Why do many parasitoids lack adult triglyceride accumulation, despite functioning fatty acid biosynthesis machinery?

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Abstract

Lipid reserves are a major energy source and play a key role in survival and reproduction. Lipid accumulation is a conserved metabolic pattern in insects, yet in most parasitoid species no adult lipid mass gain takes place, even when nutrients are consumed ad libitum. This deviating metabolic phenotype has evolved repeatedly in phylogenetically dispersed parasitoid species, and forms a conundrum as it leads to significant constraints on energy allocation toward key adult functions. Recent work on the underlying genetic and biochemical mechanisms has spurred a debate on the interpretation of results obtained using different methodologies, which is in part due to semantics. This highlights the need for a reflection of research on fat accumulation that unites new insights and terminology. In this paper, we first compile a comprehensive list of fat accumulation studies performed during the last 25 years, including 35 parasitic wasp species. Second, we clarify the distinct metabolic pathways involved in the complex process of lipogenesis and propose updated, unambiguous terminology, including 'fatty acid biosynthesis' and 'lack of adult fat accumulation'. Third, we discuss the benefits and drawbacks of the main methods available to measure fatty acid biosynthesis or adult fat accumulation. Most importantly, gravimetric/colorimetric and isotope labelling methods provide complementary information, provided that they are applied with appropriate controls and interpreted correctly. Last, we present avenues for future research that combine chemistry, ecology, and evolution into an integrative approach, which is what is now needed to understand the dynamics of fat accumulation in parasitoids.

Key words

Parasitoids; Lipid synthesis; Life histories; Metabolism

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Declaration of interests

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The evolution of a deviating metabolic phenotype in parasitoid insects

Lipids are the main energetic reserve for insects (Arrese and Soulages, 2010), yet in contrast with other insects adults of most parasitoids do not increase their lipid reserves when fed surplus carbohydrates. In the past 25 years, this lack of adult fat mass gain has not only been demonstrated in more than 29 parasitoid wasp species (Table 1), but also in a wider taxonomic range of insects, including 3 parasitoid flies and a parasitoid beetle (Kemp and Alcock, 2003; Fadamiro *et al.*, 2005), and in comparison with >90 species of non-parasitoid insects (Visser *et al.*, 2010). Finding the same atypical metabolic phenotype in evolutionary independent parasitoid lineages is evidence for convergent evolution for deviating lipid metabolism in parasitoids. Visser & Ellers (2008) proposed that lack of adult fat accumulation may be the evolutionary consequence of a parasitoid lifestyle. Parasitoid larvae can assimilate the lipids produced by their host and thus carry over large lipid stores from larval feeding into adulthood (i.e., up to 30 to 40% of the parasitoid's dry body weight; Visser *et al.*, 2018; Visser *et al.*, 2021). This may render *de novo* lipid synthesis from adult feeding either unnecessary or too energetically costly to maintain, leading to loss of the adult fat accumulation phenotype (Ellers *et al.*, 2012). Because lack of fat accumulation prevents adult parasitoids from replenishing ebbing lipid reserves, it can lead to significant constraints on energy allocation toward key adult functions, such as maintenance, dispersal, and reproduction (Jervis, Ellers and Harvey, 2008). This thus poses a conundrum as to why most parasitoids do not increase their lipid reserves as adults, which is still unresolved.

More recently, focus has shifted towards understanding the mechanistic aspects of the lack of adult fat accumulation by studying the underlying genetic and biochemical changes that can cause this deviating metabolic phenotype (Visser *et al.*, 2012, 2017, 2021; Lammers *et al.*, 2019; Prager, Bruckmann and Ruther, 2019; Ruther, Prager and Pokorny, 2021). Functionality of the genes and enzymes involved in lipid synthesis was found to be largely unimpaired, pointing towards the evolution of regulatory rather than structural changes. However, the evidence for functionally intact genetic and enzymatic machinery has also provoked contentions that the claim for widespread lack of fat accumulation in parasitoids needs to be re-evaluated. This has created an apparent contradiction between researchers working on the topic that we believe is fueled only by ambiguous semantics and misinterpretations. In this opinion paper, we aim to unite and advance research on lipid synthesis and fat accumulation in parasitoids by proposing new and unambiguous definitions and highlighting important proximate and ultimate questions for future studies.

