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Lipogenesis in *Nasonia vitripennis*: Influence of sugar chemistry, preferential production of triacylglycerides, and comparison of fatty acid biosynthetic capacity with *Drosophila melanogaster*

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ABSTRACT

Sugar consumption increases the fecundity and longevity in many species of parasitic wasps (parasitoids) but whether these insects use sugars to synthesize significant amounts of fatty acids and storage fat de novo (lipogenesis) is discussed controversially. It has long been assumed that parasitic wasps lost this ability during evolution, mainly because in several species wasps with ad libitum access to sugar did not increase teneral lipid levels. Recent studies demonstrated that many species are nonetheless capable of synthesizing fatty acids de novo from glucose. It is unclear, however, whether also other sugars are used for fatty acid biosynthesis and whether an increase of sugar concentration to levels occurring in natural sugar sources translates into higher fatty acid production. Furthermore, it has been suggested that fatty acid production in parasitoids is negligible compared to species increasing teneral fat reserves such as *Drosophila melanogaster*. Here we show by stable isotope labeling experiments that females of *Nasonia vitripennis* convert D-glucose, D-fructose, sucrose, and α,α-trehalose, major sugars consumed by adult parasitoids in nature, equally well to palmitic, stearic, oleic, and linoleic acid. Lipogenesis from D-galactose occurs as well albeit to a lesser extent. Sugar concentration is crucial for lipogenic activity, and almost 80% of de novo synthesized fatty acids were incorporated into storage fat (triacylglycerides). Comparison of fatty acid biosynthesis within a 48-h feeding period with *D. melanogaster* revealed that *N. vitripennis* produced approximately half as many fatty acids per body mass unit. Both species fed equal amounts of the glucose offered. We conclude that lipogenesis is far from negligible in *N. vitripennis* and plays an important role for the energy balance when teneral lipid reserves deplete.

1. Introduction

Parasitic wasps (parasitoids) form one of the most speciose groups of animals on earth (Forbes et al., 2018) providing significant ecosystem services as natural enemies of other arthropods and biocontrol agents (Losey and Vaughan, 2006). Parasitic wasp females lay their eggs into different stages of other invertebrates, mostly insects, and their offspring develop in or on the host eventually causing its death (Burke and Sharanowski, 2024; Godfray, 1994; Quicke, 1997). As adults, many parasitic wasp females feed on the hemolymph of their host (Jervis and Kidd, 1986) and consume carbohydrate rich liquids such as floral and extrafloral nectar or honeydew (Jervis et al., 1993; Lee et al., 2004; Rose et al., 2006; Wäckers et al., 2008) increasing both longevity and fecundity (Benelli et al., 2017; Harvey et al., 2012; Lee, 2024). The question whether parasitic wasps convert these carbohydrates into fatty acids and storage lipids (lipogenesis) is a controversial subject. In several species, it has been found that, unlike many other insects, newly emerged wasps having ad libitum access to a sugar source do not gain additional lipid mass (Visser et al., 2010). This lack of teneral lipid mass gain after sugar feeding in parasitic wasps has long been thought to be indicative of their general inability to de novo synthesize fatty acids and triacylglycerides (TAG) from sugars (Lammers et al., 2019; Visser et al., 2010, 2012, 2017, 2018). Alternative explanations for the lacking lipid accumulation in parasitic wasps were discussed already in very early studies on this topic. It has been suggested, for instance, that a higher or equal break down of lipids compared to concurrent de novo synthesis would yield the same pattern of lack of lipid accumulation (Visser et al., 2010). Hence, parasitoids may maintain a steady renewal of lipids through lipogenesis that does not translate into lipid accumulation (Giron and Casas, 2003). However, precise methods to investigate this

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alternative explanation were lacking at that time. Recent work revealed furthermore that lipogenesis in the parasitoid *Leptopilina heterotoma* is plastic, and it has been suggested that this might be true for other parasitoid species as well (Visser et al., 2021).

A series of papers has reinvestigated fatty acid biosynthesis in parasitic wasps by a stable isotope labeling approach and found for 17 species that they were all capable of synthesizing fatty acids and derivatives thereof de novo from glucose (Multerer et al., 2022; Prager et al., 2019; Ruther et al., 2021). Subsequently, the original assumption concerning the lost metabolic trait in parasitic wasps has been modified to indicate that parasitic wasps, while being able to synthesize fatty acids, do not accumulate fat with fat accumulation being defined as "bulk storage of fat in the insect body" (Visser et al., 2023). In this context, it is important what is considered as a reference point in studies investigating fatty acid biosynthesis. Many studies on the divergent lipid metabolism in parasitic wasps used the lipid status of newly emerged wasps as a reference (Lammers et al., 2022; Visser et al., 2010, 2012, 2018), i.e., only those wasps "accumulated" fat that further increased their teneral, often already ample fat reserves. This, however, bears the danger of overlooking more subtle processes. It is possible that, while parasitic wasps do not further increase their teneral lipid levels, use de novo biosynthesis to partially replenish ebbing lipid reserves later in their life by de novo biosynthesis (Multerer et al., 2022; Wang et al., 2020). After emergence, many parasitic wasps rapidly lose lipid mass, but this loss is often absent or at least decelerated when access to sugar is enabled (Ellers, 1996; Gündüz et al., 2010; Visser et al., 2010, 2012; Wang et al., 2014). This can be explained by a reduced metabolization of constitutive TAG due to the preferred use of carbohydrates for energy production. However, recent labeling and gene knock-down studies suggest that de novo biosynthesis occurs concurrently and contributes significantly to the fatty acid status of the wasps (Multerer et al., 2022; Wang et al., 2020). A prerequisite to disentangle these concurrent processes is the use of suitable methods for lipid analysis such as stable isotope labeling.

The first studies reinvestigating fatty acid biosynthesis in parasitic wasps were intended as a proof of principle and only used 10% ^{13}C-labeled α-D-glucose solution to feed the insects (Prager et al., 2019; Ruther et al., 2021). This resulted in consistent but relatively low ^{13}C-incorporation and raised the question whether de novo fatty acid biosynthesis is relevant in parasitic wasps (Visser et al., 2023). However, already an increase of glucose concentration to 20% led to significantly higher de novo biosynthesis. Flower nectar has often even higher sugar concentration than 20% with a worldwide median of 40% (Pamminger et al., 2019). It is unknown, however, whether feeding realistic sugar concentrations further increases the degree of de novo fatty acid biosynthesis.

Apart from glucose, other sugars occur in the diet of parasitic wasps such as sucrose, fructose (floral and extrafloral nectar, honeydew) (Fischer et al., 2005; Nicolson, 2022; Shenoy et al., 2012) and trehalose (host hemolymph, honeydew) (Becker et al., 1996; Fischer et al., 2005). These sugars as well as ecologically less relevant ones such as galactose have been investigated with respect their utilization by parasitic wasps. In *Cotesia glomerata*, for instance, trehalose and galactose had weaker effects on the longevity than the typical nectar sugars glucose, fructose, and saccharose (Wäckers, 2001). It is unknown, however, whether sugars other than glucose are equally well suitable for de novo fatty acid biosynthesis. Furthermore, the amounts of sugar consumed by the insects in studies investigating fatty acid biosynthesis in parasitic wasps have hardly ever been quantified.

Another important aspect for the evaluation of the relevance of lipogenesis for the energy balance of parasitic wasps is the question whether they incorporate de novo synthesized fatty acids into storage fat or use them for other purposes. Although it has been shown that newly synthesized fatty acids are used to produce TAG (Ruther et al., 2021), it is not known to what extent this occurs in comparison to other derivatives such as diacylglycerides (DAG) or free fatty acids (FFA).

