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De novo biosynthesis of linoleic acid is widespread in parasitic wasps

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Abstract

Linoleic acid (C18:2$^{\Delta 9,12}$, LA) is an important metabolite with numerous essential functions for growth, health, and reproduction of organisms. It has long been assumed that animals lack Δ12-desaturases, the enzymes needed to produce LA from oleic acid (C18:1$^{\Delta 9}$, OA). There is, however, increasing evidence that this is not generally true for invertebrates. In the insect order Hymenoptera, LA biosynthesis has been shown for only two parasitic wasp species of the so-called "*Nasonia* group," but it is unknown whether members of other taxa are also capable of synthesizing LA. Here, we demonstrate LA biosynthesis in 13 out of 14 species from six families of parasitic wasps by gas chromatography–mass spectrometry analysis using two different stable isotope labeling techniques. Females of the studied species converted topically applied fully ^{13}C-labeled OA into LA and/or produced labeled LA after feeding on fully ^{13}C-labeled α-D-glucose. These results indicate that Δ12-desaturases are widespread in parasitic Hymenoptera and confirm previous studies demonstrating that these insects are capable of synthesizing fatty acids de novo.

KEYWORDS

Δ12-desaturase, fatty acid metabolism, Hymenoptera, linoleic acid, parasitoid

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1 | INTRODUCTION

For successful growth and reproduction, animals depend on the availability of nutrients such as carbohydrates, proteins, and fat. In animals, numerous biochemical pathways enable the conversion of primary metabolites into each other to avoid temporary shortages of indispensable nutrients (Nelson & Cox, 2017). Some essential metabolites, however, need to be ingested through the diet since animals are unable to produce them de novo. Among the nutrients essential for humans and other vertebrates are polyunsaturated fatty acids (PUFA, fatty acids with ≥2 double bonds) which are needed, for instance, for the formation of biomembranes, hormones, and other signaling molecules (Blomquist et al., 1991; Saini & Keum, 2018). The crucial step for the biosynthesis of PUFA is the introduction of a second double bond into oleic acid (C18:$1^{\Delta 9}$, OA) at position 12 to form linoleic acid (C18:$2^{\Delta 9,12}$, LA) which is typically catalyzed by Δ12-desaturases (Malcicka et al., 2018). This class of enzymes has long been thought to be absent in animals (de Renobales et al., 1986). However, studies on insects (Blaul et al., 2014; Blomquist et al., 1982; Buckner & Hagen, 2003; Cripps et al., 1986, 1990; de Renobales et al., 1986; Semmelmann et al., 2019), collembolans (Malcicka et al., 2017), nematodes (Peyou-Ndi et al., 2000; Zhou et al., 2011), gastropods (Weinert et al., 1993) and mites (Aboshi et al., 2013; Brückner & Heethoff, 2020; Shimizu et al., 2014) have demonstrated that not all animals depend on nutritional supply with LA but are capable of synthesizing it de novo. Δ12-desaturases from nematodes (Peyou-Ndi et al., 2000; Zhou et al., 2011) and insects (Semmelmann et al., 2019; Zhou et al., 2008) have been heterologously expressed and functionally characterized. For insects, however, a comparative study using radioactive [1-^{14}C]-acetate as precursor and involving 32 species from 13 orders suggested that not all orders are able to synthesize LA (Cripps et al., 1986). Until now, LA biosynthesis has been shown in six insect orders (Aboshi et al., 2013; Blaul et al., 2014; Semmelmann et al., 2019) and in 10 species of the Collembola (Malcicka et al., 2017).

Evidence for the de novo biosynthesis of LA in the hyper-speciose insect order Hymenoptera (Forbes et al., 2018) is restricted to the parasitic wasps *Nasonia vitripennis* Walker (Pteromalidae) and the closely related species *Urolepis rufipes* Ashmead (Blaul et al., 2014; Semmelmann et al., 2019). For *N. vitripennis*, a pupal parasitoid of several fly species (Whiting, 1967), LA is of particular importance, because it functions as a precursor of the male sex pheromone (Blaul & Ruther, 2011; Blaul et al., 2014). This suggested that the Δ12-desaturases Nvit_D12a and Nvit_D12b that have been recently characterized in *N. vitripennis* (Semmelmann et al., 2019) have evolved in the context of chemical communication. However, this hypothesis was not supported by experiments with *U. rufipes*, which like *N. vitripennis* belongs to the so-called "*Nasonia* group" within the subfamily Pteromalinae (Burks, 2009). In this species, the male sex pheromone is synthesized independently of fatty acid metabolism via the mevalonate pathway (Ruther et al., 2019), but at least the females are nonetheless capable of synthesizing LA (Semmelmann et al., 2019). Hence, more species of parasitic Hymenoptera from different families need to be studied to get a clearer picture on the occurrence of LA biosynthesis in this group of insects.

