tricuspis* that were either sugar-starved (provided water only), fed 25% sucrose on the first day of life only, or fed sucrose continuously throughout their lifespan.

for sugar feeding in some field conditions (Jervis *et al.*, 2001; Heimpel & Jervis, 2005). Adult female phorids have also been observed feeding on freshly dead insects (Disney, 1994), indicating the possibility of host feeding, a behaviour consistent with synovigenic life-history strategy (Jervis *et al.*, 2001). Although the present study did not investigate the effect of sugar feeding on fecundity of *P. tricuspis*, the increase in lifespan that resulted from sugar feeding should, at the very least, enhance the efficacy of the phorid fly as a biological control agent by allowing the parasitoid more time to locate suitable fire ant hosts (Heimpel & Jervis, 2005). Future studies will determine the life-history strategy and the effect of sugar feeding on the fecundity of *P. tricuspis*.

Phorid fly adults fed 25% sucrose have a similar lifespan to those fed 50% sucrose. This suggests that both sucrose concentrations are within the range suitable for female and male flies. Romeis & Wäckers (2000) reported no increase in the number of *Pieris rapae* responding to sucrose in a 2 M solution compared with a 0.5 M solution. Analysis of the composition of major sugars in the nectars of several flowering plant species common in the southern U.S.A. shows that sugar concentration varies widely in different plants, with most plants having sucrose concentrations in the range of 20–50% (van Handel *et al.*, 1972). This suggests that the sucrose concentration in the nectars of several flowering plants in the region where phorid flies are being released for biological control of imported fire ants is within the range suitable to *P. tricuspis*.

Adult *P. tricuspis* emerge with maximum amount of lipids, low amounts of body sugars and glycogen, and no gut sugars. Although the levels of body sugars and glycogen decline gradually in starved flies, a single day of sugar feeding results in the accumulation of maximum amounts of gut sugars, body sugars and glycogen in female and male flies. Carbohydrate nutrient levels remain high in flies fed sucrose continuously but begin to decline on the first day of starvation in flies fed sucrose only on the first day of life,