A recent review on the role of lipogenesis in parasitic wasps has emphasized the significance of suitable positive controls (Visser et al., 2023). Studies investigating the apparent fatty acid biosynthesis in parasitic wasps (Visser et al., 2012) and the underlying molecular mechanisms (Lammers et al., 2019) used insects as positive controls that are adapted to sugar-rich diets and habitats such as *Apis mellifera* and *Drosophila melanogaster*, respectively. Summarizing comparisons of fatty acid biosynthesis in parasitic wasps and *D. melanogaster* made by Visser et al. (2023, Fig. 2 of the cited paper) suggest that de novo fatty acid biosynthesis in parasitic wasps is negligible. Data used in these comparisons, however, originated from experiments involving differing sugar sources and concentrations, feeding times, labeling techniques, and calculation methods. Hence, there is a need for carefully controlled experiments investigating the fatty acid biosynthetic capacity of parasitic wasps and a control insect under comparable conditions with suitable methods.

The model organism *Nasonia vitripennis* (Hymenoptera: Pteromalidae) is one of the species which has long been thought to lack the ability to convert sugar into fatty acids (Lammers et al., 2019; Rivero and West, 2002; Visser et al., 2012). *N. vitripennis* parasitizes the pupae of numerous fly species and is used to study all aspects of parasitoid wasp biology (Mair and Ruther, 2019; Werren et al., 2010). Stable isotope labeling experiments using 10% ^{13}C-labeled α-D-glucose solution revealed that *N. vitripennis* and three other species of the so-called *Nasonia* group (Burks, 2009) incorporated glucose-derived ^{13}C-labeled carbon into fatty acids (Prager et al., 2019). Increase of sugar concentration fostered lipogenesis and de novo synthesized fatty acids were incorporated by *N. vitripennis* females into storage fat and eggs (Multerer et al., 2022; Ruther et al., 2021). In the present study, we use *N. vitripennis* to answer important open questions concerning the significance of de novo fatty acid biosynthesis in parasitic wasps. We investigate whether increasing the sugar concentration to levels found in nectar (Pamminger et al., 2019) further promotes de novo production of fatty acids and whether sugars other than glucose are converted equally well into fatty acid derivatives. Furthermore, we quantify the percentage of de novo synthesized fatty acids being used to produce storage fat (TAG). Finally, we compare the fatty acid biosynthetic capacity of *N. vitripennis* with *D. melanogaster* under standardized conditions and quantify the amounts of selected sugars being ingested by the insects.

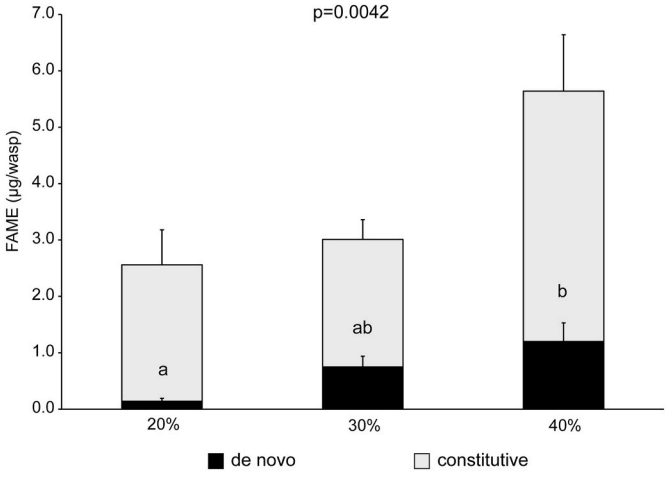

Fig. 1. Impact of glucose concentration on de novo biosynthesis of fatty acids in *Nasonia vitripennis* females. Stacked columns show mean amounts (+SEM) of de novo synthesized (black) and constitutive (grey) amounts of fatty acid methyl esters (FAME). Females had 48-h access to 20–40% solutions of ^{13}C-labeled α-D-glucose. Different lowercase letters indicate significant differences of de novo synthesized FAME at $p < 0.05$ (Kruskal-Wallis test and Bonferroni-corrected Mann-Whitney *U* test ($n = 10$).

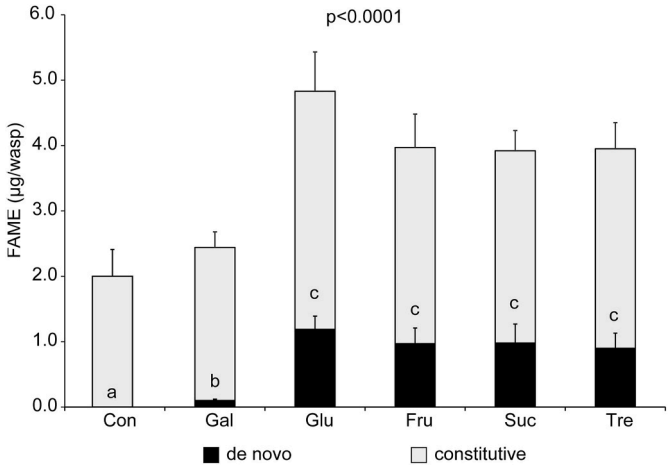

Fig. 2. Impact of sugar chemistry on de novo biosynthesis of fatty acids in *Nasonia vitripennis* females. Stacked columns show mean amounts (+SEM) of de novo synthesized (black) and constitutive (grey) amounts of fatty acid methyl esters (FAME). Females had 48-h access either to water (Con) or 30% solutions of fully ^{13}C-labeled D-galactose (Gal), α-D-glucose (Glu), D-fructose (Fru), sucrose (Suc), or α,α-trehalose (Tre). Different lowercase letters indicate significant differences of de novo synthesized FAME at $p < 0.05$ (Kruskal-Wallis test and Bonferroni-corrected Mann-Whitney *U* test, n = 10).

2. Materials and methods

2.1. Insects

The *N. vitripennis* used in this study were from an inbred strain originally collected from bird nests in northern Germany. Wasps were reared as previously reported (Steiner et al., 2006) on freeze-killed pupae of the green bottle fly, *Lucilia caesar* (Diptera: Calliphoridae). Host larvae were purchased from a commercial supplier (b.t.b.e. Insektenzucht GmbH, Schnürpflingen, Germany). Insect rearing and feeding experiments were performed at 25 °C, 60% relative humidity, and a 16:8 h light-dark regime. Wasps of defined age and mating status were obtained by isolating wasp pupae from parasitized hosts two days before the expected emergence date (generation time at 25 °C is ca. 14–15 d). Newly emerged females were left for two days with a male to mate and were then exposed for two days to unparasitized hosts. Females used for the comparison with *D. melanogaster* (section 2.5) were mated and directly exposed for two days to unparasitized hosts. The purpose of this pretreatment was to allow the females to lay eggs and consume some of their fat reserves prior to the feeding experiment.

D. melanogaster were reared on feeding medium (agar, 0.7%; cornmeal, 6.4%; yeast,1.4%; soymeal, 0.8%; malt extract, 6.4%; molasses, 1.8%, nipagin, 0.2%, and water, 82.3%) at 25 °C, 60% relative humidity, and a 16:8 h light-dark regime. To ensure comparability with *N. vitripennis* in the feeding experiment (section 2.5), mated females were allowed to oviposit on feeding medium for two days before being used in the experiment.

2.2. Influence of glucose concentration on fatty acid biosynthesis in *N. vitripennis*

Females pre-treated as described in section 2.1 were exposed in groups of five in 1.5-ml microcentrifuge tubes for 48 h to 30 µl of 20, 30, and 40% solutions (w/v) of fully ^{13}C-labeled α-D-glucose (Sigma-Aldrich, Taufkirchen, Germany). During the experiment, microcentrifuge tubes were stored horizontally in a climate chamber (25 °C, 16:8 h light-dark regime) with the bottom oriented towards a desk light, to facilitate the wasps' access to the sugar solutions. After the feeding period, residues of the sugar solutions were clearly visible in the vials. Therefore, ad libitum supply of the wasps with sugar solution was assumed during the experiment. In a few cases, wasps drowned in viscous sugar solutions before the end of the feeding experiment. These wasps were excluded from further analysis. After the feeding period, wasps were frozen and kept at −20 °C until being used for fatty acid analysis in sections 2.7 and 2.8.