Table 1: List of hymenopteran parasitoid species for which studies have looked at three different measures: fat accumulation, gene transcription of genes involved in fatty acid synthesis and/or fatty acid synthesis in adults. “Yes” refers to an increase in accumulated lipids, gene transcripts or fatty acids; “no” refers to similar or lower amounts. Studies on the same species are cited within the same line when having similar results and using similar methodologies or in separate lines otherwise. At the bottom of the table, bold numbers before the slash symbol indicate the number of species tested for each measure whereas numbers after the slash symbol indicate the number of studies that have tested each measure.

Species	Fat accumulation	Gene transcription	Fatty acid synthesis	Reference
Acrolyta nens	yes	-	-	(Visser *et al.*, 2010)
Anisopteromalus calandrae	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Apanteles aristoteliae	no	-	-	(Lightle, Ambrosino and Lee, 2010)
Aphelinus abdominalis	no	-	-	(Visser *et al.*, 2010)
Aphidius ervi	no	-	-	(Visser *et al.*, 2010)
Aphidius rhopalosiphi	no	-	-	(Visser *et al.*, 2010; Le Lann *et al.*, 2012)
Aphidius picipes (=avenae)	no	-	-	(Visser *et al.*, 2010; Le Lann *et al.*, 2012)

Aphidius platensis	no	-	-	(Souza *et al.*, 2018)
Asobara tabida	no	-	-	(Ellers, 1996; Visser *et al.*, 2010; Le Lann *et al.*, 2014)
Asobara tabida	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Baryscapus tineivorus	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Cephalonomia tarsalis	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Cotesia glomerata	no	-	-	(Visser *et al.*, 2010)
Cotesia rubecula	no	-	-	(Visser *et al.*, 2010)
Diadegma insulare	no	-	-	(Lee, Heimpel and Leibee, 2004)
Dibrachys cavus	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Diglyphus isaea	no	-	-	(Zhang *et al.*, 2011)
Eupelmus vuilletti	no	-	-	(Casas *et al.*, 2015; Muller *et al.*, 2017)
Eupelmus vuilletti	-	-	no	(Giron and Casas, 2003; (Visser *et al.*, 2017)
Exersites comstockii	-	-	yes	(Thompson and Barlow, 1972)
Gelis agilis	yes	-	-	(Visser *et al.*, 2010)
Gelis agilis	-	-	yes	(Visser *et al.*, 2017)
Gelis areator	-	-	yes	(Visser *et al.*, 2017)
Goniozus nephantidis	no	-	-	(Visser *et al.*, 2010)
Goniozus legneri	no	-	-	(Visser *et al.*, 2010)
Habrobracon hebetor	no	-	-	(Akman Gündüz *et al.*, 2010; Varer Işitan, Akman Gündüz and Gülel, 2011)
Habrobracon hebetor	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Lariophagus distinguendus	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Leptopilina boulardi	no	-	-	(Visser *et al.*, 2010)
Leptopilina boulardi	yes + no	-	-	(Moiroux *et al.*, 2010, 2012)
Leptopilina heterotoma	no	-	-	(Eijs, Ellers and van Duinen, 1998; Visser *et al.*, 2018)
Leptopilina heterotoma	yes	-	-	(Visser *et al.*, 2010)
Leptopilina heterotoma	yes + no	-	-	(Le Lann *et al.*, 2014)
Leptopilina heterotoma	yes + no	-	yes + no	(Visser *et al.*, 2021)
Leptopilina heterotoma	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Lysibia nana	yes	-	-	(Visser *et al.*, 2010)
Macrocentrus grandii	no	-	-	(Olson *et al.*, 2000)
Meteorus pulchricornis	no	-	-	(Sheng *et al.*, 2019)
Meteorus pulchricornis	no	yes	-	(Wang *et al.*, 2020)
Microplitis mediator	no	-	-	(Luo *et al.*, 2010)
Muscidifurax raptorellus	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Muscidifurax uniraptor	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Nasonia vitripennis	no	-	-	(Rivero and West, 2002)
Nasonia vitripennis	no	no	no	(Visser *et al.*, 2012)