The most straightforward method to demonstrate LA biosynthesis in insects is the use of isotope labeling techniques. In earlier studies, radiolabeled acetate was injected into insects (Blomquist et al., 1982; Cripps et al., 1986) which, however, requires specialized lab equipment. Alternatively, stable isotope-labeled precursors can be used which can be either fed (Aboshi et al., 2013; Blaul et al., 2014; Brückner & Heethoff, 2020; Malcicka et al., 2017; Shimizu et al., 2014) or topically applied to the study organism using nontoxic solvents such as acetone (Blaul et al., 2014; Jatsch & Ruther, 2021). Fully ^{13}C-labeled OA is commercially available for this purpose which, however, is very expensive. A cheaper alternative is the use of labeled precursors related to de novo fatty acid biosynthesis from carbohydrates such as glucose (Shimizu et al., 2014), a process commonly referred to as lipogenesis (Bullón-Vela et al., 2018; Segner & Böhm, 1994). Glucose is metabolized to pyruvate during glycolysis, and pyruvate is oxidatively decarboxylated to acetyl coenzyme A, the building block used by the multienzyme complex fatty acid synthase to produce fatty acids (Nelson & Cox, 2017). Hence, the detection of ^{13}C-carbon in LA from organisms fed ^{13}C-labeled glucose or acetate indicates that the studied organisms possess all enzymes necessary for LA biosynthesis including a Δ12-desaturase (Brückner & Heethoff, 2020; Shimizu et al., 2014). Parasitic wasps

have long been assumed to lack lipogenesis (Visser & Ellers, 2008; Visser et al., 2010), but recent studies have demonstrated that this is not the case (Prager et al., 2019; Ruther et al., 2021). Hence, stable isotope labeling experiments with ^{13}C-labeled glucose should be generally suitable to demonstrate LA biosynthesis in parasitic wasps. The incorporation of stable isotope-labeled precursors into LA can be detected through gas chromatography–mass spectrometry (GC/MS) analysis by monitoring the abundance of certain diagnostic ions that indicate the incorporation of heavier isotopes into LA (Blaul et al., 2014; Malcicka et al., 2017; Semmelmann et al., 2019; Shimizu et al., 2014). Additionally, molecules with incorporated heavier isotopes such as deuterium or ^{13}C-carbon elute slightly before the unlabeled analogues due to the inverse isotope effect (Matucha et al., 1991). Hence a retention time shift of labeled mass spectrometric ions can be used as a second criterion to infer the incorporation of labeled precursors by the studied organisms (Prager et al., 2019).

In the present study, we investigate the ability of 14 parasitic wasp species from six families with respect to LA biosynthesis. In stable isotope labeling experiments, we administered ^{13}C-labeled OA by topical application of acetone solutions and offering ^{13}C-labeled α-D-glucose as food source. Subsequently, we analyzed transesterified lipid extracts of the treated wasps by GC/MS. We demonstrate LA biosynthesis in all but one of the studied species by at least one of the two labeling methods. Our results suggest that the ability to synthesize LA and thus the occurrence of Δ12-desaturases is not restricted to species of the *Nasonia* group but is widespread in parasitic Hymenoptera.