2.3. Use of other sugars for de novo fatty acid biosynthesis in *N. vitripennis*

Females pretreated as described in section 2.1 were exposed in groups of five in 1.5-ml microcentrifuge tubes for 48 h to 30 µl of 30% solutions of fully ^{13}C-labeled D-fructose (Sigma-Aldrich), D-galactose, sucrose, and α,α-trehalose (Santa Cruz Biotechnology, Heidelberg, Germany). Water-fed control wasps were used as control. Preliminary experiments using unlabeled 30% glucose as a feeding substrate revealed no detectable effects on the abundance of diagnostic ions used in our mass spectrometric analysis (results not shown). Therefore, no control experiments with unlabeled sugars were performed. After the feeding period, wasps were frozen and kept at −20 °C until being used for fatty acid analysis as described in sections 2.7 and 2.8.

2.4. Distribution of de novo synthesized fatty acids in different lipid classes

To investigate the distribution of de novo synthesized fatty acids in TAG, DAG and FFA, we pooled four ^{13}C-glucose fed *N. vitripennis* females (see section 2.2) and extracted their raw lipids with 400 µl dichloromethane as described in section 2.7 (n = 7 pools). The raw extracts were concentrated under nitrogen to approximately 20 µl and separated by size exclusion high performance liquid chromatography (Sperling et al., 2015) on an Agilent 1260 Infinity HPLC system (Agilent Technologies, Waldbronn, Germany). The separation was performed on a 300 × 7.5 mm PLGel-column (particle size 5 µm, pore size 100 Å) using dichloromethane as eluent (1 ml/min). This equipment enables the separation of TAG, DAG, and C_{16}-C_{18} FFA as single chromatographic peaks. The separation was monitored at 230 nm with an Infinity 1260 multi wavelength detector and three fractions (TAG 5.85–6.34 min, DAG 6.36–6.65 min, and FFA 7.65–8.20 min) were collected using an Infinity 1260 fraction collector (Fig. S1 in the electronic supplementary material. The expected retention times of TAG, DAG, and FFA were determined by analyzing synthetic samples of triolein, diolein, and palmitic acid (Sigma-Aldrich). To each fraction, 100 µl dichloromethane containing 3000 ng (TAG) or 750 ng (DAG and FFA) 2-methyloctadecanoic acid (Sigma-Aldrich) were added as an internal standard. Subsequently, the fractions were dried under nitrogen, transesterified and analyzed by GC/MS as described in sections 2.7 and 2.8.

2.5. Comparison of fatty acid biosynthetic capacity between *N. vitripennis* and *D. melanogaster*

To compare the fatty acid biosynthetic capacity of *N. vitripennis* and *D. melanogaster* females, we gave them first the opportunity to lay eggs for two days (see section 2.1) and then fed individual females (*N. vitripennis* n = 23, *D. melanogaster* n = 15) for 48 h 30 µl of a 30% ^{13}C-glucose solution in microcentrifuge tubes. Control females were fed water (*N. vitripennis* n = 18, *D. melanogaster* n = 12). After this feeding period, females were frozen and lipid analysis was performed as described in sections 2.7 and 2.8. Given the different size of the two species, we determined the wet mass (referred to as body mass in the following) of individual wasps and flies with two days oviposition experience (n = 20 for each species) using a Sartorius type SC2 microbalance (range 0.01–2.1 g, readability 0.1 µg, Sartorius, Göttingen, Germany). Prior to weighing, live insects were anesthetized by CO_2. Mean mass values of the two species were used to calculate the de novo synthesized lipid mass and consumed sugar amounts per mg body mass.

2.6. Gravimetric quantification of sugars consumed by the insects

To investigate whether the higher de novo biosynthesis in *D. melanogaster* (see section 3.4) was due to increased sugar ingestion by this species, we fed females of both species (n = 15, pretreated as described in section 2.1) for 48 h with 30 µl of unlabeled 30% glucose solution. Control tubes (n = 15) were equipped with the same amount of glucose solution and treated in the same way without adding insects. To investigate whether the reduced fatty acid biosynthesis from galactose in *N. vitripennis* (see section 3.2) was due to a lower ingestion of this sugar, we performed the same experiment with *N. vitripennis* females offering a 30% solution of galactose. Prior to being used, the lids of all microcentrifuge tubes were perforated four times with a needle to supply the insects with air, and tubes were dried for 1 h at 100 °C. After cooling, tubes were weighed on the Sartorius SC2 microbalance. After the feeding experiment, the remaining sugar solutions in the tubes were freeze-dried overnight, and the tubes were additionally dried for 1 h at 100 °C. After cooling to room temperature, the tubes containing the dry sugar residues were weighed again. The mass of sugar residues in each tube was determined by subtracting the respective tare mass. The mass of sugar residues in the tubes having contained insects was subtracted from the mean mass of sugar in the control tubes to determine the amount of sugar consumed by individual insects. Sugar amounts ingested by individual insects were additionally corrected for body mass.

2.7. Preparation of fatty acid methyl esters (FAME)

After thawing, individual females were carefully homogenized with a Teflon pestle in 1.5 ml microcentrifuge tubes after adding 100 µl dichloromethane containing 0.75 µg of 2-methyloctadecanoic acid as an internal standard. Crushed wasps were extracted at room temperature for 30 min. Subsequently, the extracts were transferred to 1.5-ml glass vials and wasp remains were washed a second time with 100 µl dichloromethane. Combined extracts were dried under a stream of nitrogen. Subsequently 200 µl of methanol and 20 µl of a 10% solution of acetyl chloride (Sigma-Aldrich) in methanol were added to the vials which were then transferred to a dry-block thermostat (VWR International, Darmstadt, Germany). Extracts were transesterified for 2 h at 75 °C. After cooling, 200 µl of a 5%-solution of sodium hydrogen carbonate (Sigma-Aldrich) in deionized water and 200 µl of hexane were added. Vials were vortexed for 10 s to extract the FAME. The hexane phase was concentrated to approximately 20 µl under nitrogen and used for GC/MS analysis.

2.8. GC/MS analysis and calculation of de novo synthesized fatty acids

Chemical analyses were done on a Shimadzu QP2010 SE GC/MS system (Shimadzu GmbH, Duisburg, Germany) equipped with a 60 m × 0.25 mm inner diameter BPX5 capillary column (film thickness 0.25 µm, SGE Analytical Science Europe, Milton Keynes, UK). FAME samples (2 µl) were injected in spitless mode at 300 °C using a Shimadzu AOC 20i auto sampler. The MS was operated in the EI mode at 70 eV, the mass range was m/z 35–350. The ion source was kept at 200 °C. Helium was used as carrier gas at a constant velocity of 30 cm s^{-1}. The temperature program started at 150 °C, increased at 3 °C min^{-1} to 300 °C and was held at this temperature for 15 min. FAME were identified by comparison of retention times and mass spectra with those of authentic reference chemicals (reference mixture of 37 FAME, Sigma-Aldrich). Total amounts of FAME in the *N. vitripennis* samples were determined by integrating the peak areas of the four most abundant FAME palmitic acid methyl ester (C16-ME), stearic acid methyl ester (C18-ME), oleic acid methyl ester (C18-ME:1), and linoleic acid methyl ester (C18-ME:2) in the total ion current chromatograms (TIC) and relating them to the peak area of the internal standard 2-methyloctadecanoic acid methyl ester. For quantification of FAME in *D. melanogaster*, additionally peak areas of lauric acid methyl ester (C12-ME), myristic acid methyl ester (C14-ME), and palmitoleic acid methyl ester (C16-ME:1) were considered which were major components in *D. melanogaster* but only trace components in *N. vitripennis* (Fig. S2 in the electronic supplementary material). In both cases, the selected compounds accounted for >90% of the total FAME in the samples and were therefore considered indicative for the overall fatty acid status of the insects. The percentage of de novo synthesized fatty acids was calculated by using the cluster of fully and partially labeled molecular ions resulting from the varying number of sugar-derived, labeled acetyl-CoA units being incorporated into the fatty acid chain as (Table 1). We did not use the M+2 satellites, because the M+2 ion originating from the naturally occurring ^{18}O in the ester function interfered with the M+2 signal resulting from the incorporation of one labeled acetyl-CoA. By this means, we also excluded labeled C18 fatty acids from the analysis that originated from mere elongation of C16 fatty acids. Hence, each compound containing at least two labeled acetyl-CoA units were considered being synthesized de novo. Extracted ion chromatograms of the diagnostic ions were integrated at the retention times of the respective FAME. Subsequently, percentages of de novo synthesized fatty acids were calculated by relating the sum of peak areas of the isotope cluster ions for each compound to the total peak areas of isotope cluster ions plus unlabeled molecular ion. The amounts of de novo synthesized fatty acids (in µg) were calculated by relating the calculated individual percentages of de novo synthesized C16-ME, C18-ME, C18-ME:1, and C18-ME:2 (*N. vitripennis*) as well as C12-ME, C14:ME, C16-ME, C16-ME:1, C18-ME, C18-ME:1, and C18-ME:2 (*D. melanogaster*), respectively, to the total amounts of the respective FAME obtained by integration of the TIC. The constitutive FAME, i.e. the unlabeled amounts remaining from teneral lipids, were calculated by subtracting the amounts of de novo FAME from the total amounts of FAME determined for each female.