Nasonia vitripennis	-	no	-	(Lammers *et al.*, 2019)
Nasonia vitripennis	-	-	yes	(Prager, Bruckmann and Ruther, 2019)
Nasonia vitripennis	no	-	yes	(Ruther, Prager and Pokorny, 2021)
Nasonia giraulti	-	-	yes	(Prager, Bruckmann and Ruther, 2019)
Nasonia longicornis	-	-	yes	(Prager, Bruckmann and Ruther, 2019)
Neochrysocharis formosa	no	-	-	(Wang *et al.*, 2014)
Orthopelma mediator	no	-	-	(Visser *et al.*, 2010; Visser *et al.*, 2013))
Pachycrepoideus vindemmiae	no	-	-	(Visser *et al.*, 2010)
Pimpla turionellae	no	-	-	(Ortel, 1991)
Pteromalus bedeguaris	no	-	-	(Visser *et al.*, 2013)
Pteromalus puparum	yes	-	-	(Visser *et al.*, 2010)
Spalangia erythromera	no	-	-	(Visser *et al.*, 2010)
Spalangia cameroni	no	-	-	(Taylor, King and Burgess, 2022)
Tachinaephagus zealandicus	no	-	-	(Lammers *et al.*, 2020)
Tachinaephagus zealandicus	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Trichogramma evanescens	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Trichopria drosophilae	no	-	-	(Visser *et al.*, 2010)
Urolepis rufipes	-	-	yes	(Ruther, Prager and Pokorny, 2021)
Venturia canescens	no	-	-	(Casas *et al.*, 2003; Gomes *et al.*, 2021)
No. of species/No. of studies	**35/31**	**2/3**	**20/7**	

Fat synthesis in parasitoids versus other insects

Within the insect fat body, more than 90% is composed of triglycerides (i.e., fat)(Arrese and Soulages, 2010). Triglycerides, consisting of three fatty acids and a glycerol molecule, are the most efficient form in which energy can be stored in insects. Fat or triglycerides can be synthesized from dietary carbohydrates, fatty acids or proteins (Arrese and Soulages, 2010). Typically, the consumption of sugars and other carbohydrates, which are a major part of the diet of insects, leads to the synthesis of fatty acids that are subsequently used for the synthesis of triglycerides (Arrese and Soulages, 2010). Other direct precursors of triglycerides are diglycerides, which are esterified by the enzyme diacylglycerol acyltransferase using fatty acyl-coA (Figure 1). The synthesis of tri- and diglycerides from monoglycerides is referred to as the monoglyceride synthesis pathway. The monoglyceride pathway can readily use lipid molecules produced during catabolism, such as degraded phospholipids or deacylated triglycerides through the action of lipases. Triglycerides can also be synthesized *de novo* through the glycerophosphate pathway (or the so-called 'Kennedy pathway'). Here, glycerol-3-phosphate (G3P) is converted to triglyceride by catalysis in four steps by glycerophosphate acyltransferase (GPAT), acylglycerophosphate acyltransferase (AGPAT), lipid phosphate phosphohydrolase (LPP) and diacylglycerol acyltransferase (DGAT). In insects, both pathways can be used to synthesize triglycerides, in the gut, as well as the fat body (Beenakkers, Van der Horst and Van Marrewijk, 1985; Soulages *et al.*, 2015). In adult parasitoids, fatty acid synthesis in response to sugar-feeding has been observed in some species, yet accumulation of triglycerides is lacking for most species (Table 1), even though the metabolic pathways and associated genes are present and functional (Werren *et al.*, 2010; Visser *et al.*, 2012, 2021; Kraaijeveld *et al.*, 2019; Lammers *et al.*, 2019). For parasitoid larvae, early work revealed that, compared to typical triglyceride synthesis, only little triglycerides were synthesized *de novo* through the glycerophosphate pathway, while the majority of triglycerides were produced through the monoglyceride pathway (Barlow and Jones, 1981; Jones, Barlow and Thompson, 1982). For larvae of the parasitoids *Exeristes roborator* and *Itoplectis conquisitor*, respectively 75% and 97% of triglycerides are formed from diglycerides through the action of the monoglyceride pathway. Substantial inhibition of the *de novo* triglyceride synthesis pathway could explain the apparent lack of triglyceride accumulation, also this still remains to be investigated in adults.