2 | MATERIALS AND METHODS

2.1 | Insects

Dibrachys cavus Walker (Dc, originally collected from empty bird's nests near Hamburg in Northern Germany), *N. giraulti* Darling (Ng, kindly provided by T. Schmitt, University of Würzburg), *N. longicornis* Darling (Nl, kindly provided by B. Pannebakker, University of Wageningen), *Muscidifurax raptorellus* Kogan and Legner and *M. uniraptor* Kogan and Legner (Mr and Mu, kindly provided by E. Verhulst, University of Wageningen), *Tachinaephagus zealandicus* Ashmead (Tz, collected from carcass baits near Gießen, Germany), *U. rufipes* Ashmead (Ur, kindly provided by B. H. King, University of Northern Illinois), and *Exoristobia philippinensis* Ashmead (Ep, collected from carcass baits in Davie, Florida, USA) were reared on juvenile stages of the green bottle fly (*Lucilia caesar* L.). Dc, Ng, Nl, Nv, Mr, Mu, and Ur were reared on pupae that were freeze-killed 2 days after pupation and thawed on demand (Ruther et al., 2019, 2011; Steiner et al., 2006). After thawing and drying the hosts for 2 h at 30°C, females were exposed in Petri dishes to newly emerged wasps and kept in an incubator at 25°C and approximately 50% relative humidity. Tz and Ep females were provided with live final-instar larvae (Tz) or live pupae (0–2 days after pupation, Ep), and thereafter reared under identical conditions as mentioned above. A starter culture of *Lariophagus distinguendes* Förster (Ld) was provided by a local company (Biologische Beratung Prozell und Schöller GmbH) and reared on larvae of the granary weevil (*Sitophilus granarius* L.) as described previously (Ruther et al., 2000). *Anisopteromalus calandrae* Howard (Ac), reared on larvae of *S. granarius*, *Baryscapus tineivorus* Ferriere (Bt), reared on larvae of the clothing moth (*Tineola bisselliella* Hummel), *Cephalonomia tarsalis* Ashmead (Ct), reared on larvae of the sawtooth grain beetle (*Oryzaephilus surinamensis* L.), *Trichogramma evanescens* Westwood (Te), reared on eggs of the Mediterranean flour moth (*Ephestia kuehniella* Zeller), and *Habrobracon hebetor* Say (Hh), reared on larvae *E. kuehniella*, were also obtained from Biologische Beratung Prozell und Schöller GmbH and used as delivered without prior oviposition. Before treatment with ^{13}C-labeled precursors, newly emerged females of Dc, Ep, Mr, Mu, Ng, Nl, Nv, Tz, Ld, and Ur were offered hosts ad libitum for oviposition for two days (Tz: 1 day). We assumed that this pretreatment caused a partial depletion of the LA reserves and enhanced the need for de novo biosynthesis of LA. Females of all other species were used as delivered by the commercial provider.

2.2 | Application of ^{13}C-labeled oleic acid

To investigate whether OA is converted by the parasitoids to LA, we performed ^{13}C-labeling experiments. Single parasitoid females of each species were cold-sedated on an ice bath and ca. 200 nl of a solution containing 100 μg/μl of fully ^{13}C-labeled OA (>99%, Campro Scientific, free of ^{13}C-labeled LA as checked by GC/MS, see Figure S1) dissolved in acetone was applied to the abdominal tip using a 10-μl microsyringe designed for GC on-column injection (Hamilton) as described in detail elsewhere (Blaul et al., 2014). For the precise application of the precursor solution, the procedure was performed with help of a stereomicroscope. After keeping the treated females for 20–24 h at 25°C, they were frozen and stored at −20°C until being used for chemical analysis. Preliminary tests had revealed that application of the pure solvent acetone per se has no impact on the wasps' longevity and survival and does not impact the abundance of ^{13}C in the fatty acids of treated wasps (Jatsch & Ruther, 2021). Therefore, we used 2-day-old untreated females of either species as controls for both this experiment and the ^{13}C-glucose feeding experiment (see Section 2.3).

2.3 | Feeding of ^{13}C-labeled α-D-glucose

Two-day-old females (Tz: 1-day-old) of either species were kept for two more days in groups of five wasps (n = 3 for each species, exception Ur: n = 5) 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). The microcentrifuge tubes were stored horizontally, to facilitate the wasps' access to the labeled glucose solution. Control wasps were kept under the same conditions without providing glucose solution. After the feeding period, wasps were frozen and kept at −20°C until being used for chemical analyses.

2.4 | Preparation of fatty acid methyl esters

After thawing, wasps were pooled for the extraction of raw lipids and the preparation of fatty acid methyl esters (FAME). Females applied with ^{13}C-labeled OA were extracted in groups of three individuals while those fed ^{13}C-labeled glucose were extracted in groups of five (Hh: 2, Bt: 10). Due to the extremely small size of Te, 50–100 individuals of both sexes were used per sample in this case. Three replicates per species and treatment were done (exception Ur: n = 5). For lipid extraction, wasps were homogenized with a glass pestle in 1.5 ml glass vials after adding 200 μl dichloromethane. The crushed wasps were extracted at room temperature for 30 min. Subsequently, the extracts were transferred to new 1.5 ml glass vials. The wasp residues were washed a second time with 200 μl dichloromethane and the combined extracts were dried under a stream of nitrogen. The lipids were 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. Subsequently, 200 μl of a solution of sodium hydrogen carbonate (5% in deionized water) and 200 μl of hexane were added. FAME were extracted by vortexing the vials for 10 s. The hexane phase was concentrated to 25 μl under nitrogen and used for GC/MS analysis.