2.9. Statistical analysis

Statistical analysis was performed with PAST 4.03 scientific software (Hammer et al., 2001). Data did not meet the assumptions of parametric testing (test for normality and homogeneity of variances by Shapiro-Wilks test and Levene's test, respectively) and were thus analyzed by non-parametric methods. Percentages of de novo synthesized FAME from ^{13}C and control treatments as well as amounts of constitutive and de novo synthesized FAME in the samples were compared by a Kruskal-Wallis H-test. If this test was significant, individual comparisons were done by Mann-Whitney U-tests. Percentages of de novo synthesized fatty acids in TAG, DAG, and FFA were compared by a Friedman-test followed by Wilcoxon matched pairs test for individual comparisons. In Tests including multiple individual comparisons both uncorrected and Bonferroni-corrected values were calculated. Amounts of sugar consumed by individual females was determined by subtracting the amounts of sugar recovered after the feeding period from the mean amount determined in the control experiment (no wasp feeding). Three negative values resulting from this procedure were interpreted as females that did not feed and thus set 0. Resulting data (both uncorrected and normalized to body mass) were analyzed by a Mann-Whitney *U* test.

3. Results

3.1. Influence of glucose concentration on de novo fatty acid biosynthesis

Females exposed for 48 h to differently concentrated solutions of ^{13}C-labeled α-D-glucose exhibited significantly different amounts of de novo biosynthesized FAME (C16-ME, C18-ME, C18:1-ME, and C18:2-ME, Kruskal-Wallis-test: H = 10.95, p = 0.0042, Fig. 1, Table S1 in the electronic supplementary material). Females fed 30% or 40% glucose solutions showed significantly higher de novo biosynthesis than those fed 20% solution. After Bonferroni-correction, however, the 30%

Table 1
Diagnostic ions (m/z) used to analyze ^{13}C-incorporation from sugars into lauric acid methyl ester (C12-ME), myristic acid methyl ester (C14-ME), palmitoleic acid methyl ester (C16:1-ME), linoleic acid methyl ester (C18:2-ME), oleic acid methyl ester (C18:1-ME) and stearic acid methyl ester (C18-ME). Given are the nominal masses of the unlabeled molecular ion (M) and the masses resulting from the incorporation of 2–9 ^{13}C-labeled acetyl-CoA units (M+4 to M+18). The M+2 ions (incorporation of one acetyl-CoA unit) were not included due to the interference with the ^{18}O-signal of the ester function.

Compound	M	M+4	M+6	M+8	M+10	M+12	M+14	M+16	M+18
C12-ME	214	218	220	222	224	226	–	–	–
C14-ME	242	246	248	250	252	254	256	–	–
C16:1-ME	268	272	274	276	278	280	282	284	–
C16-ME	270	274	276	278	280	282	284	286	–
C18:2-ME	294	298	300	302	304	306	308	310	312
C18:1-ME	296	300	302	304	306	308	310	312	314
C18-ME	298	302	304	306	308	310	312	314	316

treatment was neither significantly different from 20% nor 40%. Irrespective of this procedure, there were no significant differences between the two higher doses. Thus, the feeding experiments with the other sugars were performed with 30% sugar solutions.

3.2. Influence of sugar chemistry on de novo fatty acid biosynthesis

Females exposed for 48 h to 30% solutions of ^{13}C-labeled galactose, glucose, fructose, sucrose, or trehalose exhibited significantly higher de novo biosynthesis of C16-ME, C18-ME, C18:1-ME, and C18:2-ME than water-fed control wasps (total amounts, Kruskal-Wallis test: $H = 39.6$, $p < 0.0001$, Fig. 2; percentage de novo synthesized, Kruskal-Wallis test C16-ME: $H = 36.68$, $p < 0.0001$; C18-ME: $H = 38.45$, $p < 0.0001$; C18:1-ME: $H = 38.52$, $p < 0.0001$; C18:2-ME: $H = 39.23$, $p < 0.0001$, Fig. S3). For all FAME, de novo biosynthesis was detectable after feeding ^{13}C-labeled galactose, but it was significantly lower than for the other sugars, which did not differ from each other. Bonferroni-correction rendered the differences in the relative amounts (percentage de novo synthesized) between galactose and sucrose (C16-ME, C18-ME, C18:1-ME) and fructose (C18-ME), respectively, non-significant (Fig. S3 and Table S2 in the electronic supplementary material).

3.3. Distribution of de novo synthesized fatty acids in different lipid classes

HPLC analysis of raw lipids revealed that 78.3 ± 3.0%, 2.9 ± 0.3%, and 18.8 ± 2.8% (mean ± SEM) of de novo synthesized fatty acids were present in the TAG-, DAG-, and FFA-fraction, respectively (Fig. 3). These differences were statistically significant (Friedman-ANOVA: $Chi^2 = 14$, $p < 0.0001$, Wilcoxon: TAG vs. DAG, TAG vs. FFA, and DAG vs. FFA $p = 0.02326$). Due to the low sample size ($n = 7$), Bonferroni-correction rendered individual comparisons non-significant ($p = 0.0698$).

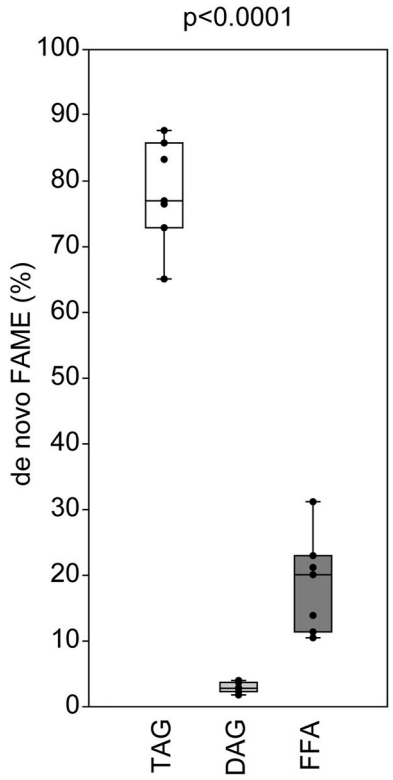

Fig. 3. Percentage distribution of fatty acids de novo synthesized from ^{13}C-α-D-glucose-fed *Nasonia vitripennis* females in the triacylglyceride (TAG), diacylglyceride (DAG) and free fatty acid (FFA) fraction of extracted raw lipids (Friedman-test, $n = 7$).

