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De novo biosynthesis of fatty acids from α-D-glucose in parasitoid wasps of the *Nasonia* group

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ABSTRACT

Fatty acids are indispensable primary metabolites for virtually any organism on earth and thus enzymatic machinery enabling de novo production of fatty acids from carbohydrates is highly conserved. A series of studies has questioned the ubiquity of lipogenesis in parasitoid wasps suggesting that the vast majority of species have lost the ability to synthesize fatty acids de novo. One such species is *Nasonia vitripennis*, which, like the congeneric species *N. giraulti* and *N. longicornis*, uses a fatty acid-derived male sex pheromone for sexual communication. Here we demonstrate by feeding fully ^{13}C-labeled α-D-glucose and analyzing insect-derived fatty acid methyl esters and the male sex pheromone by coupled gas chromatography/mass spectrometry that both males and females of *N. vitripennis* as well as *N. giraulti* and *N. longicornis* are capable of synthesizing fatty acids de novo. We furthermore show by a proteomics approach that predicted fatty acid synthase, ATP-citrate synthase, and acetyl-CoA carboxylase, key enzymes of lipogenesis, are expressed in the male pheromone gland of *N. vitripennis* and *N. giraulti*. Labeling experiments with *Urolepis rufipes*, a closely related species producing a male sex pheromone independently of fatty acids via the mevalonate pathway, revealed that both sexes are likewise able to synthesize fatty acids de novo. We conclude that the parasitoid wasp species studied here, irrespective of the biosynthetic origin of their sex pheromones, are capable of responding flexibly to lipid shortage during their adult life by keeping enzymatic machinery for lipogenesis running.

1. Introduction

Fatty acids and their derivatives are of central importance for survival and reproduction of virtually any organism on earth. They play crucial roles in the storage and activation of metabolic energy, thermal insulation, cell function as components of biomembranes and as precursors for the biosynthesis of signaling molecules such as eicosanoids (Devlin, 1997; Koolman and Röhm, 2012). Therefore, highly conserved enzymatic machinery is present in the vast majority of organisms enabling the de novo production of fatty acids from carbohydrates and other nutritional resources. During glycolysis, glucose is metabolized to pyruvate which is converted in the mitochondrion to acetyl coenzyme A (AcCoA), the central building-block of fatty acid biosynthesis. For the transport to the cytosol, where lipogenesis occurs, AcCoA is condensed with oxaloacetate to citrate. In the cytosol AcCoA is released from citrate by the ATP-citrate lyase (= ATP-citrate synthase, ATPCS). For fatty acid biosynthesis, AcCoA is then carboxylated by the AcCoA carboxylase (ACC) to form malonyl-CoA, which is used for the formation of fatty acids by the Type 1 fatty acid synthase (FAS), a multifunctional enzyme complex catalyzing the different steps of fatty acid synthesis in animals and fungi. The resulting primary product of this process is palmitic acid which is further processed by elongases and fatty acid desaturases to form saturated and unsaturated longer-chain fatty acids. These can be incorporated into mono-, di- and triglycerides as well as phospholipids and used for energy storage and the formation of biomembranes (Beld et al., 2015; Koolman and Röhm, 2012; Visser et al., 2012).

In insects, apart from the general functions mentioned above, fatty acids are indispensable for the production of protective cuticular waxes, defensive compounds and pheromones for intraspecific communication (Blomquist, 2010; Blomquist et al., 2012; Moriconi et al., 2019; Pei et al., 2019; Stanley-Samuelson et al., 1988; Stanley-Samuelson and

Abbreviations: FAME, fatty acid methyl ester; PAME, palmitic acid methyl ester; SAME, stearic acid methyl ester; *RR*-HDL, (4*R*,5*R*)-5-hydroxy-4-decanolide; *RS*-HDL, (4*R*,5*S*)-5-hydroxy-4-decanolide; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; ATPCS, ATP-citrate synthase; GC/MS, gas chromatography/mass spectrometry; LC-MS/MS, liquid chromatography/tandem mass spectrometry

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Nelson, 1993). Hence, it has long been assumed that, besides the uptake of nutritional lipids, the ability to synthesize fatty acids de novo is ubiquitous also in insects (Stanley-Samuelson et al., 1988). This view has been recently challenged by a number of studies reporting that several insects have lost the capability to synthesize fatty acids de novo because of their parasitic lifestyle (Visser et al., 2010, 2012, 2017). It has been argued that parasitoid wasps developing in or on arthropod hosts get sufficient lipids from their host resulting in a loss of lipogenesis during evolution in most parasitoid wasps due to environmental compensation (Visser et al., 2010). In fact, parasitoids can even manipulate their host to produce increased amounts of lipids to the benefit of the developing offspring (Nakamatsu and Tanaka, 2004; Rivers and Denlinger, 1994, 1995). However, this benefit is restricted to the juvenile stages and whether even well-fed adults can afford to waive entirely a metabolic pathway of central importance such as lipogenesis is questionable. Early studies suggesting a lack of lipogenesis in parasitoid wasps were based on rough gravimetric and colorimetric methods (Eijs et al., 1998; Giron and Casas, 2003; Olson et al., 2000; Rivero and West, 2002; Visser et al., 2010). Wasps exposed for several days to a carbohydrate source nevertheless showed decreased lipid levels with increasing age. However, decreasing lipid levels in sugar-fed parasitoid wasps were not found in all species (Visser et al., 2010) and it remained often unclear whether and to which extend the insects actually consumed the offered carbohydrates. Furthermore, measuring the total lipids does not rule out the possibility that anabolic and catabolic processes occur simultaneously and that lipid resources are partially replenished though the amount of total lipids decreases. Hence, gravimetric and colorimetric techniques are unsuitable to demonstrate unequivocally the presence or lack of lipogenesis in a species. Few studies employing more sophisticated isotope labeling techniques led to contrasting results. Feeding experiments with radiolabeled ^{14}C-glucose revealed the incorporation of up to 7% of total radioactivity in the raw lipids of the parasitoid wasp *Eupelmus vuilleti* (Hymenoptera: Eupelmidae) suggesting the ability to synthesize lipids from sugars in this species (Giron and Casas, 2003). However, individual lipids were not characterized by chromatographic techniques in this study leaving the possibility that glycolipids rather than de novo synthesized fatty acids were responsible for this result. Studies involving feeding experiments with unlabeled glucose dissolved in deuterium oxide (D_2O) and analysis of the fatty acid methyl esters (FAME) by coupled gas chromatography/mass spectrometry (GC/MS) revealed the incorporation of deuterium into fatty acids for the parasitoid wasps *Gelis agilis* and *G. areator* (Hymenoptera: Ichneumonidae) whereas in *E. vuilleti* no labeling could be shown (Visser et al., 2017). Hence, more species need to be investigated with combined isotope labeling and high resolution chromatography techniques to answer the question whether the vast majority of parasitoid wasps indeed lack lipogenesis or not.