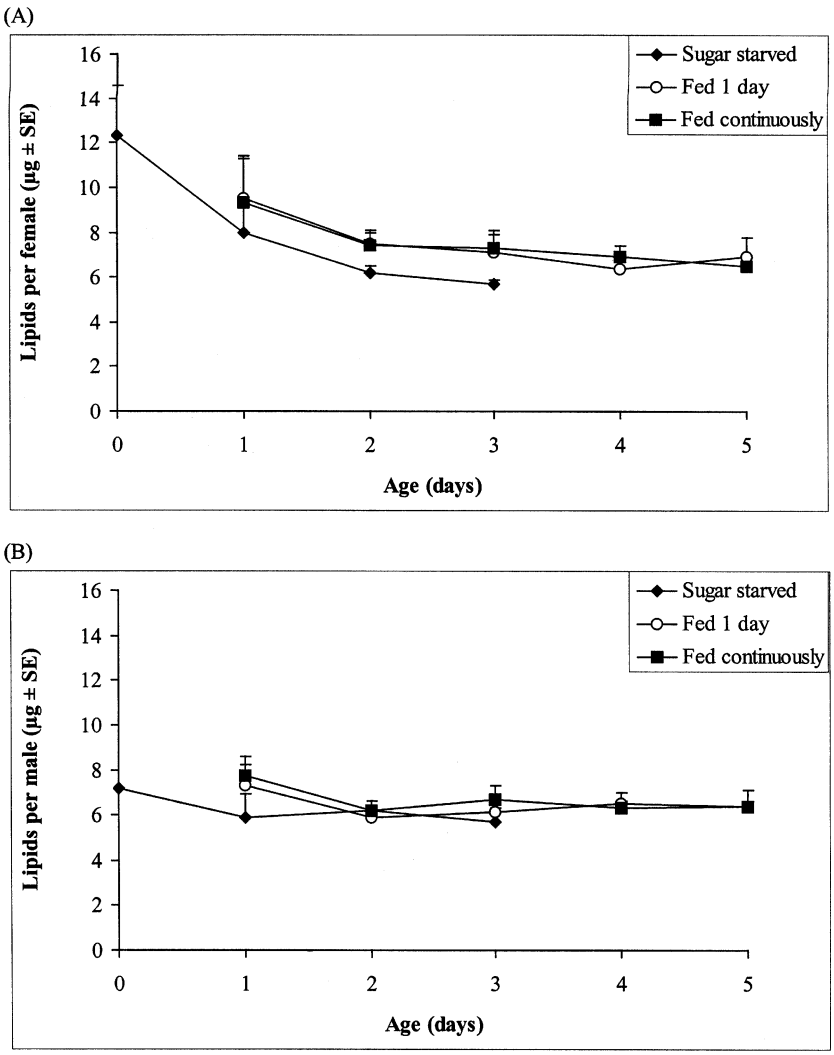

Fig. 5. Amounts (µg ± SE) of lipids in (A) female and (B) male *Pseudacteon tricuspis* that were either sugar-starved (provided water only), fed 25% sucrose on the first day of life only, or fed sucrose continuously throughout their lifespan.

suggesting rapid mobilization of these nutrients during fasting. However, sugar feeding does not result in increased lipid levels, suggesting that phorid flies are capable of rapidly converting dietary sucrose to body sugars and glycogen, but not to lipids. Although several hymenopteran parasitoids have also been shown to synthesize body sugars and glycogen from dietary sucrose, conversion of dietary sugars to lipid is rare among parasitoids (Olson

Table 4. Diet-specific linear-regression analyses testing for the effect of wing length on nutrient amounts in female and male *Pseudacteon tricuspis*.

		Females				Males			
Nutrient	Diet	*P*	r^2	Slope	Intercept	*P*	r^2	Slope	Intercept
Gut sugars	Starved	0.93	–	–	–	0.19	–	–	–
	Fed 1 day	<0.001	0.28	4.56	−3.73	0.14	–	–	–
	Fedcontinuously	0.35	–	–	–	0.002	0.17	3.34	−1.86
Body sugars	Starved	0.053	–	–	–	0.86	–	–	–
	Fed 1 day	0.09	–	–	–	0.09	–	–	–
	Fed continuously	0.02	0.08	2.37	−1.90	0.14	–	–	–
Glycogen	Starved	–	–	–	–	0.001	0.34	3.80	0.23
	Fed 1 day	–	–	–	–	0.17	–	–	–
	Fed continuously	–	–	–	–	0.02	0.09	2.38	0.45

Regression parameters are only reported if *P* for the slope parameter is <0.05.

et al., 2000; Fadamiro & Heimpel, 2001; Giron & Casas, 2003a; Lee *et al*., 2004). By contrast, lipogenesis (conversion of sugar to lipid) is common in many nonparasitoid dipterans (van Handel, 1984; Warburg & Yuval, 1996; Naksathit *et al*., 1999), as well as other insect taxa (Brown & Chippendale, 1974; Downer & Matthews, 1976). Nevertheless, sugar-fed female *P. tricuspis* are able to maintain their teneral lipid levels, whereas lipid levels decline slightly in starved females, probably indicating that lipids are mobilized to some extent by starved females, but not by sugar-fed females and that sucrose-fed flies exclusively use sugars to preserve their lipid reserves (Giron & Casas, 2003a). Similar results in which sugar feeding slows the decline rate of lipids compared with starved adults have been reported for several hymenopteran parasitoids, including *Asobara tabida* (Ellers, 1996), and *Diadegma insulare* (Lee *et al*., 2004), as well nonparasitoid dipterans (Nestel *et al*., 1985; Jacome *et al*., 1995). The reduced rate of decline in lipid levels in sugar-fed females relative to starved females is crucial given that lipid reserves are critical for egg production in insects (Ellers & van Alphen, 1997), and may suggest an additional potential benefit of sugar feeding. Boggs (1997) postulated that sugar nutrients are utilized in preference to stored nutrients when the former are abundant in adult diet.