3.4. Comparison of fatty acid biosynthetic capacity between N. vitripennis and D. melanogaster

Diagnostic ions indicating the incorporation of ^{13}C-labeled acetyl-CoA units into FAME were detectable in all samples from ^{13}C-glucose-fed *N. vitripennis* and *D. melanogaster* but absent in the extracted ion chromatograms from water-fed control insects (Figs. S4a–d in the electronic supplementary material). However, the fatty acid composition of *D. melanogaster* and *N. vitripennis* females differed in that *D. melanogaster* produced significant amounts of lauric acid, myristic acid, and palmitoleic acid which were only trace components in *N. vitripennis* (Fig. S2 in the electronic supplementary material). On the other hand, de novo production of C18:2-ME occurred only in *N. vitripennis* (Figs. 4 and 5). Hence, statistical comparison between the two species with respect to the percentages of de novo synthesized fatty acids were done only for C16-ME, C18:1-ME, and C18-ME which were major components in both species. After the 48-h feeding period, 49.9 ± 3.2%, 56.3 ± 2.6%, and 30.8 ± 1.8% of C12-ME, C14-ME, and C16:1-ME, respectively, had been synthesized de novo from ^{13}C-glucose by *D. melanogaster* females (Fig. 4). Furthermore, the percentage of de novo synthesized C16-ME was significantly higher in *D. melanogaster* (38.3 ± 1.8%) than in *N. vitripennis* (11.9 ± 2.2%, Mann-Whitney: $U = 18$, $p < 0.0001$). In contrast, only *N. vitripennis* synthesized C18:2-ME (10.1 ± 1.7%) while in *D. melanogaster* C18:2-ME was present but not synthesized de novo. The percentage of de novo synthesized C18:1-ME (*D. melanogaster*: 11.9 ± 0.6, *N. vitripennis*: 23.5 ± 3.9%; Mann-Whitney: $U = 124{,}5$, $p = 0.1558$) and C18-ME (*D. melanogaster*: 24.2 ± 1.6, *N. vitripennis*: 29.1 ± 2.7%; Mann-Whitney: $U = 146$, $p = 0.4375$) did not differ between the two species. The mean body mass of *D. melanogaster* females (1.166 ± 0.043 mg) was almost twice as high as of *N. vitripennis* (0.632 ± 0.041 mg; Mann-Whitney: $U = 0$, $p < 0.0001$). These values were used to calculate the absolute amounts of de novo synthesized FAME per mg body mass. Accordingly, *D. melanogaster* synthesized significantly higher amounts of C12-ME, C14-ME, C16:1-ME (trace components in *N. vitripennis*, not analyzed), and C16-ME, while *N. vitripennis* produced higher amounts of C18:2-ME, C18:1-ME, and C18-ME (Fig. 5, Table S3 in the electronic supplementary material). As for the total amount of de

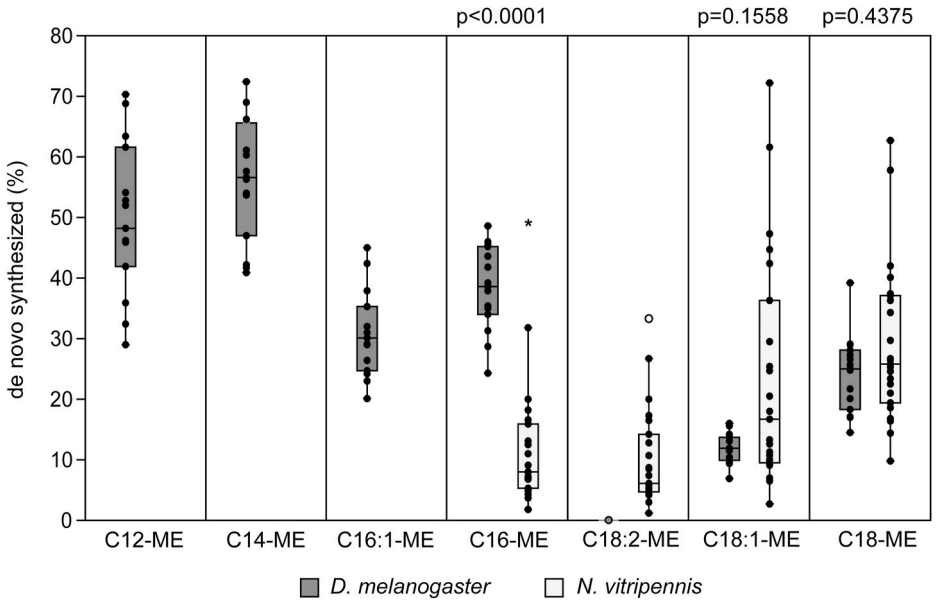

Fig. 4. Comparison of fatty acid biosynthetic capacity of *Nasonia vitripennis* and *Drosophila melanogaster* females. Shown is the calculated percentage of de novo biosynthesized lauric acid methyl ester (C12-ME), myristic acid methyl ester (C14-ME), palmitoleic acid methyl ester (C16:1-ME), palmitic acid methyl ester (C16-ME), linoleic acid methyl ester (C18:2-ME), oleic acid methyl ester (C18:1-ME) and stearic acid methyl ester (C18-ME). Females had 48-h access to 30% solutions of fully ^{13}C-labeled α-D-glucose. De novo biosynthesis of C12-ME, C14-ME and C16:1-ME were not calculated for *N. vitripennis*, because these compounds were absent or trace components in this species (Fig. S2 in the electronic supplementary material). Comparison for C18:2-ME was not done, because *D. melanogaster* does not produce it. Box-and-whisker plots show median (horizontal line), 25–75% quartiles (box), maximum/minimum range (whiskers) and outliers (° > 1.5 x upper quartile; * > 3 x upper quartile). Statistical analysis by Mann-Whitney *U* test (n = 23 for *N. vitripennis* and n = 15 for *D. melanogaster*).

novo synthesized FAME, *N. vitripennis* females synthesized approximately half the amount (4.9 ± 1.3 μg/mg body mass) produced by *D. melanogaster* females (10.0 ± 1.4 μg/mg body mass; Mann-Whitney: U = 69, p = 0.0021). In both species, 48 h glucose feeding resulted in significantly higher constitutive amounts of FAME (*D. melanogaster*: 19.5 ± 2.1 μg/mg body mass; *N. vitripennis*: 22.5 ± 3.2 μg/mg body mass) when compared to the water-fed controls (*D. melanogaster*: 6.0 ± 0.9 μg/mg body mass; Mann-Whitney: U 12 = , p = 0.0002; *N. vitripennis*: 13.3 ± 2.5 μg/mg body mass; Mann-Whitney: U = 113, p = 0.0140; Fig. 5, Table S3 in the electronic supplementary material). The differences between these values in the two species (*D. melanogaster*: 13.5 μg/mg body mass, *N. vitripennis*: 9.2 μg/mg body mass) allow a rough estimation of the effect that the decelerated metabolization of teneral lipids has on the lipid status of both species.

3.5. Quantification of consumed sugars

After the 48-h feeding experiment, the control tubes without insects contained 9.279 ± 0.066 mg (mean ± SEM) glucose. Feeding by both, *N. vitripennis* (8.789 ± 0.170 mg) and *D. melanogaster* (8.702 ± 0.068 mg) females resulted in significantly decreased amounts of glucose (Kruskal-Wallis: H = 20,71, p < 0.0001; Mann-Whitney: *N. vitripennis* vs. control, U = 28, p < 0.001; *D. melanogaster* vs. control, U = 9, p < 0.0001; between species, U = 105, p = 0.7716). Hence there was no significant difference in the average amount of sugar consumed by both species, but there was a large variation in individual quantity consumed (Fig. 6). Control tubes without insects contained after 48 h 9.419 ± 0.083 mg galactose. This amount was significantly decreased by feeding *N. vitripennis* females (8.789 ± 0.170 mg, Mann-Whitney: U = 43, p = 0.0042)). The amounts of glucose and galactose consumed by *N. vitripennis* females were not significantly different (Mann-Whitney: U = 108, p = 0.8682).