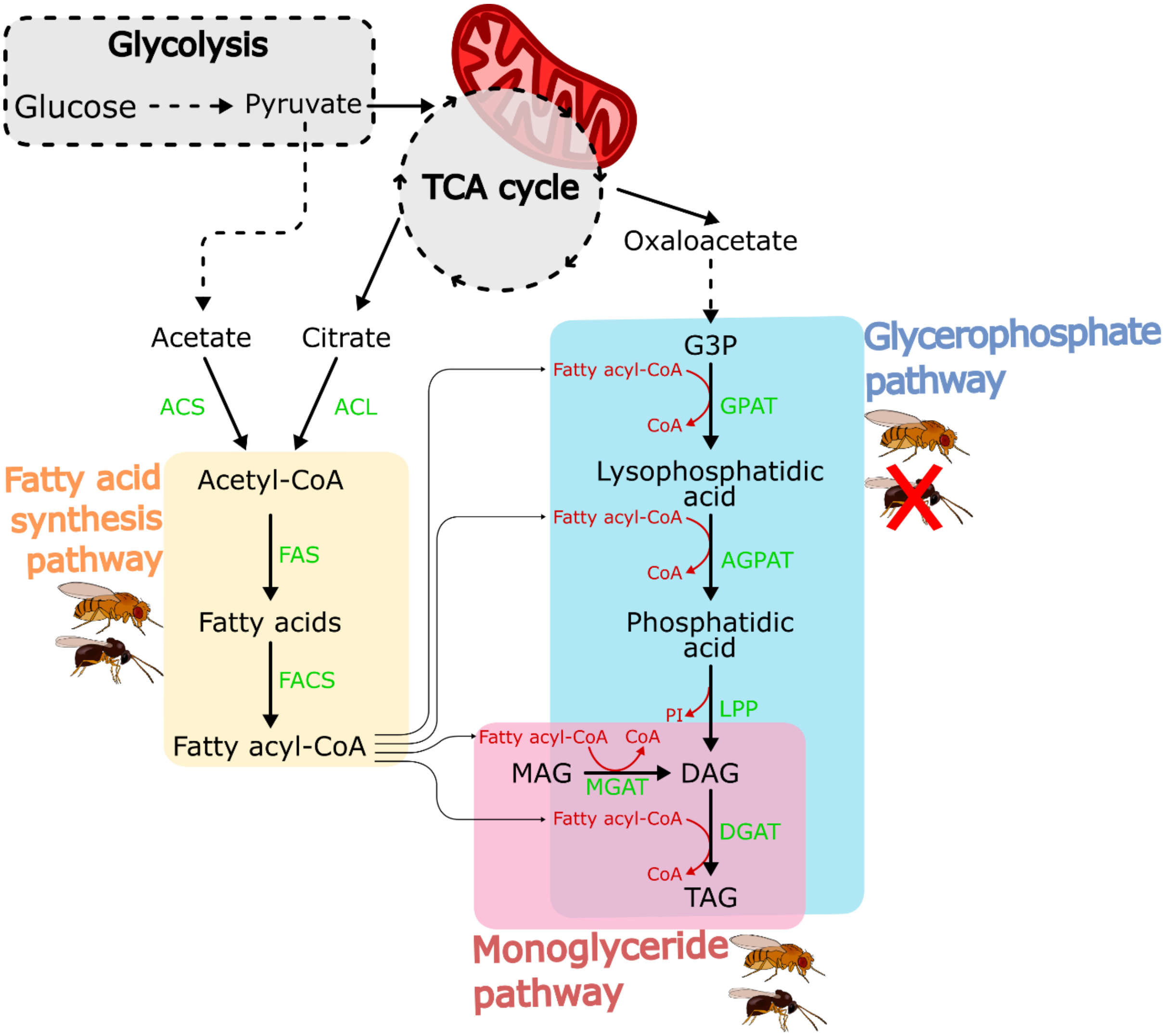

Figure 1. Lipid synthesis in adult parasitoids and other insects. Ingested sugars are converted to pyruvate through glycolysis, after which pyruvate is whether converted to acetate or used in the tricarboxylic acid (TCA) cycle to produce substrates for triglyceride (TAG) synthesis (citrate and glycerol-3-phosphate, i.e. G3P). Acetate and citrate are converted to acetyl-CoA that enters the fatty acid synthesis pathway (left), and oxaloacetate is used to produce (G3P) that enters the glycerophosphate pathway (right). Triglycerides are also synthesized through the monoglyceride pathway. All three pathways are functional and active in insects in general. In contrast, parasitoids do not seem to synthesize triglycerides *de novo* through the glycerophosphate pathway, leading to the lack of triglyceride accumulation. Depicted metabolic pathways are simplified with enzymes highlighted in green, and reactions shown in red. Abbreviations: ACL, ATP-citrate lyase; ACS, Acetyl-coA synthetase; FAS, Fatty acid synthase; FACS, Fatty acyl-coA synthetase; GPAT, glycerophosphate acyltransferase; AGPAT, acylglycerophosphate acyltransferase; LPP, lipid phosphate phosphohydrolase; DGAT, diacylglycerol acyltransferase; MGAT, monoacylglycerol acyltransferase; MAG, mono(acyl)glycerides; DAG, di(acyl)glycerides; TAG, tri(acyl)glycerides; TCA cycle: tricarboxylic acid cycle.

Semantics in research on parasitoid lipid metabolism

For over a decade, the term 'lack of lipogenesis' has been used in research to describe the deviating lipid metabolism of insect parasitoids (Visser and Ellers, 2008). Using the term lipogenesis has been a poor choice, however, because lipogenesis consists of two equally important parts: first, the conversion of glucose or other substrates into fatty acids, and second subsequent biosynthesis of triglycerides (Kersten, 2001). We propose to use the more accurate terms 'fatty acid biosynthesis' and 'lack of adult fat accumulation' to refer to separate and specific processes. 'Fat accumulation' is the process of bulk synthesis and storage of triglycerides across a broad range of taxa (e.g., Pullin, 1987; Teixeira *et al.*, 2003; Schmid *et al.*, 2005; Guo *et al.*, 2008; Lu *et al.*, 2018; Sinclair and Marshall, 2018; Didion *et al.*, 2021). In contrast, the term 'fatty acid biosynthesis' more narrowly defines and denotes the conversion of acetyl-CoA and malonyl-CoA into the fatty acid palmitate (i.e., a substrate for triglyceride synthesis; Figure 1). A clear distinction between these terms is crucial to reveal the mechanisms by which parasitoids accumulate, or not, substantial fat reserves as adults.