2.5 | GC/MS analysis

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). Samples (1 μl, Te: 2 μl) were injected splitless at 300°C using a Shimadzu AOC 20i autosampler. The MS was operated in the electron impact ionization 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}. The temperature program started at 50°C, increased at 3°C min^{-1} to 280°C, and was kept at this temperature for 22 min. Identification of linoleic acid methyl ester (LAME) was done by comparison of the retention time and the mass spectrum with an authentic reference chemical (Sigma-Aldrich) which was analyzed four times to ensure the absence of the diagnostic ion *m/z* 312 and to calculate the relative abundance of diagnostic ion *m/z* 72 (RA_{72}) in the unlabeled reference (see below). We furthermore analyzed the methyl ester of the fully ^{13}C-labeled OA used for the application experiment to ensure the absence of fully ^{13}C-labeled LA in the precursor (Figure S1). In another control experiment, we analyzed a 1:1 blend of fully ^{13}C-labeled and unlabeled esterified linoleic acid to monitor the differing retention behavior of labeled and unlabeled diagnostic ions (see below). To conclude the conversion of ^{13}C-labeled OA to LA by the wasps in the application experiment, we monitored the diagnostic ions *m/z* 312 and 72 and calculated their relative abundances in relation to the unlabeled ions (*m/z* 294 and 67, respectively). Ions *m/z* 312 and 294 represent the molecular ions. The mass spectrum of LAME is dominated by a series of fragments of the general formula $[C_nH_{n-3}]^+$ (Fahy et al., 2007) explaining the shift of the base peak from *m/z* 67 $[C_5H_7]^+$ to *m/z* 72 $[^{13}C_5H_7]^+$ in the ^{13}C-labeled compound (Figure 1a,b). We extracted the respective ion traces and manually integrated their peak areas at the retention time of LAME. The RA_{72} and RA_{312} of the labeled ions were calculated by relating their peak areas to the added peak areas of the respective unlabeled and labeled ions. The diagnostic ion *m/z* 312 was neither detected in the mass spectra of LAME obtained from untreated control wasps nor in mass spectra of unlabeled synthetic LAME and RA_{72} never exceed 0.5% (Table 1). We therefore concluded that the conversion of OA to LA had occurred when RA_{312} was >0% and/or when RA_{72} was >0.5%. As for the feeding experiment with ^{13}C-labeled α-D-glucose, we detected the fully ^{13}C-labeled molecular ion only occasionally since the labeled ^{13}C-glucose-derived and unlabeled acetyl CoA-units are incorporated stochastically by the wasps during fatty acid biosynthesis. Therefore, we focused on RA_{72} to infer de novo biosynthesis of LA in ^{13}C-glucose-fed wasps. Apart from the abundance of diagnostic ions, the chromatographic behavior of labeled LAME can be used as a second criterion for their detection. Due to the inverse isotope effect of heavier isotopes (Matucha et al., 1991), ^{13}C-labeled LAME elutes ca. 1.5 s before unlabeled LAME on the high-performance 60 m capillary GC-column used in this study (Figure S2). Hence, we used a shift of the diagnostic ions *m/z* 312 and 72 in relation to the unlabeled ions as a second criterion to conclude incorporation of the labeled precursors into wasp-derived LAME.

2.6 | Statistical analysis

Statistical analysis was performed with PAST 4.03 scientific software (Hammer et al., 2001). RA_{72} and RA_{312} values in LAME from differently treated wasps were compared across species with Kruskal–Wallis tests followed by Bonferroni-corrected Mann–Whitney *U*-tests for individual comparisons.

3 | RESULTS

Females of 7 out of 8 species (all except Dc), that had been treated with an acetone solution of fully ^{13}C-labeled OA converted the labeled precursor partially into linoleic acid as indicated by the appearance of the labeled molecular ion *m/z* 312 in the mass spectra of LAME (RA_{312} mean ± *SE*: 1.35 ± 0.22%, Table 1, Figures 1c and 2a). This diagnostic ion was neither detectable in the mass spectra of synthetic LAME (Figure 1b) nor in LAME of the untreated control wasps of either species (Figure 2c, Mann–Whitney *U*-test across species: $p < 0.0001$). Apart from this, the production of LA from OA was detectable by an increase of the relative abundance of the diagnostic ion *m/z* 72 (RA_{72} mean ± *SE*: 1.6 ± 0.18%) when compared to the untreated controls (RA_{72} 0.29 ± 0.01, Mann–Whitney *U*-test across species: $p < 0.0001$, Table 1, Figures 1c and 2a). Both labeled diagnostic ions eluted 1.5 s earlier than the respective unlabeled ions further supporting the conversion of ^{13}C-labeled OA to LA by the wasps