4. Discussion

Investigating the model organism *N. vitripennis*, the present study provides additional evidence for the biological significance of de novo fatty acid biosynthesis in parasitic wasps. About 10 % (C18:2-ME) to 40 % (C18:1-ME) of the available fatty acid derivatives were synthesized de novo by females that had consumed parts of their teneral lipid reserves and were exposed to field-realistic sugar concentrations for 48 h. All sugars dominating in naturally occurring carbohydrate sources such as nectar, honeydew, and host hemolymph (Becker et al., 1996; Nicolson, 2022; Nicolson and Thornburg, 2007), i.e., sucrose, glucose, fructose, and trehalose were equally well metabolized. Even galactose, a monosaccharide rarely reported in sugar sources used by parasitic wasps (Nicolson, 2022; Nicolson and Thornburg, 2007; Wäckers, 2001), was converted by *N. vitripennis* females into fatty acids albeit to a lesser extent. Almost 80% of the newly synthesized fatty acids were used to produce storage fat (TAG). A comparison of the fatty acid biosynthetic capacity between *N. vitripennis* and *D. melanogaster* females with a similar pre-feeding history showed that wasps produced about half the amounts of fatty acids per mg body mass as the flies, while ingesting approximately the same amount of the sugar solution offered. Thus, *D. melanogaster* has in fact a higher efficacy for de novo biosynthesis of fatty acids (Visser et al., 2023). The average amounts of glucose consumed by *N. vitripennis* (0.836 mg/mg body mass) and *D. melanogaster* (0.493 mg/mg body mass) in our experiments can theoretically be converted to 314 μg and 185 μg C16-ME, respectively. Thus, only approximately 1.6% and 5.4% of the glucose consumed by *N. vitripennis* and *D. melanogaster*, respectively, was utilized for de novo biosynthesis. Our analytical approach allowed us to disentangle the effects of sugar feeding on de novo biosynthesis of fatty acids and decelerated lipid metabolization and demonstrates that both processes occur simultaneously in either species. The amounts of constitutive lipids saved by the insects when having access to sugar were in a similar range to the amounts synthesized de novo. This suggests that both species, when supplied with sugar ad libitum in the lab, utilize considerable proportions of the carbohydrates for other purposes or excrete them unutilized. This deserves further research in the future. *D. melanogaster* produced higher amounts of shorter-chain fatty acids (C12-C16) while *N. vitripennis* produced mainly C18 fatty acids. This emphasizes that

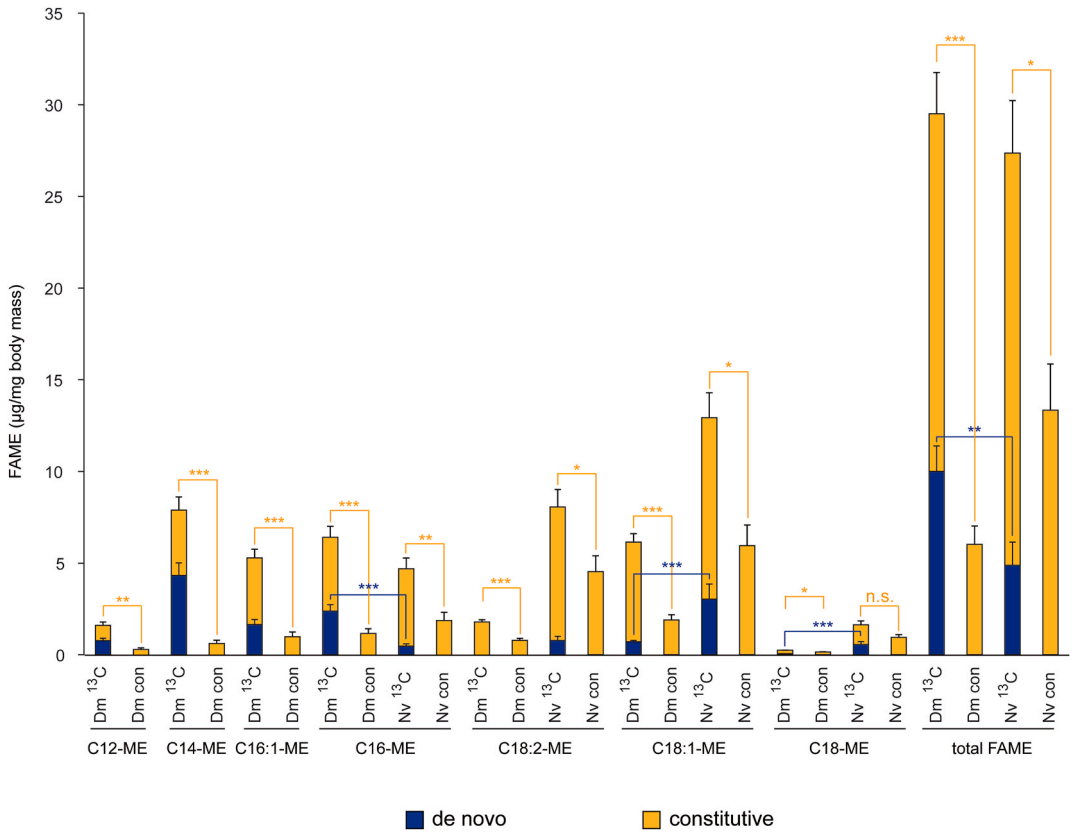

Fig. 5. Comparison of fatty acid biosynthetic capacity of *Nasonia vitripennis* (Nv) and *Drosophila melanogaster* (Dm) females. Stacked columns show mean amounts (+SEM) of de novo synthesized (blue) and constitutive (orange) amounts of lauric acid methyl ester (C12-ME), myristic acid methyl ester (C14-ME), palmitoleic acid methyl ester (C16:1-ME), palmitic acid methyl ester (C16-ME), linoleic acid methyl ester (C18:2-ME), oleic acid methyl ester (C18:1-ME) and stearic acid methyl ester (C18-ME) per mg insect body mass. Females had 48-h access to 30% solutions of fully ^{13}C-labeled α-D-glucose (n = 23 for *N. vitripennis* and n = 15 for *D. melanogaster*) or water (con, n = 18 for *N. vitripennis* and n = 12 for *D. melanogaster*). De novo biosynthesis of C12-ME, C14-ME and C16:1-ME were not calculated for *N. vitripennis*, because these compounds were absent or trace components in this species (Fig. S2 in the electronic supplementary material). Comparison for C18:2 was not done, because *D. melanogaster* does not produce it. Asterisks indicate statistical differences between columns of the same color (*** = $p < 0.001$, ** = $p < 0.01$, *$p < 0.05$), statistical analysis by Mann-Whitney *U* test.

comparison of the fatty acid biosynthetic capacity of the two species needs to consider all major fatty acids and should not rely on palmitic acid only (Visser et al., 2023).

Interestingly, *N. vitripennis* can produce fatty acids from galactose, a monosaccharide rarely reported in nectar or honeydew. De novo synthesis from galactose, however, was lower than from the ecologically more relevant sugars glucose, sucrose, fructose, and trehalose. This difference is not due to decreased galactose ingestion, because *N. vitripennis* females ingested equal amounts of galactose and glucose in our feeding experiment. Galactose is metabolized after epimerization to glucose within the Leloir pathway (Frey, 1996). This conversion appears to limit the use of galactose by *N. vitripennis*, although genes coding all three enzymes involved (galactokinase, UDP-galactose 4-epimerase (=UDP-glucose 4-epimerase), and galactose-1-phosphate uridylyl transferase) (Frey, 1996) are expressed in *N. vitripennis* (Wang et al., 2015). There are three gustatory receptors in the genome of *N. vitripennis* that are orthologues of the *A. mellifera* sugar receptors AmGR1, AmGR2, and AmGR3 (Robertson et al., 2010). Interestingly, neither of these receptors or a combination of them is sensitive to galactose in *A. mellifera* (Değirmenci et al., 2023). Furthermore, galactose did not trigger a feeding response in the parasitic wasp *Cotesia glomerata* (Hymenoptera: Braconidae) but just as little deterred water-deprived wasps from drinking (Wäckers, 1999). Unlike glucose, fructose, and sucrose, ingestion of galactose prolonged the lifetime of *C. glomerata* only marginally (Wäckers, 2001). These results raise the question, whether galactose ingestion in *N. vitripennis* occurred following stimulation of gustatory receptors or was merely a side effect of water ingestion.