One species of parasitoid wasps for which the lack of lipogenesis has been concluded is *Nasonia vitripennis* (Hymenoptera: Pteromalidae) (Visser et al., 2012). *N. vitripennis* parasitizes the pupae of numerous fly species and has become a model organism for the study of all aspects of parasitoid wasp biology not least because of the availability of a fully sequenced genome (Werren and Loehlin, 2009; Werren et al., 2010). An initial colorimetric approach to study lipogenesis in *N. vitripennis* revealed that aging females, albeit having ad libitum access to honey solution, showed decreasing lipid levels (Rivero and West, 2002). This result was confirmed later by a gravimetric approach (Visser et al., 2012) and in another study significant amounts of deuterium were not detected in FAME from *N. vitripennis* fed unlabeled glucose dissolved in D_2O (Visser et al., 2012). Furthermore, gene expression analysis revealed that sugar feeding did not induce FAS and some other key genes involved in lipogenesis (Visser et al., 2012). These findings led to the conclusion that *N. vitripennis* is lacking the ability to synthesize fatty acids de novo from carbohydrates.

Sexual communication in the genus *Nasonia* depends widely on the fatty acid metabolism. Males of *N. vitripennis* produce a mixture of (4*R*,5*R*)- and (4*R*,5*S*)-5-hydroxy-4-decanolides (*RR*- and *RS*-HDL) in their rectal vesicle which act together with the synergistic minor compound 4-methylquniazoline and is highly attractive for virgin females (Abdel-Latief et al., 2008; Ruther et al., 2007, 2008). Mated females, however, do not respond to the pheromone anymore (Lenschow et al., 2018; Ruther and Hammerl, 2014; Ruther et al., 2010). Males of the congeneric species *N girault, N. longicornis,* and *N. oneida* produce only *RS*-HDL in their pheromone glands (Niehuis et al., 2013). *RR*-HDL, the exclusive pheromone component used by *N. vitripennis*, is produced by epimerization of *RS*-HDL catalyzed in the pheromone gland by short chain dehydrogenase/reductases (Niehuis et al., 2013; Ruther et al., 2016; Semmelmann et al., 2019a). Stable isotope labeling experiments using fully ^{13}C-labeled precursors revealed that the biosynthesis of *RR*- and *RS*-HDL involves the unsaturated fatty acids oleic acid (OA) and linoleic acid (LA) (Blaul and Ruther, 2011; Blaul et al., 2014). Males express a functional Δ12-desaturase (Nvit_D12a) in the pheromone gland converting OA into LA (Semmelmann et al., 2019b). Also the behavioral switch in the pheromone response shown by *N. vitripennis* females after mating is related to fatty acid metabolism. Contact of female antennae with male-derived fatty acid ethyl esters during courtship renders them unresponsive to the male sex pheromone (Ruther and Hammerl, 2014). Nutritional supply with OA or LA increases male sex pheromone titers (Blaul and Ruther, 2011; Brandstetter and Ruther, 2016), but whether the fatty acids used for pheromone production can be additionally produced de novo from nutritional carbohydrates is unknown.

The genus *Nasonia* is closely related to the genera *Trichomalopsis* and *Urolepis* and has been suggested to form a monophyletic taxon within the Pteromalinae, the so-called “Nasonia group” (Burks, 2009). While *T. sarcophagae*, the only *Trichomalopsis* species studied so far, uses also (4*R*,5*S*)-5-hydroxy-4-decanolide as a male sex pheromone component (Niehuis et al., 2013), males of *Urolepis rufipes* release 2,6-dimethyl-7-octene-1,6-diol, to attract virgin females. This compound is synthesized independently of fatty acid metabolism via the mevalonate pathway (Ruther et al., 2019).

Given the prominent additional role of fatty acids in the sexual communication of *Nasonia* species we hypothesized in the present study that they do not lack the ability to synthesize fatty acids de novo. We performed feeding experiments with three *Nasonia* species involving fully ^{13}C-labeled α-D-glucose and analyzed FAME by GC/MS to investigate whether ^{13}C is incorporated into fatty acids from both males and females of all species as well as in the sex pheromone of *N. vitripennis* males. By a proteomics approach, we performed a targeted search for key enzymes of fatty acid biosynthesis in the sex pheromone glands of *N. vitripennis* and *N. girault* males. Finally, we performed comparative ^{13}C-labeling experiments with *U. rufipes* to investigate whether lipogenesis is restricted to those species of the *Nasonia group* that depend on fatty acids for pheromone communication.

2. Materials and methods

2.1. Insects

N. vitripennis (strain Phero01, originally collected from birds’ nests in northern Germany), *N. longicornis* (strain NLMN8510, provided by B. Pannebakker, Wageningen University, The Netherlands), *N. girault* (strain NGVA2, provided by T. Schmitt, University of Würzburg, Germany) and *U. rufipes* (provided by B.H. King, Northern Illinois University, DeKalb, IL) were reared on freeze-killed puparia of the green bottle fly *Lucilia caesar* (Diptera: Calliphoridae) as described previously (Mair et al., 2018; Ruther et al., 2014, 2019; Steiner et al., 2006).