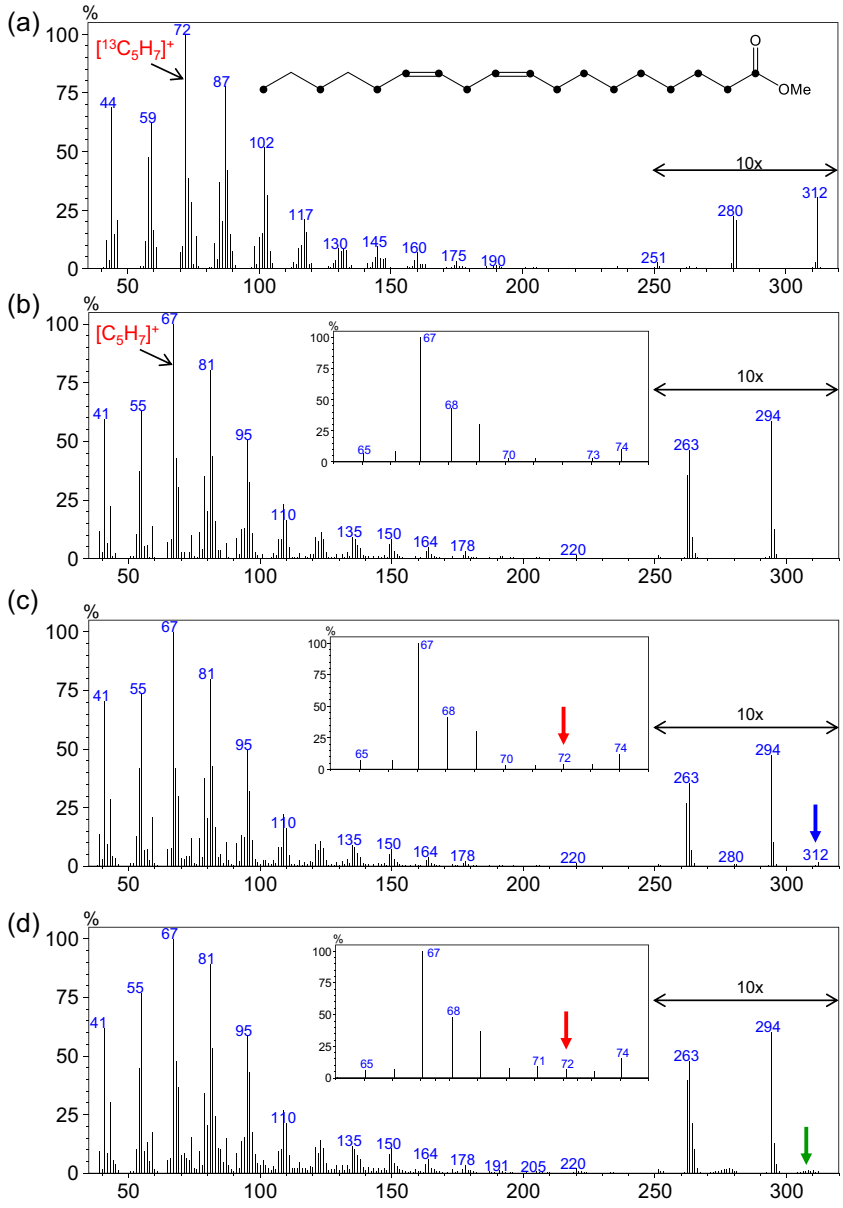

FIGURE 1 Mass spectra of (a) synthetic fully ^{13}C-labeled linoleic acid methyl ester (LAME), (b) synthetic unlabeled LAME, (c) LAME obtained by transesterification of lipid extracts from *Nasonia longicornis* females treated with an acetone solution of fully ^{13}C-labeled oleic acid, and (d) LAME obtained by transesterification of lipid extracts from *Habrobracon hebetor* females fed fully ^{13}C-labeled α-D-glucose. Inserts in panels (b–d) show the magnified region containing the diagnostic ion m/z 72 (red arrows) representing the fragment $[^{13}C_5H_7]^+$ that indicates the incorporation of ^{13}C into LAME. The blue arrow in panel (b) shows the diagnostic ion *m/z* 312 $[M]^+$ that indicates the conversion of the ^{13}C-labeled oleic acid precursor by the wasps. The green arrow in panel (c) indicates clusters of the respective fully and partially labeled diagnostic ions resulting from the incorporation of a varying number of ^{13}C-labeled glucose-derived acetate units into the fatty acid chain of LAME

TABLE 1 Relative abundance (RA) of ^{13}C-labeled diagnostic ions *m/z* 72 $[^{13}C_5H_7]^+$ and *m/z* 312 $[^{13}M]^+$ in mass spectra of synthetic linoleic acid methyl ester (LAME) and LAME from transesterified lipids of differently treated parasitic wasp females