Considerations when measuring fatty acid synthesis and fat accumulation in parasitoids

Different methods can be used to measure lipid accumulation in insects when adults have access to a dietary source of sugars and/or proteins, but the different methods do not necessarily provide the same information about the phenotype. Gravimetric and colorimetric methods (based on weight, as in Visser *et al.*, 2010, 2012, 2018, 2021, or conversion of chromophores measured by light absorbance, as in Muller *et al.*, 2017, respectively) focus on bulk fat accumulation compared between a group of individuals measured at emergence and after feeding on sugar. Bulk methods are relatively straightforward to apply without the need for high-tech equipment and, more importantly, they provide information about the impact of (lack of) fat accumulation on the energy stores of individuals. However, observing a lack of fat accumulation using bulk methods cannot be taken as proof for absence of fatty acid or triglyceride synthesis in adults, because the net increase in fat reserves hinges on the rate at which lipids are metabolized (Giron and Casas, 2003; Visser *et al.*, 2010, 2012). Alternatively, isotope labelling methods (deuterium, ^{13}C) allow tracing the dietary conversion of sugars into fatty acids that can subsequently be used for triglyceride synthesis (Giron & Casas, 2003; Prager et al., 2019; Ruther et al., 2021; Visser et al., 2012, 2017, 2021). The isotope methods are powerful tools to provide insight into the mechanisms of fatty acid synthesis, but are not suitable to assess the lipid accumulation phenotype as it does not provide information about the total energy stores of individuals. This is because in typical stable isotope studies only a subset of fatty acids is analyzed (but see Wood, Harlow and Lambremont, 1969 showing that in a lepidopteran 95% of the fatty acids used in triglycerides are C16 and C18 fatty acids) and incorporation levels of isotopes is what is most commonly quantified. Moreover, there are several methodological considerations that need to be taken into account with the isotope method to avoid spurious results and interpretations.

First, using isotope labelling methods to show that fatty acid synthesis is occurring or not requires not only an estimation of incorporation levels of tracers into fatty acids in different parasitoid species, but also a positive control of incorporation using an insect that has typical high fatty acid synthesis and high adult fat accumulation (as in Visser *et al.*, 2012, 2017). Including an insect that synthesizes and accumulates substantial fat stores, allows an estimation as to how much the parasitoid under investigation deviates from a typical lipid metabolic model. Second, isotope tracing studies on fat accumulation require appropriate negative controls. Incorporation rates should be compared between wasps that are fed with and without the isotope. If one compares, for example, isotope-fed individuals with starved individuals, two different and potentially confounding factors are varied at the same time: isotope labelling (yes or no) and feeding status (fed vs starved)(Prager, Bruckmann and Ruther, 2019; Ruther, Prager and Pokorny, 2021; Multerer, Wendler and Ruther, 2022). Third, in isotope labeling studies, the choice of isotope matters. For example, there is some controversy with regard to the use of ^{13}C labeling techniques for measuring fatty acid synthesis, because discrimination against ^{13}C incorporation into the lipid fraction increases with dietary ^{13}C concentration (Wessels and Hahn, 2010). The advantage of using the equally stable hydrogen isotope, deuterium, is that it is the cheapest and easiest method to measure fatty acid synthesis from acetate, glucose or other precursors (i.e., proteins) and that it can provide insights also into other metabolic processes (Murphy, 2006). In parasitoids, the tracing of deuterium was indeed shown to detect biologically relevant variation in fatty acid incorporation across treatments (Visser *et al.*, 2021). An alternative to using deuterated water is to use deuterated glucose (Wallace *et al.*, 2018) or deuterated acetate (Votruba, Zeddun and Schoeller, 2001), which have so far not been used in parasitoid studies.

The overall conclusion is that there is no singular suitable method to study the lack of fat accumulation in parasitoids. Both type of methodologies (gravimetry/colorimetry and isotope labelling) offer valuable and complementary information, provided that they are applied and interpreted appropriately. Ideally, these two methodologies should be used concomitantly to understand how they relate to each other and whether estimated rates of fatty acid synthesis could be interpreted as a good estimate of total triglyceride accumulation.