2.2. Feeding experiments

For each species, newly emerged wasps were kept together in Petri

dishes for 2 days at 25 °C without providing an additional carbohydrate source. Groups of 10–20 males and females each were provided with ca. 30 host pupae and allowed to court, mate and oviposit to ensure that both males and females could metabolize parts of existing fat reserves. After this pre-treatment, wasps were separated according to their sex and kept for two more days in groups of 5 wasps in 1.5 ml microcentrifuge tubes the bottom of which was covered by 40 µl of a 10% solution of fully ^{13}C-labeled α-D-(+)-glucose (99% ^{13}C, Sigma-Aldrich, Taufkirchen, Germany). After one day, the labeled glucose solution was renewed when necessary. Control wasps were kept under identical conditions without providing water or glucose solution. After a feeding period of 48 h, wasps were frozen and kept at −20 °C until being used for chemical analyses.

2.3. Transesterification of lipids for GC/MS analysis

After thawing, groups of five male or female wasps were put in a 1.5 ml glass vial. 200 µl dichloromethane were added and wasps were homogenized using a glass stick and extracted for 30 min. The extract was transferred to another 1.5 ml glass vial and the residue was washed with 200 µl dichloromethane. Extracts were put together and the solvent was removed under a gentle stream of nitrogen. The residue of raw lipids was re-suspended in 200 µl of methanol and 20 µl of acetyl chloride (10%, dissolved in methanol) and transesterified for 3 h at 80 °C. Afterwards, 200 µl of a solution of sodium hydrogen carbonate (5%) were added and FAME were extracted with 200 µl hexane. The hexane phase was concentrated to 25 µl under nitrogen and used for GC/MS analysis. For each species/sex, n = 3 samples of treatment and control wasps were prepared.

2.4. Preparation of pheromone extracts for GC/MS analysis

Abdomens of individual *N. vitripennis* males from the feeding experiment (n = 5) and control males (n = 5), respectively, were dissected and extracted for 30 min with 50 µl dichloromethane. Vials were rinsed with another 50 µl of dichloromethane and unified extracts were concentrated under nitrogen to 25 µl and used for GC/MS analysis.

2.5. Chemical analysis by GC/MS

Chemical analyses were performed using a Shimadzu QP2010 Plus GC/MS system 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). Samples (1 µl) were injected splitless at 300 °C using a Shimadzu AOC 20i auto sampler. The MS was operated in the electron impact mode at 70 eV; the mass range was m/z 35–500. Helium was used as carrier gas at a constant velocity of 40 cm s^{-1}. For FAME analysis, the initial oven temperature was 50 °C, the temperature was programmed at 3 °C/min to 280 °C and held at this temperature for 22 min. For pheromone analysis, the GC program started at 80 °C and was programmed at 5 °C to 280°C and held at this temperature for 15 min. Identification of unlabeled compounds was done by comparison of retention times and mass spectra with those of authentic reference chemicals (HDL: see (Ruther et al., 2007); FAME: reference mixture of 37 FAME, Sigma-Aldrich, Deisenhofen, Germany). For the detection of incorporated ^{13}C in insect-derived fatty acids and HDL we checked the mass spectra at the expected retention times of selected FAME as well as *RR*- and *RS*-HDL for the appearance of diagnostic ions. As for FAME, we focused on the fully ^{13}C-labeled methyl esters of the saturated fatty acids palmitic (PAME) and stearic acid (SAME), because mass spectra of the unlabeled compounds are less complex than those of unsaturated FAME facilitating the interpretation of the results. Mass spectra for ^{13}C-labeled and unlabeled SAME and structures of selected diagnostic ions are shown in Fig. 1 A-B. To infer ^{13}C-incorparation into saturated fatty acids, we selected the diagnostic ions m/z 87 (fully labeled: m/z 90) and m/z 143 (fully labeled: m/z 150), because these occur in mass spectra of both PAME and SAME (Figs. 1B and 2A). Additionally, we monitored the molecular ions m/z 270 (fully labeled: m/z 286) and m/z 298 (fully labeled: m/z 316) for PAME and SAME, respectively. Incorporation of ^{13}C into the male sex pheromone can be inferred by the occurrence of the diagnostic ion m/z 90 (fully labeled base peak, Fig. 5C), which is absent in the mass spectrum of unlabeled HDL (Blaul and Ruther, 2011; Blaul et al., 2014). Labeling rates were calculated by relating the peak area of the labeled diagnostic ion (HDL, PAME/SAME: m/z 90) to the added peak areas of the respective unlabeled (HDL: m/z 86; PAME/SAME: m/z 87) and labeled diagnostic ions. Please note that m/z 90 is coincidentally diagnostic for ^{13}C-incorporation in both HDL and PAME/FAME although the underlying molecule structures are different as shown (Figs. 1B and 5C).

2.6. Protein analysis of the pheromone gland of Nasonia males by LC/MS/MS

To investigate whether predicted proteins involved in the fatty acid biosynthesis are present in the pheromone glands of *N. vitripennis* and *N. giraulti* males, we re-analyzed proteomics data obtained in a previous study (Ruther et al., 2016). In this study, we extracted proteins from 10 male pheromone glands (rectal vesicles) and analyzed the in-gel digested proteins by coupled liquid chromatography/tandem mass spectrometry (LC-MS/MS) (n = 2 replicates, for methodical details see original study).

2.7. Statistical analysis

Incorporation rates of ^{13}C in *RS*-HDL from *N. vitripennis* males fed ^{13}C-glucose and unfed control males were compared by a Mann-Whitney *U* test.