			Control		^{13}C-OA		^{13}C-Glu
Family	LAME origin	Replicate	RA_{312}	RA_{72}	RA_{312}	RA_{72}	RA_{72}
	Synthetic	1	0.00	0.32	–	–	–
		2	0.00	0.30	–	–	–
		3	0.00	0.29	–	–	–
		4	0.00	0.30	–	–	–
Pteromalidae	Ac	1	0.00	0.25	–	–	**1.34**
		2	0.00	0.29	–	–	**0.55**
		3	0.00	0.26	–	–	0.41
	Dc	1	0.00	0.29	0.00	0.48	**0.41**
		2	0.00	0.34	0.00	**0.64**	**2.53**
		3	0.00	0.32	0.00	**0.54**	**1.95**
	Ld	1	0.00	0.28	0.00	**1.11**	**1.07**
		2	0.00	0.27	0.00	**1.48**	0.40
		3	0.00	0.28	0.00	**0.95**	0.39
	Mr	1	0.00	0.33	**0.66**	**0.78**	**0.59**
		2	0.00	0.31	**1.18**	**1.25**	0.31
		3	0.00	0.29	**0.54**	**0.77**	0.30
	Mu	1	0.00	0.00	**0.85**	**0.86**	0.35
		2	0.00	0.27	**0.76**	**0.75**	0.41
		3	0.00	0.27	**1.44**	**1.52**	**1.40**
	Ng	1	0.00	0.38	**2.16**	**1.97**	**6.63**
		2	0.00	0.00	**2.35**	**2.40**	**5.23**
		3	0.00	0.31	**2.34**	**1.86**	**3.29**
	Nl	1	0.00	0.00	**3.62**	**3.97**	**0.70**
		2	0.00	0.28	**2.71**	**2.63**	**3.59**
		3	0.00	0.26	**4.67**	**3.59**	**0.58**
	Ur	1	0.00	0.31	0.00	**0.74**	**0.52**
		2	0.00	0.39	**1.71**	**1.43**	**1.99**
		3	0.00	0.30	**1.97**	**2.05**	**2.25**
		4	–	–	**2.04**	**2.34**	–
		5	–	–	**1.37**	**1.54**	–
Encyrtidae	Ep	1	0.00	0.29	–	–	**0.97**
		2	0.00	0.30	–	–	**0.89**

(Continues)

TABLE 1 (Continued)

Family	LAME origin	Replicate	Control		^{13}C-OA		^{13}C-Glu
			RA_{312}	RA_{72}	RA_{312}	RA_{72}	RA_{72}
		3	0.00	0.32	–	–	**0.86**
	Tz	1	0.00	0.43	**1.67**	**1.87**	**3.35**
		2	0.00	0.44	**1.28**	**1.81**	**1.47**
		3	0.00	0.44	**1.36**	**1.60**	**1.50**
Bethylidae	Ct	1	0.00	0.33	–	–	0.30
		2	0.00	0.31	–	–	0.30
		3	0.00	0.32	–	–	0.33
Eulophidae	Bt	1	0.00	0.35	–	–	0.36
		2	0.00	0.29	–	–	0.39
		3	0.00	0.32	–	–	**0.75**
Trichogrammatidae	Te	1	0.00	0.29	–	–	**2.32**
		2	0.00	0.29	–	–	**1.82**
		3	0.00	0.31	–	–	**1.83**
Braconidae	Hh	1	0.00	0.42	–	–	**0.52**
		2	0.00	–	–	–	**6.07**
		3	0.00	0.36	–	–	**6.48**

Note: Females were either treated with fully ^{13}C-labeled oleic acid (OA), fed fully ^{13}C-labeled α-D-glucose (Glu), or left untreated (Control). RA was calculated by relating the peak areas of the diagnostic ions *m/z* 72 and 312 to the added peak areas of the diagnostic ions and the respective unlabeled ions *m/z* 67 and 294. $RA_{312} > 0.0\%$ and $RA_{72} > 0.05\%$ indicate the incorporation of ^{13}C-labeled OA and ^{13}C-glucose-derived acetyl-CoA units, respectively. These samples are printed in bold. Species names as follows: *Dibrachys cavus* (Dc), *Muscidifurax raptorellus* (Mr), *M. uniraptor* (Mu), *Nasonia giraulti* (Ng), *N. longicornis* (Nl), *Tachinaephagus zealandicus* (Tz), *Urolepis rufipes* (Ur), *Anisopteromalus calandrae* (Ac), *Barsiscapus tineivorus* (Bt), *Cephalonomia tarsalis* (Ct), *Exoristobia philippinensis* (Ep), *Habrobracon hebetor* (Hh), *Lariophagus distinguendus* (Ld), and *Trichogramma evanescens* (Te).