The results of the present study together with the finding that sugar-fed *N. vitripennis* females produce more eggs and incorporate de novo synthesized fatty acids into their eggs (Multerer et al., 2022), suggest that de novo synthesis of fatty acids from sugar is a fitness-relevant process even if this species does not increase teneral lipid levels upon sugar feeding but shows a continuous decrease of lipids in the course of its lifetime (Rivero and West, 2002; Ruther et al., 2021; Visser et al., 2012). In the braconid wasp *Meteorus pulchricornis*, a species also losing lipid mass in the adult stage despite access to carbohydrates, honey feeding resulted in an upregulation of four fatty acid synthase (*fas*) genes. Knock-down of *fas* genes resulted in a significant decline of lipid content at the adult stage. (Wang et al., 2020). Six *fas* genes (*fas1-6*) have been identified in the genome of *N. vitripennis* with *fas5* being involved in the biosynthesis of cuticular hydrocarbons (Sun et al., 2023). Hence, gene knock-down experiments with the remaining *fas* genes are a promising approach to further investigate the fitness relevance of fatty acid biosynthesis in *N. vitripennis*.

Another important question is when sugar feeding becomes relevant in *N. vitripennis*. In order to mature their eggs, females of synovigenic species such as *N. vitripennis* (Rivero and West, 2002) are dependent on feeding prior to oviposition (Jervis et al., 2001). It is therefore assumed that females of many species visit flowers to feed nectar before foraging for oviposition sites (Jervis et al., 1993; Russell, 2015). In *N. vitripennis*, nutrients taken up by host feeding are sufficient for egg maturation (Rivero and West, 2005) and thus, they do not depend on nectar uptake but can start host search immediately after mating. This is supported by their olfactory preferences. Newly emerged, virgin females of

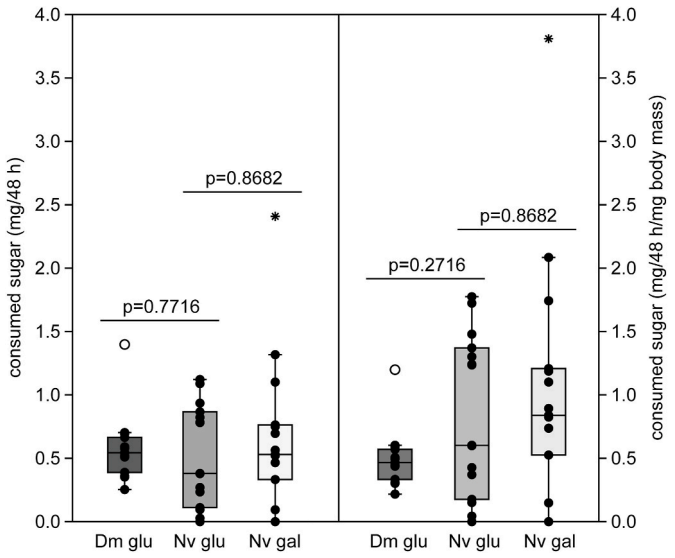

Fig. 6. Amounts of glucose ingested by *N. vitripennis* (Nv) and *Drosophila melanogaster* (Dm) females and amounts of galactose consumed by Nv females (left panel) and the same values corrected for body mass (right panel). Females had 48-h access to 30 µl of 30% sugar solutions, and sugar residues were determined gravimetrically. Box-and-whisker plots show median (horizontal line), 25–75% quartiles (box), maximum/minimum range (whiskers) and outliers (° > 1.5 x upper quartile; * > 3 x upper quartile). Statistical analysis by Mann-Whitney *U* test (n = 20 for each species).

N. vitripennis prefer the male pheromone over host odor, but olfactory preferences switch immediately after mating (Steiner and Ruther, 2009). This switch is accompanied by an increased locomotor and flight activity causing a rapid dispersal from the natal patch (King et al., 2000; Ruther et al., 2014). This suggests that the primary motivation of newly emerged *N. vitripennis* females is mating and oviposition and that the search for carbohydrates occurs rather random during host finding or after oviposition. It is therefore rather unlikely that newly emerged females have ad libitum access to sugar in natural habitats and further increase their teneral lipid reserves by lipogenesis. Rather, it can be assumed that they only utilize this metabolic pathway after they have used up some of their teneral fat reserves.

N. vitripennis emerges with approximately 16% fat (related to the dry mass) (Visser et al., 2012). An early study on the lipid metabolism of parasitic wasps compared the lipid levels of parasitic wasps and other insects at emergence and after several days of feeding (Visser et al., 2010). A review of the data in the light of today's knowledge reveals an interesting trend: Several parasitoids emerging with low levels of teneral fat reserves (<10% of the dry mass) increased lipid mass when having access to a sugar source while especially those emerging with high teneral fat reserves showed significant losses. This observation deserves further investigation considering also phylogenetic relationships. Furthermore, *L. heterotoma* shows a plastic fat accumulation depending on the lipid status of the host they developed in (Visser et al., 2021). This suggests that parasitic wasps use lipogenesis whenever they need to, i.e., when lipid reserves fall below a certain level, be it, because they emerge with low fat reserves or, like *N. vitripennis*, after having partially consumed teneral fat reserves. We therefore argue that in future studies researchers should free themselves from the paradigm that lipogenesis is only biologically relevant if ad libitum access of newly emerged parasitic wasps to carbohydrates leads to an increase in teneral fat reserves.

CRediT authorship contribution statement

Joachim Ruther: Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Julian Hoheneder:** Writing – review & editing, Investigation, Formal analysis. **Vera Koschany:** Writing – review & editing, Investigation, Formal analysis.

Data availability

All data are available in the electronic supplementary material.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibmb.2024.104179.

References

Becker, A., Schloder, P., Steele, J.E., Wegener, G., 1996. The regulation of trehalose metabolism in insects. Experientia 52, 433–439.
Benelli, G., Giunti, G., Tena, A., Desneux, N., Caselli, A., Canale, A., 2017. The impact of adult diet on parasitoid reproductive performance. J. Pest. Sci. 90, 807–823.
Burke, G.R., Sharanowski, B.J., 2024. Parasitoid wasps. Curr. Biol. 34, R483–R488.
Burks, R.A., 2009. Phylogenetics of Pteromalidae and Eulophidae (Hymenoptera: Chalcidoidea) with a Study of Cranial Bridges in Chalcidoidea. University of California Riverside, Riverside.
Değirmenci, L., Rogé Ferreira, F.L., Vukosavljevic, A., Heindl, C., Keller, A., Geiger, D., Scheiner, R., 2023. Sugar perception in honeybees. Front. Physiol. 13, 1089669.
Ellers, J., 1996. Fat and eggs: an alternative method to measure the trade-off between survival and reproduction in insect parasitoids. Neth. J. Zool. 46, 227–235.
Fischer, M.K., Völkl, W., Hoffmann, K.H., 2005. Honeydew production and honeydew sugar composition of polyphagous black bean aphid, *Aphis fabae* (Hemiptera: Aphididae) on various host plants and implications for ant-attendance. Eur. J. Entomol. 102, 155–160.
Forbes, A.A., Bagley, R.K., Beer, M.A., Hippee, A.C., Widmayer, H.A., 2018. Quantifying the unquantifiable: why Hymenoptera, not Coleoptera, is the most speciose animal order. BMC Ecol. 18, 21.
Frey, P.A., 1996. The Leloir pathway: a mechanistic imperative for three enzymes to change the stereochemical configuration of a single carbon in galactose. Faseb. J. 10, 461–470.
Giron, D., Casas, J., 2003. Lipogenesis in an adult parasitic wasp. J. Insect Physiol. 49, 141–147.
Godfray, H.C.J., 1994. Parasitoids, 1. Princeton University Press, Chichester.
Gündüz, E.A., Gülel, A., Isitan, Ö., Boz, A., Cesur, Ö., 2010. Effects of sugar feeding on lipid, glycogen, and total sugar levels of a female parasitoid, *Bracon hebetor* (Say) (Hymenoptera: Braconidae). Turk. J. Agric. For. 34, 343–347.
Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 4.
Harvey, J.A., Cloutier, J., Visser, B., Ellers, J., Wäckers, F.L., Gols, R., 2012. The effect of different dietary sugars and honey on longevity and fecundity in two hyperparasitoid wasps. J. Insect Physiol. 58, 816–823.
Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A., Kidd, N.A.C., 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. J. Anim. Ecol. 70, 442–458.
Jervis, M.A., Kidd, N.A.C., 1986. Host-feeding strategies in hymenopteran parasitoids. Biol. Rev. 61, 395–434.
Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T., Dawah, H.A., 1993. Flower-visiting by hymenopteran parasitoids. J. Nat. Hist. 27, 67–105.
King, B.H., Grimm, K.M., Reno, H.E., 2000. Effects of mating on female locomotor activity in the parasitoid wasp *Nasonia vitripennis* (Hymenoptera : Pteromalidae). Environ. Entomol. 29, 927–933.
Lammers, M., Kraaijeveld, K., Ellers, J., 2022. Lack of lipid accumulation in two species of chalcidoid wasps with secondarily evolved phytophagy. Entomol. Exp. Appl. 170, 460–467.
Lammers, M., Kraaijeveld, K., Marien, J., Ellers, J., 2019. Gene expression changes associated with the evolutionary loss of a metabolic trait: lack of lipogenesis in parasitoids. BMC Genom. 20, 309.
Lee, J.C., 2024. Flourishing with sugars - following the fate of parasitoids in the field. Curr. Opin. Ins. Sci. 61, 101158.
Lee, J.C., Heimpel, G.E., Leibee, G.L., 2004. Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp. Entomol. Exp. Appl. 111, 189–199.
Losey, J.E., Vaughan, M., 2006. The economic value of ecological services provided by insects. Bioscience 56, 311–323.
Mair, M.M., Ruther, J., 2019. Chemical ecology of the parasitoid wasp genus *Nasonia* (Hymenoptera, Pteromalidae). Front. Ecol. Evol. 7, 184.
Multerer, M.-T., Wendler, M., Ruther, J., 2022. The biological significance of lipogenesis in *Nasonia vitripennis*. Proc. R. Soc. B-Biol. Sci. 289, 20220208.