3. Results

3.1. FAME analysis of wasps fed ^{13}C-labeled α-D-glucose and unfed control wasps

GC/MS analysis of transesterified lipid extracts from both sexes of all *Nasonia* species as well as *U. rufipes* revealed the incorporation of ^{13}C into PAME and SAME if these wasps had been fed ^{13}C-labeled glucose (Figs. 1–4, Table 1). The diagnostic ion m/z 90 at the retention times of PAME and SAME was detected in all samples from wasps fed ^{13}C-labeled glucose. Calculated incorporation rates differed both within and between species and sexes, respectively, ranging between 0.74 and 23.23%. In samples with high ^{13}C-incorporation, also the diagnostic ion m/z 150 and even the fully labeled molecular ions (PAME: m/z 286; SAME: m/z 316) were detectable (Figs. 1C and 2B). Magnification of the molecular ion region of the mass spectra revealed furthermore the presence of clusters of partially labeled molecular ions (PAME: m/z 278, 280, 282, 284; SAME: m/z 310, 312, 314) resulting from the incorporation of a varying number of ^{13}C-labeled acetate units. ^{13}C-labeling of the diagnostic ions was confirmed by retention times in the respective ion traces that were slightly decreased by ca. 1 s in comparison to the unlabeled ions (Fig. 3 A) due to the inverse isotope effect of heavier isotopes on the chromatographic behavior of labeled compounds (Matucha et al., 1991).

3.2. Pheromone analysis of N. vitripennis males fed ^{13}C-labeled α-D-glucose and unfed control wasps

The ion traces of the diagnostic ion m/z 90 (Fig. 5A–B) showed a peak in all five replicates at the retention time of *RS*-HDL for *N. vitripennis* males fed ^{13}C-labeled α-glucose. This peak was absent in unfed control males indicating that the ^{13}C-labeled precursor had been incorporated into the pheromone component of males fed ^{13}C-labeled α-D-glucose. Incorporation rates ranged from 0.22 to 3.31% (Mann-

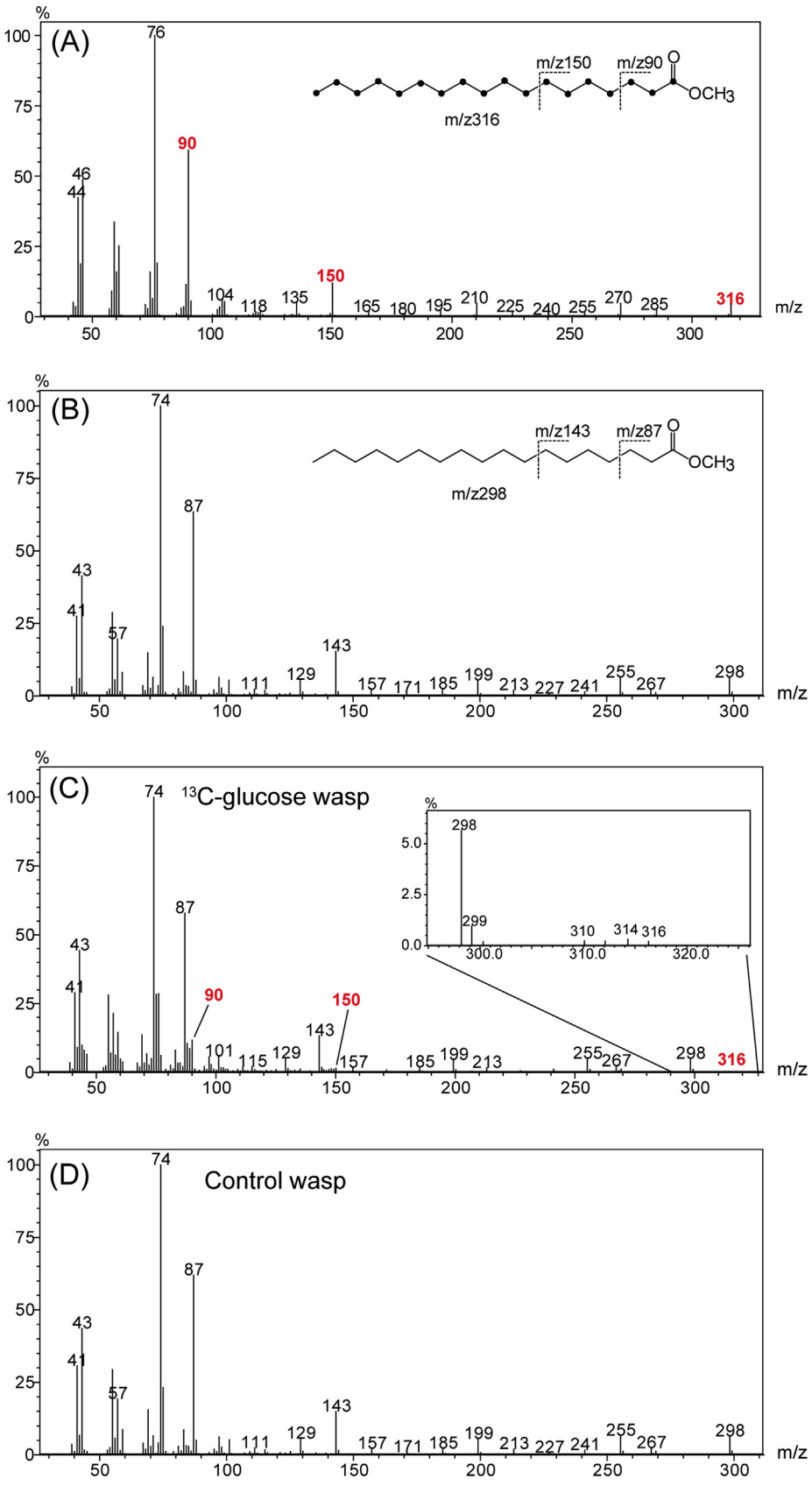

Fig. 1. Mass spectra of synthetic (A) fully ^{13}C-labeled stearic acid methyl ester (SAME), (B) unlabeled SAME, (C) SAME obtained by transesterification of lipid extracts from *Nasonia girault*i females fed fully ^{13}C-labeled α-D-glucose, and (D) unfed control females. Diagnostic ions used to detect the ^{13}C-labeling in the wasp-derived compound are indicated (bold red). Cut-out in panel (C) shows the magnified region with the unlabeled (m/z 298), the fully ^{13}C-labeled (m/z 316) and a cluster of partially labeled molecular ions resulting from the incorporation of a varying number of ^{13}C-labeled acetate units into the fatty acid chain. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