(Figures 2a and S2). Increased RA_{72} in the mass spectra of LAME (2.04 ± 0.49%, Mann–Whitney *U* test across species: $p < 0.0001$) in combination with the expected retention time shift was also detected in 1–3 samples per species in 13 out of the 14 species fed ^{13}C-labeled glucose (Table 1, Figures 1d and 2b) demonstrating that the tested species are not only able to produce fatty acids de novo but also to introduce double bonds at positions Δ9 and Δ12. In some samples, magnification of the molecular ion region of the mass spectra furthermore revealed not only the presence of the fully ^{13}C-labeled molecular ion *m/z* 312 but also clusters of partially ^{13}C-labeled molecular ions (*m/z* 300, 302, 304, 306, 308, and 310) resulting from the incorporation of a varying number of ^{13}C-labeled acetate units (Figure 1d).

4 | DISCUSSION

The present study demonstrated the ability to synthesize LA in 13 out of the 14 species studied (exception: Ct) belonging to six families by at least one of the two labeling techniques. These results indicate, that Δ12-desaturases are widespread among parasitic Hymenoptera and neither restricted to those species of the *Nasonia*

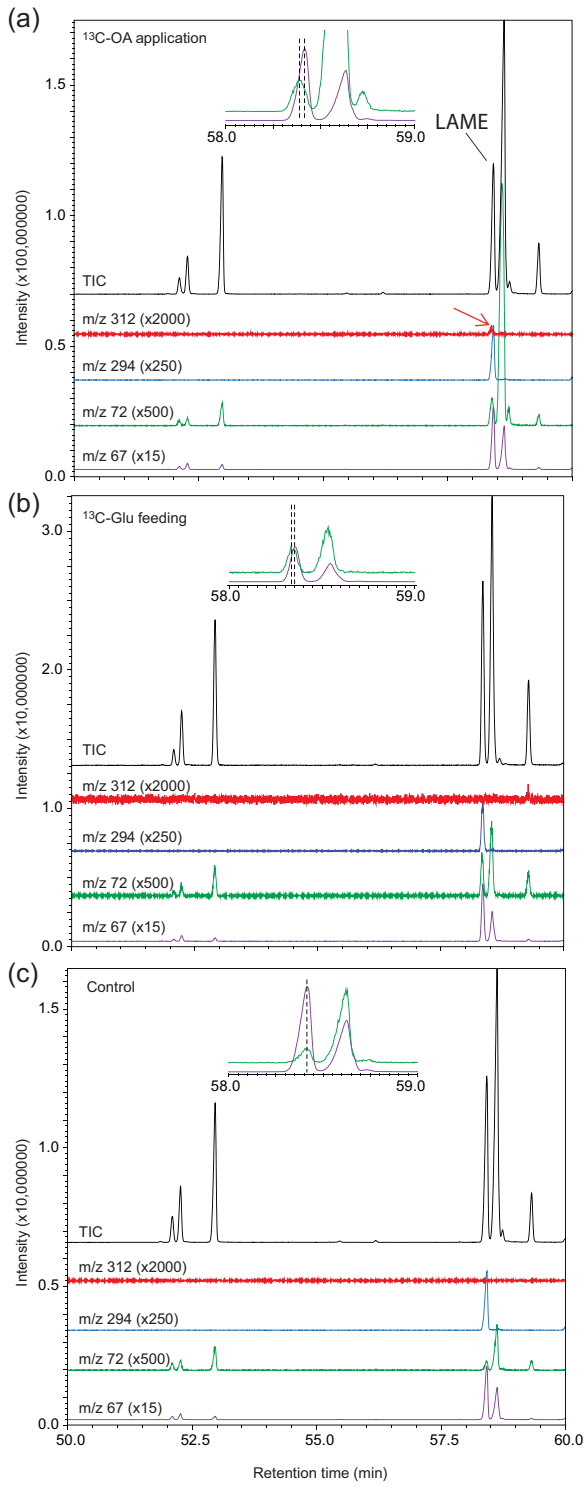

FIGURE 2 (See caption on next page)

group that depend on LA for pheromone biosynthesis nor to members of the Pteromalidae in general. Feeding experiments with non-sterile insects cannot completely rule out that symbiotic microorganisms are involved. Experiments with wasps cured from symbionts by antibiotics might shed light on this question (Brückner et al., 2020). However, the two Δ12-desaturases functionally characterized in *N. vitripennis* suggest that the enzymes synthesizing LA are insect-derived. The availability of sequence information for Nvit_D12a and Nvit_D12b from *N. vitripennis* opens the door for the identification of other Δ12-desaturases from parasitic wasps and for the study of their phylogenetic relationships. This will help to answer the question whether Δ12-desaturases have evolved once or several times in this group of insects. Comparison of the Nvit_D12a/b sequences with functionally characterized Δ12-desaturases from beetles and crickets (Haritos et al., 2014; Zhou et al., 2008) suggested that insect-derived Δ12-desaturases have evolved independently from each other from ancestral Δ9-desaturases of the respective genomes (Semmelmann et al., 2019; Zhou et al., 2008).