No significant increase in nutrient accumulation is recorded after the first day of feeding in phorid flies fed sucrose throughout their lifetime. Fadamiro & Heimpel (2001) also recorded maximum levels of gut sugars, body sugars and glycogen from a single day of sugar feeding in *M. grandii*. Similar results have also been reported for other insects in which peak nutrient accumulation occurs from a single day of sugar feeding (Naksathit *et al*., 1999). The apparent lack of increase in the amounts of sugar resources accumulated after 1 day of sugar feeding may indicate a decrease in the amounts of sucrose ingested after the first day, possibly implying that the amounts acquired during a single day are close to the maximum limits of acquisition for each nutrient as determined physiologically or morphologically (van Handel, 1984). An alternative hypothesis that the rate of sugar metabolism increases with age, and balancing the rate of sugar ingestion is unlikely because the flies in this study were in conditions of minimal activity. These data suggest that adult phorid flies do not necessarily need to sugar-feed daily for continuity of life, provided that a single day meal is large enough for accumulation of adequate amounts of nutrients.

Nutrient levels and the patterns of metabolism appear to be slightly different between the two sexes. Female and male *P. tricuspis* emerge with similar amounts of sugar nutrients, but teneral lipid levels are higher in females than in males. Energy utilized during the first day of starvation by males fed sucrose only for 1 day comes mainly from gut sugars, whereas glycogen also is mobilized by females of the same treatment during the first day of starvation. Although glycogen levels decline steadily postfeeding in females fed sucrose only on the first day of life, glycogen is rarely mobilized by starved males, or by males fed sucrose for 1 day only. Lipid reserves decline gradually in starved females, but remain stable in starved males, indicating the possibility that nutrient reserves (lipids and glycogen) may be used by female phorid flies in egg production. Several insects are known to utilize lipids for egg production, including hymenopteran parasitoids (Ellers & van Alphen, 1997; Giron & Casas, 2003b) and nonparasitoid flies (Warburg & Yuval, 1996; Naksathit *et al*., 1999). Generally, sugar-fed males appear to accumulate more gut sugars and body sugars than fed females, whereas fed females accumulate more glycogen than fed males, suggesting the possibility of a higher rate of conversion of dietary sucrose to glycogen reserves by females.

The positive relationship between wing length and the amounts of gut and body sugars in sugar-fed female and male phorid flies, and also with glycogen levels in males, may suggest a higher rate of sugar intake in larger flies, or a relatively higher rate of sugar metabolism in smaller flies. Fadamiro & Heimpel (2001) found no relationship between wasp size and gut sugar amounts, but a positive relationship between size and storage sugars in adult *M. grandii*, and suggested a relatively size-invariant gut volume in the species. Although larger male flies appear to have higher levels of glycogen reserves, no relationship between wing length and lipid amounts is recorded for female and male flies, suggesting that the amount of lipid reserves at emergence is not a function of size. By contrast, a significant relationship between wing length and lipid reserves is observed in the parasitoid wasp, *M. grandii* (Olson *et al*., 2000). Naksathit *et al*. (1999) also recorded a positive effect of size on lipid levels of sucrose fed female mosquitoes with smaller mosquitoes increasing their lipid reserves much sooner than larger mosquitoes. It is not entirely clear why starved adult flies do not significantly mobilize their teneral nutrient reserves for conversion to body sugars, but this may indicate thresholds of minimal irreducible amounts for each nutrient reserve (Rivero & Casas, 1999). Alternatively, it may suggest some physiological problems or energetic costs associated with conversion of reserves to body sugars in starved flies. van Handel (1969) reported that trehalose, the main sugar in insect haemolymph and body sugars (Wyatt, 1967), is not an intermediate in the interconversion between the simple sugars and glycogen. It appears that the interconversion between the different sugars, from monosaccharides to reserves and vice-versa, is a rapid process under the regulation of hormones from the median neurosecretory cells (Nayar & Sauerman, 1975; Clements, 1992).

These results provide an insight into the survival and temporal patterns of nutrient metabolism in the phorid fly *P. tricuspis*. The rapid decline in gut sugar and body sugar levels, and the relative stability in teneral amounts of glycogen and lipid reserves in sugar-starved adult phorid flies over time, may suggest that the moderate amounts of nutrient reserves in teneral female and male phorid flies are not sufficient to maintain life beyond a few days. Accumulation of additional nutrients postemergence is necessary to maintain adequate body sugar levels, and this appears to be vital to the continuity of life in the species.

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