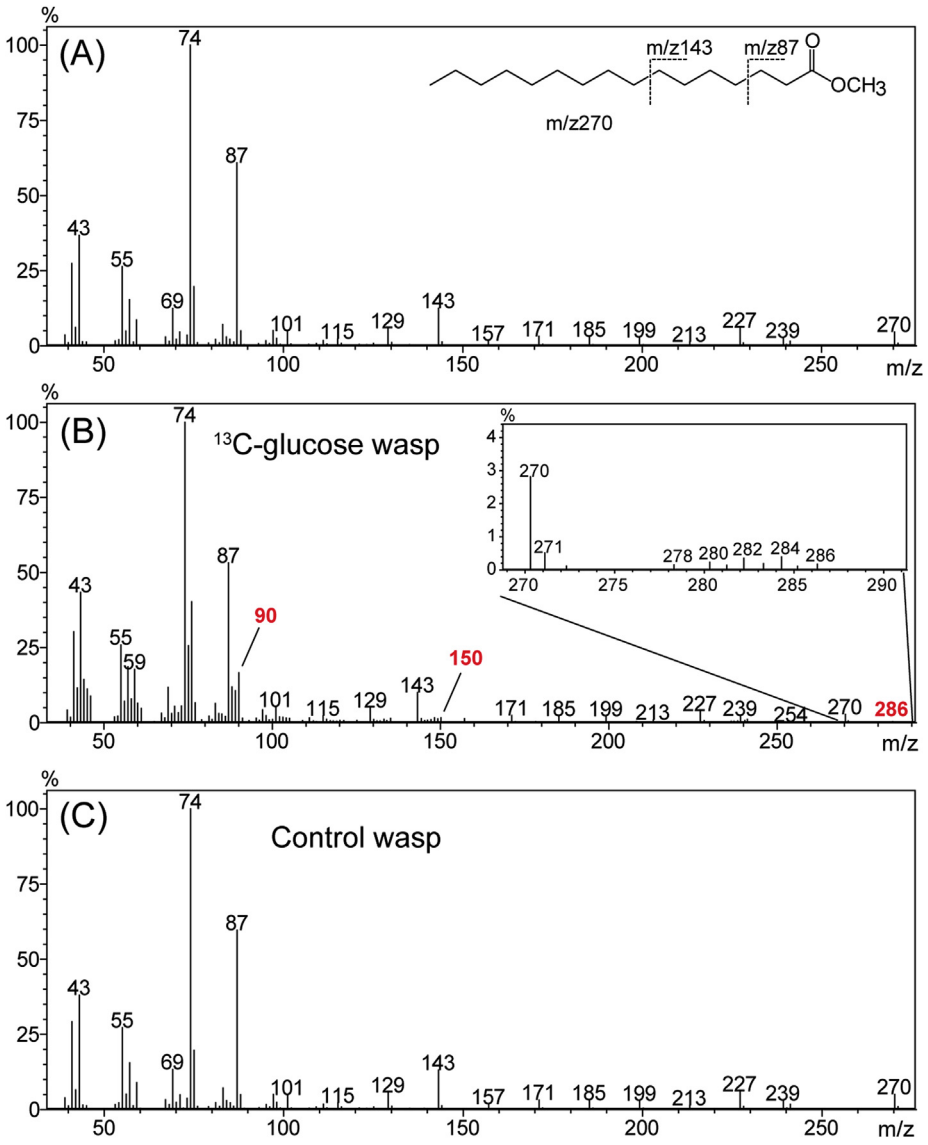

Fig. 2. Mass spectra of synthetic (A) unlabeled palmitic acid methyl ester (PAME), (B) PAME obtained by transesterification of lipid extracts from *Nasonia girault* females fed fully ^{13}C-labeled α-D-glucose, and (C) unfed control females. Diagnostic ions used to detect the ^{13}C-labeling in the wasp-derived compound are indicated (bold red). Cut-out in panel (B) shows the magnified region with the unlabeled (m/z 270), the fully ^{13}C-labeled (m/z 286) and a cluster of partially labeled molecular ions resulting from the incorporation of a varying number of ^{13}C-labeled acetate units into the fatty acid chain. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Whitney *U* test: p = 0.0075). Like with the labeled FAME, the retention time of the labeled ion was slightly decreased confirming the presence of ^{13}C. Incorporation of ^{13}C into the second pheromone component *RR*-HDL was detectable but too low to be quantified (Fig. 5 B).

3.3. Protein analysis of the pheromone gland of Nasonia males by LC-MS/MS

Analysis of our proteomics data revealed that two predicted FAS were detectable in both replicates in the pheromone gland of *N. vitripennis* males and *N. girault* males, respectively. One of these predicted FAS (XP_008203901.1) was among the most abundant proteins in the pheromone gland at all with sequence coverages > 40% (*N. vitripennis*) and > 30% (*N. girault*), respectively (Table 2). In both species, we detected furthermore predicted ATPCS and two isoforms of ACC, further key enzyme of lipogenesis.