The fact that parasitic wasps are capable of synthesizing LA from glucose confirms that these insects do not lack lipogenesis as several previous studies had suggested (Visser & Ellers, 2008; Visser et al., 2010, 2012). Due to their parasitic lifestyle, parasitic wasps were assumed to receive sufficient amounts of lipids from their hosts during larval development making the ability to synthesize fatty acids redundantly and finally have led to an evolutionary trait loss (Visser et al., 2010). Labeling studies involving feeding experiments with ^{13}C-labeled glucose, however, have demonstrated that parasitic wasps are able to convert glucose into palmitic and stearic acid (Prager et al., 2019; Ruther et al., 2021). The present study demonstrates that parasitic wasps possess the enzymatic machinery to not only synthesize these primary products of fatty acid biosynthesis but also to introduce double bonds at positions 9 and 12 to produce OA and LA. In some insects, LA has been shown to be further processed into C_{20}-PUFA by the interplay of elongases and desaturases (Jurenka et al., 1987, 1988) which in turn can be converted to multifunctional eicosanoid hormones such as prostaglandins (Stanley & Kim, 2019; Stanley, 2006; Stanley et al., 2009). Hence, parasitoids can not only independently replenish ebbing reserves of saturated and unsaturated fatty acids but might also produce these fatty acid-derived signaling molecules. A recent study on the production of the LA-derived sex pheromone in *N. vitripennis*, however, suggested that adults, despite possessing the ability to produce LA, benefit from nutritional supply with LA as larvae (Brandstetter & Ruther, 2016).

Our study showed that both topical application of ^{13}C-labeled OA and the feeding of ^{13}C-labeled glucose are suitable techniques for the detection of LA biosynthesis in insects and that the two techniques can complement each other very well. In some species of parasitic wasps, both methods worked equally well, while in others either OA application or glucose feeding turned out to be superior. The big advantage of glucose feeding is the relatively low price of labeled precursors. However, some parasitoids are reluctant to ingest larger amounts of sugar solutions and some insect species do not feed at all as adults. In these cases, OA-application is probably the better choice. LA biosynthesis after ^{13}C-glucose feeding was not detectable in Ct. Why this species differs from the others with respect to LA biosynthesis needs further investigation. We can exclude, however, that Ct rejected the offered glucose solution, because the glucose-derived ^{13}C-label was detectable in the saturated fatty acids of the same Ct samples. Both labeling techniques reported here are convenient to perform and straightforward to interpret. They might be used to reinvestigate species from those insect orders, for which the use of radiolabeling techniques had

FIGURE 2 GC/MS analysis of transesterified lipid extracts from *Muscidifurax uniraptor* females (a) treated with an acetone solution of fully ^{13}C-labeled oleic acid (OA), (b) fed fully ^{13}C-labeled α-D-glucose, and (c) unfed control wasps. Each panel shows the total ion current chromatograms (TIC) and the extracted ion chromatogram of the labeled/unlabeled molecular ions *m/z* 312/294 $[M]^+$ and the diagnostic ion pair *m/z* 67/72 $[C_5H_7]^+$ (magnification factors given in brackets). The red arrow in panel (a) indicates the peak of the ^{13}C-labeled linoleic acid methyl ester (LAME) in females treated with ^{13}C-labeled OA. The inserts show a magnification of the elution profile of LAME showing the slightly decreased retention time of the ^{13}C-labeled diagnostic ion *m/z* 72 due to the inverse isotope effect of heavier isotopes (for more details see text). GC/MS, gas chromatography–mass spectrometry

failed to demonstrate the LA biosynthesis so far (Cripps et al., 1986), thus enabling a comprehensive picture on the ability of insects to synthesize this multifunctional metabolite.

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AUTHOR CONTRIBUTIONS

Bastian Broschwitz: Formal analysis (supporting); investigation (equal); writing review & editing (supporting). **Lorena Prager**: Investigation (supporting); writing review & editing (supporting). **Tamara Pokorny**: Resources (supporting); writing review & editing (supporting). **Joachim Ruther**: Conceptualization (lead); formal analysis (lead); investigation (equal); methodology (lead); project administration (lead); supervision (lead); visualization (lead); writing original draft (lead).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author on reasonable request.

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