Nicolson, S.W., 2022. Sweet solutions: nectar chemistry and quality. Phil. Trans. Biol. Sci. 377, 20210163.
Nicolson, S.W., Thornburg, R.W., 2007. Nectar chemistry. In: Nicolson, S.W., Nepi, M., Pacini, E. (Eds.), Nectaries and Nectar. Springer, Netherlands, Dordrecht, pp. 215–264.
Pamminger, T., Becker, R., Himmelreich, S., Schneider, C.W., Bergtold, M., 2019. The nectar report: quantitative review of nectar sugar concentrations offered by bee visited flowers in agricultural and non-agricultural landscapes. PeerJ 7, e6329.
Prager, L., Bruckmann, A., Ruther, J., 2019. De novo biosynthesis of fatty acids from α-D-glucose in parasitoid wasps of the *Nasonia* group. Insect Biochem. Mol. Biol. 115, 103256.
Quicke, D.L.J., 1997. Parasitic Wasps, 1. Chapman & Hall, London.
Rivero, A., West, S.A., 2002. The physiological costs of being small in a parasitic wasp. Evol. Ecol. Res. 4, 407–420.
Rivero, A., West, S.A., 2005. The costs and benefits of host feeding in parasitoids. Anim. Behav. 69, 1293–1301.
Robertson, H.M., Gadau, J., Wanner, K.W., 2010. The insect chemoreceptor superfamily of the parasitoid jewel wasp *Nasonia vitripennis*. Insect Mol. Biol. 19, 121–136.
Rose, U.S.R., Lewis, J., Tumlinson, J.H., 2006. Extrafloral nectar from cotton (*Gossypium hirsutum*) as a food source for parasitic wasps. Funct. Ecol. 20, 67–74.
Russell, M., 2015. A meta-analysis of physiological and behavioral responses of parasitoid wasps to flowers of individual plant species. Biol. Control 82, 96–103.
Ruther, J., McCaw, J., Böcher, L., Pothmann, D., Putz, I., 2014. Pheromone diversification and age-dependent behavioural plasticity decrease interspecific mating costs in *Nasonia*. PLoS One 9, e89214.
Ruther, J., Prager, L., Pokorny, T., 2021. Parasitic wasps do not lack lipogenesis. Proc. R. Soc. B-Biol. Sci. 288, 20210548.
Shenoy, M., Radhika, V., Satish, S., Borges, R.M., 2012. Composition of extrafloral nectar influences interactions between the Myrmecophyte *Humboldtia brunonis* and its ant associates. J. Chem. Ecol. 38, 88–99.
Sperling, S., Kühbandner, S., Engel, K.C., Steiger, S., Stökl, J., Ruther, J., 2015. Size exclusion high performance liquid chromatography: re-discovery of a rapid and versatile method for clean-up and fractionation in Chemical Ecology. J. Chem. Ecol. 41, 574–583.
Steiner, S., Hermann, N., Ruther, J., 2006. Characterization of a female-produced courtship pheromone in the parasitoid *Nasonia vitripennis*. J. Chem. Ecol. 32, 1687–1702.
Steiner, S., Ruther, J., 2009. How important is sex for females of a haplodiploid species under local mate competition? Behav. Ecol. 20, 570–574.
Sun, W., Lange, M.I., Gadau, J., Buellesbach, J., 2023. Decoding the genetic and chemical basis of sexual attractiveness in parasitic wasps. Elife 12, e86182.
Visser, B., et al., 2021. Phenotypic plasticity explains apparent reverse evolution of fat synthesis in parasitic wasps. Sci. Rep. 11, 7751.
Visser, B., Hance, T., Noel, C., Pels, C., Kimura, M.T., Stokl, J., Geuverink, E., Nieberding, C.M., 2018. Variation in lipid synthesis, but genetic homogeneity, among *Leptopilina* parasitic wasp populations. Ecol. Evol. 8, 7355–7364.
Visser, B., Le Lann, C., den Blanken, F.J., Harvey, J.A., van Alphen, J.J.M., Ellers, J., 2010. Loss of lipid synthesis as an evolutionary consequence of a parasitic lifestyle. Proc. Natl. Acad. Sci. USA 107, 8677–8682.
Visser, B., et al., 2023. Many parasitoids lack adult fat accumulation, despite fatty acid synthesis: a discussion of concepts and considerations for future research. Curr. Res. Insect. Sci. 3, 100055.
Visser, B., Roelofs, D., Hahn, D.A., Teal, P.E.A., Mariën, J., Ellers, J., 2012. Transcriptional changes associated with lack of lipid synthesis in parasitoids. Genome Biol. Evol. 4, 864–874.
Visser, B., Willett, D.S., Harvey, J.A., Alborn, H.T., 2017. Concurrence in the ability for lipid synthesis between life stages in insects. R. Soc. Open Sci. 4, 160815.
Wäckers, F.L., 1999. Gustatory response by the hymenopteran parasitoid *Cotesia glomerata* to a range of nectar and honeydew sugars. J. Chem. Ecol. 25, 2863–2877.
Wäckers, F.L., 2001. A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. J. Insect Physiol. 47, 1077–1084.
Wäckers, F.L., van Rijn, P.C.J., Heimpel, G.E., 2008. Honeydew as a food source for natural enemies: making the best of a bad meal? Biol. Control 45, 176–184.
Wang, J., Shen, L.W., Xing, X.R., Xie, Y.Q., Li, Y.J.C., Liu, Z.X., Wang, J., Wu, F.A., Sheng, S., 2020. Lipid dynamics, identification, and expression patterns of fatty acid synthase genes in an endoparasitoid, *Meteorus pulchricornis* (Hymenoptera: Braconidae). Int. J. Mol. Sci. 21, 6228.
Wang, W., Lu, S.L., Liu, W.X., Cheng, L.S., Zhang, Y.B., Wan, F.H., 2014. Effects of five naturally occurring sugars on the longevity, oogenesis, and nutrient accumulation pattern in adult females of the synovigenic parasitoid *Neochrysocharis formosa* (Hymenoptera: eulophidae). Neotrop. Entomol. 43, 564–573.
Wang, X., Werren, J.H., Clark, A.G., 2015. Genetic and epigenetic architecture of sex-biased expression in the jewel wasps *Nasonia vitripennis* and *girault*i. Proc. Natl. Acad. Sci. USA 112, E3545–E3554.
Werren, J.H., et al., 2010. Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. Science 327, 343–348.