4. Discussion

The present study clearly demonstrates that both sexes of the studied *Nasonia* species and the closely related species *U. rufipes* are capable of synthesizing fatty acids de novo from α-D-glucose. Feeding a fully ^{13}C-labeled precursor resulted in the occurrence of specific ^{13}C-labeled ions in the mass spectra of PAME and SAME which showed the typical shift in retention times shown by labeled compounds in comparison to the unlabeled analogues (Matucha et al., 1991). Incorporation of glucose-derived ^{13}C into fatty acids was furthermore confirmed by the occurrence of ^{13}C-labeled fragment ions in the mass spectra of the male sex pheromone of *N. vitripennis* which is synthesized from fatty acids (Blaul and Ruther, 2011; Blaul et al., 2014). Finally, key enzymes of fatty acid biosynthesis such as FAS, ACC, and ATPCS were abundant in the pheromone glands of *N. vitripennis* and *N. girault* males. Hence, for pheromone production *Nasonia* males do not entirely depend on fatty acids taken up from the host during larval development, although this is likely still the most important lipid source (Blaul and Ruther, 2011; Brandstetter and Ruther, 2016). Additionally, they may replenish, at least partially, their demand of fatty acids by de novo synthesis from carbohydrate sources such as floral and extrafloral nectar or honeydew which is taken up by many parasitoid wasps (Benelli et al., 2017; Heimpel, 2019; Wäckers et al., 2008). Further work is necessary to investigate whether and if so in which context adult parasitoid wasps make use of lipogenesis under natural conditions.

Our results raise the question whether the lack of lipogenesis in actually as widespread in parasitoid wasps as previously thought and whether fatty acid production in *Nasonia* is one of few exceptions

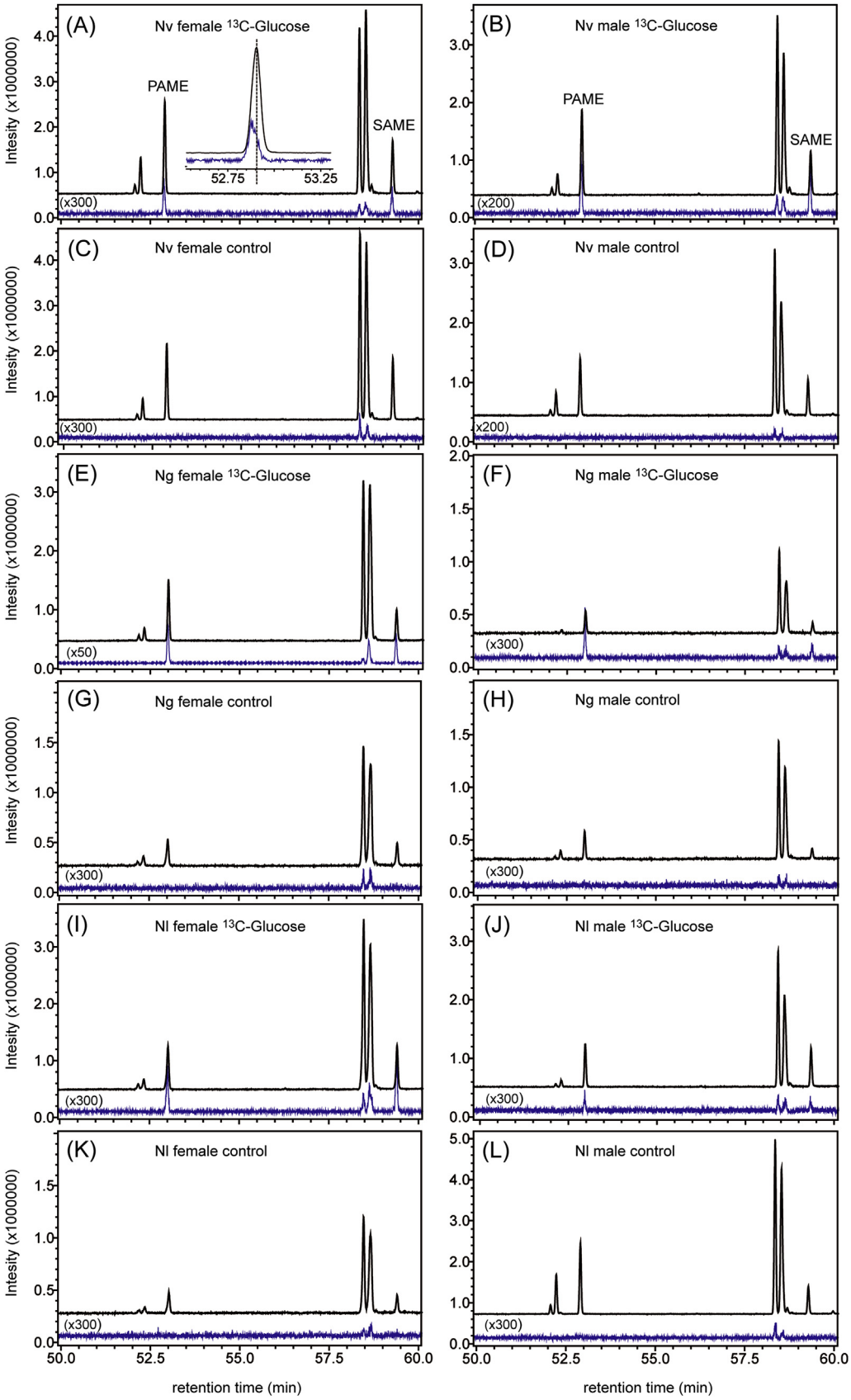

Fig. 3. GC/MS analysis of transesterified lipid extracts from (A, B) female and male *Nasonia vitripennis* (Nv) wasps fed fully ^{13}C-labeled α-D-glucose, and (C, D) unfed control wasps of either sex. The respective analyses for *Nasonia giraulti* (Ng) are shown in panels E, F (^{13}C-labeling) and G, H (controls) as well as for *Nasonia longicornis* (Nl) in panels I, J (^{13}C-labeling) and K, L (controls), respectively. Each panel shows the total ion chromatograms (upper trace) and the extracted ion chromatogram of the diagnostic ion m/z 90 (lower trace, magnification factors given in brackets). The peaks of palmitic acid methyl ester (PAME) and stearic acid methyl ester (SAME) are indicated. The cut-out in panel (A) is a magnification of the elution profile of PAME showing the slightly decreased retention time of the diagnostic ion due to the inverse isotope effect of heavier isotopes (for more details see text).

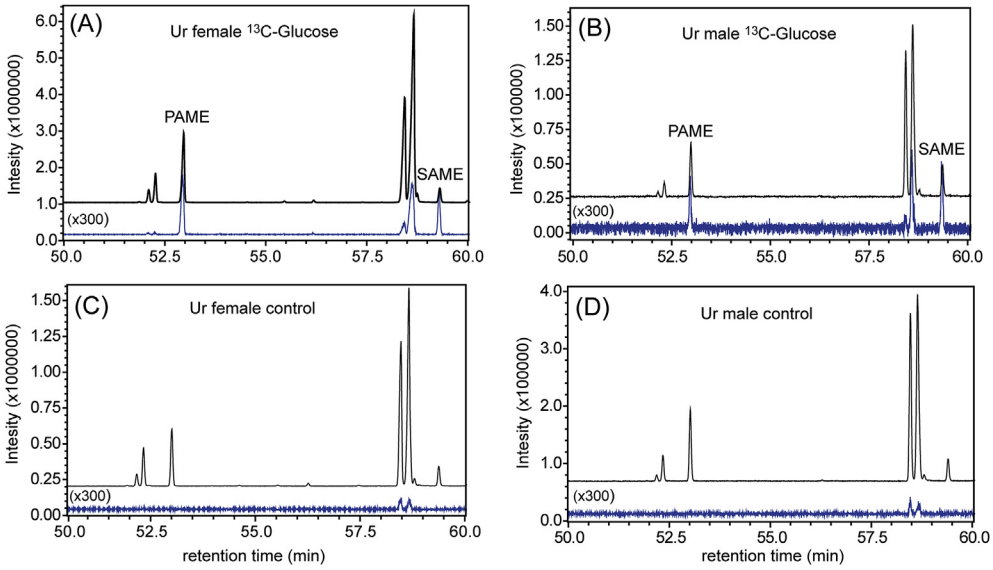

Fig. 4. GC/MS analysis of transesterified lipid extracts from (A, B) female and male *Urolepis rufipes* (Ur) wasps fed fully ^{13}C-labeled α-D-glucose, and (C, D) unfed control wasps of either sex. Each panel shows the total ion chromatograms (upper trace) and the extracted ion chromatogram of the diagnostic ion m/z 90 (lower trace, magnification factors given in brackets). The peaks of palmitic acid methyl ester (PAME) and stearic acid methyl ester (SAME) are indicated.

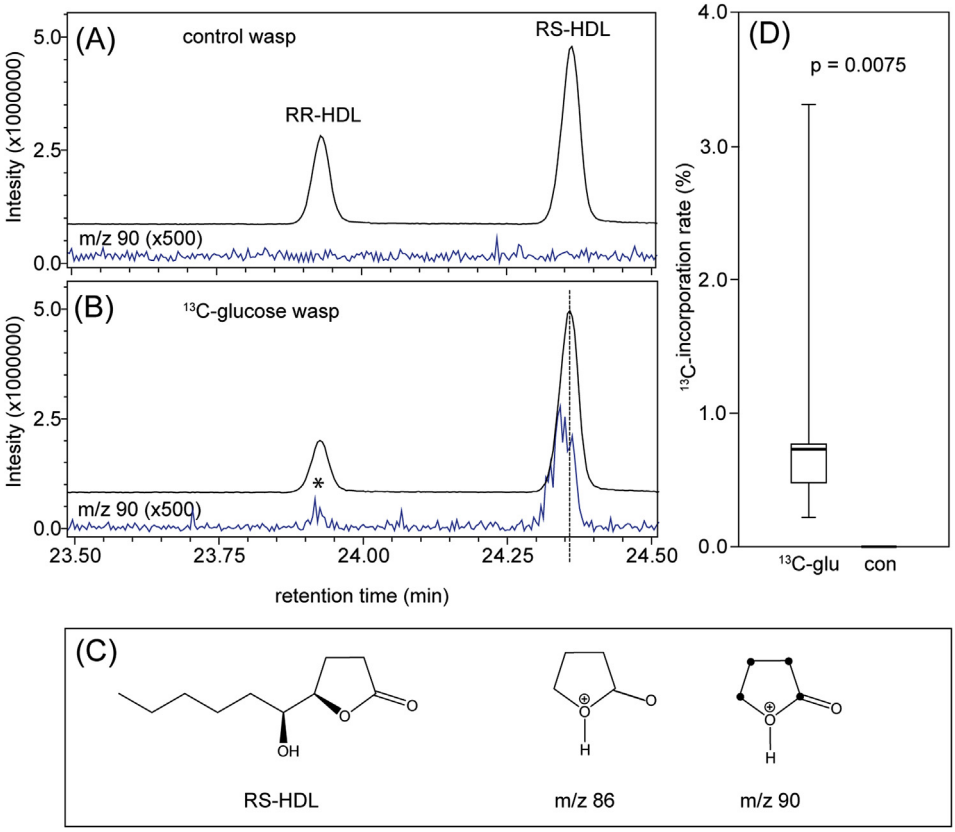

Fig. 5. GC/MS analysis of pheromone extracts from (A) male *Nasonia vitripennis* wasps fed fully ^{13}C-labeled α-D-glucose and (B) unfed control males. Shown are the total ion chromatograms (upper trace) and the extracted ion chromatogram of the diagnostic ion m/z 90 indicating the incorporation of the ^{13}C-labeled precursor into the sex pheromone component (4*R*,5*S*)-5-hydroxy-4-decanolide (*RS*-HDL). Asterisk indicates a peak at the retention time of *RR*-HDL, which, however, was too small to be quantified. (C) Structures of *RS*-HDL as well as the unlabeled (m/z 86) and fully ^{13}C-labeled (m/z 90) diagnostic ion (black dots indicate ^{13}C-atoms). (D) Incorporation rate of ^{13}C into *RS*-HDL estimated by the peak areas of the ion chromatograms m/z 90 and 86, respectively (Mann-Whitney *U* test, n = 5).

because of the important role fatty acids play for sexual communication in these species. We propose that this is not the case, because also (a) females of the *Nasonia* species (not producing the pheromone) and (b) males of *U. rufipes* (producing their pheromone via the mevalonate pathway) are capable of synthesizing fatty acids de novo. Furthermore, the evidence for lacking lipogenesis in parasitoid wasps is based for many species on methods such as gravimetry or colorimetry (Eijs et al., 1998; Giron and Casas, 2003; Olson et al., 2000; Rivero and West, 2002; Visser et al., 2010) which fail to detect the general capability of lipogenesis if fatty acid degradation and de novo synthesis are concurrent processes or in species that feed only small amounts of the offered sugar source. For instance, *N. vitripennis* females starved for three days had lower total lipid levels than those that had access to sugar solution (Visser et al., 2012). This result suggests a reduced fat degradation due to sugar availability but might as well be explained by the (partial) de novo synthesis of lipids or both processes occurring concurrently. Even isotope labeling techniques seem to vary in their performance to detect lipogenesis, because the glucose/D_2O-technique (Zhang et al., 2017), while successfully applied to some parasitoid wasp species (Visser et al., 2017), failed to detect lipogenesis in *N. vitripennis* (Visser et al., 2012). In general, these techniques depend on the amount of sugar solution taken up by the study organism which is often difficult to control. While working fine with insects adapted to forage sugar solutions such as honey bees (Visser et al., 2012), they might fail in

Table 1
Incorporation rates of ^{13}C into palmitic acid methyl ester (PAME) and stearic acid methyl ester (SAME) from transesterified lipids of *Nasonia vitripennis* (*Nv*), *N. giraulti* (*Ng*), *N. longicornis* (*Nl*), and *Urolepis rufipes* (*Ur*) wasps fed fully ^{13}C-labeled α-D-glucose. Incorporation was calculated by relating the peak area of the ^{13}C-labeled diagnostic ion m/z 90 to the added peak areas of m/z 90 and the respective unlabeled ion m/z 87. Unfed wasps of either species or sex, respectively, were analyzed for control (con). Asterisks indicate control samples in which m/z 90 was detected in traces. The respective peaks, however, did not show the typical decreased retention times due to the inverse isotope effect.

species	replicate	PAME				SAME			
		females		males		females		males	
		^{13}C-glu	con	^{13}C-glu	con	^{13}C-glu	con	^{13}C-glu	con
Nv	1	0.94	0.00	2.35	0.00	0.92	0.00	2.98	0.00
	2	1.57	0.00	2.62	0.00	1.99	0.00	4.72	0.00
	3	0.74	0.04*	0.53	0.04*	1.02	0.00	0.58	0.00
Ng	1	23.23	0.00	6.90	0.00	16.53	0.00	6.18	0.00
	2	10.10	0.00	7.86	0.00	13.85	0.00	10.05	0.00
	3	4.66	0.04*	19.49	0.00	12.67	0.00	32.41	0.00
Nl	1	2.18	0.00	9.78	0.00	3.08	0.00	8.98	0.00
	2	14.88	0.05*	0.56	0.00	11.10	0.00	0.71	0.00
	3	1.32	0.00	0.75	0.00	1.67	0.00	1.81	0.00
Ur	1	0.57	0.00	1.94	0.00	2.45	0.00	6.91	0.00
	2	4.39	0.00	2.31	0.04*	7.05	0.00	5.73	0.02*
	3	2.57	0.00	0.13	0.00	9.11	0.00	0.00	0.00

Table 2
Results of the mass spectrometric analysis of lipogenesis-related proteins in the rectal vesicles of *N. vitripennis* (*Nv*) and *N. giraulti* (*Ng*) males. Roman numbers indicate the individual replicates. Ten rectal vesicles of 1–2 day old wasps were pooled per replicate and subjected to SDS-PAGE. In-gel trypsin digested proteins were analyzed by LC-MS/MS. Protein database searching of the resulting mass spectra was performed using the Mascot search engine.

Protein name	GenBank accession no.	Species	Mascot score		Sequence coverage (%)		Number of peptides	
			I	II	I	II	I	II
fatty acid synthase-like	XP_008203901.1	*Nv*	6956.8	4674.8	48.9	43.2	108	77
		Ng	4452.3	3585.0	37.2	30.2	78	61
fatty acid synthase	XP_003423914.1	*Nv*	1354.9	523.3	18.6	7.4	32	12
		Ng	1705.9	1216.3	19.0	14.7	38	24
acetyl-CoA carboxylase	XP_016838051.1	*Nv*[a]	619.0	n.d.	7.4	n.d.	12	n.d.
	XP_008215455.1	*Ng*[b]	1135.1	1025.8	15.4	12.5	27	19
ATP-citrate synthase	XP_003425261	*Nv*	1461.2	1360.4	33.9	36.2	27	24
		Ng	1326.5	1432.3	28.2	30.8	23	22

[a] Isoform X4.
[b] Isoform X7; n.d. = not detected.

those species feeding only occasionally little amounts of the offered sugar source. Also results from gene expression experiments might not necessarily reflect the abundance of gene products since *N. vitripennis* did not respond to sugar feeding with increased gene expression of some lipogenesis-related genes (except for ATP-citrate lyase) (Visser et al., 2012) while in our protein analyses FAS and ACC were among the most abundant proteins in the pheromone glands of *N. vitripennis* and *N. giraulti* males. Hence, we propose that more species from different families need to be investigated with ^{13}C-based labeling techniques to re-evaluate the proposed lack of lipogenesis in most parasitoid wasps. As a starting point, we have studied six additional species of parasitoid wasps with the technique described here in all of which at least one sex was capable of producing fatty acids (Prager & Ruther, unpublished results). This suggests that parasitoids, while getting the bulk of lipids necessary for growth and reproduction from their host during larval development, face the costs of keeping the lipogenesis machinery running to respond flexibly to lipid shortage during their adult life.

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