Current research and perspectives

More than 25 years after the first discovery of lack of adult triglyceride accumulation in a parasitoid species (Ellers, 1996), the question still stands - why do most adult parasitoids show atypically low triglyceride accumulation and how can parasitoids cope with such low amounts of newly synthesized fat in adulthood? What we know is that several species of hyperparasitoids, that show adult triglyceride accumulation, carry over low amounts of lipids into adulthood from the host (4-8% lipids at emergence; Visser *et al.*, 2010, 2017). Also, when development occurs on very lean hosts (Visser *et al.*, 2021), *de novo* fatty acid synthesis and triglyceride accumulation can occur in significant quantities in an adult parasitoid that consume excess carbohydrates. Moreover, in the parasitoid *Leptopilina heterotoma,* there appears to be genetic variation in how much fatty acids are synthesized, as well as how much fat is accumulated by adults among wasp lines (Visser *et al.*, 2021). However, this species belongs to only few that were found to accumulate substantial fat as adults (Table 1).

Some recent studies focusing on fatty acid synthesis (but not fat accumulation) in parasitoids have found that fatty acids are produced in some species (Prager, Bruckmann and Ruther, 2019; Ruther, Prager and Pokorny, 2021; Multerer, Wendler and Ruther, 2022). This is consistent with the fact that the fatty acid synthase (*fas*) gene is intact in several parasitoid species (Kraaijeveld et al., 2019; Visser et al., 2012, 2021) and its multiple paralogs are constitutively expressed in other species (Lammers et al., 2019; Visser et al., 2012; Wang et al., 2020), suggesting that *fas* plays a functional role in several aspects of the insect's biology, for instance pheromone synthesis (Blaul *et al.*, 2014) and production of cuticular hydrocarbons (Moriconi *et al.*, 2019; Holze, Schrader and Buellesbach, 2021). An alternative hypothesis is that the insect is not synthesizing these fatty acids themselves but that microbes are involved. The (gut) microbiota could contribute to the production of some fatty acids that are subsequently absorbed by the wasps' digestive tract or produced in other tissues. Bacteria use type II dissociate fatty acid synthesis and possess all the necessary fatty acid biosynthetic genes (Rock and Jackowski, 2002). Nevertheless, it has so far remained unclear what role the parasitoid microbiota plays. Conclusive evidence of the role played by microbiota in producing fatty acids *de novo* requires experiments using germ-free and endosymbiont-free parasitoids. Another way to increase our understanding of the mechanisms underlying lack of triglyceride accumulation is to use inhibitors for key enzymes in the lipid synthesis pathway (e.g., Ventura *et al.*, 2015), or manipulate expression of the underlying genes using RNA interference (e.g., Guo *et al.*, 2008) or CRISPR-Cas9 (Sander and Joung, 2014).

Future studies on the evolution of fat accumulation should include parasitoids from distinct families, e.g., the Cryptinae, where some hyperparasitoid species were previously shown to accumulate fat (Visser *et al.*, 2010). This would provide a higher resolution of the occurrence of (lack of) fat accumulation within phylogenetic lineages and whether there is a link with the hyperparasitoid lifestyle or typical parasitoid life history syndromes (Visser *et al.*, 2010). An important consideration is to take into account plasticity of adult triglyceride accumulation, generated through variation in parasitoid genotype (Visser *et al.*, 2021) and environmental conditions (Moiroux *et al.*, 2010, 2012; Le Lann *et al.*, 2014), including host lipid content (Visser *et al.*, 2021) as well as potential effects of the microbiome (Cronan and Thomas, 2009; Jiménez *et al.*, 2019). At the mechanistic level, more physiological studies are needed in adults to fully understand how lack of fat accumulation is brought about and whether the same mechanisms evolved in distinct parasitoids. Furthermore, molecular studies focusing on tissue-specific gene expression involving the monoglyceride and *de novo* triglyceride synthesis pathways can be used to identify when and how fat accumulation is shut off.

Conclusion

We urge that future work on lipid metabolism in parasitoids uses more precise definitions for fat/triglyceride accumulation and the process of fatty acid biosynthesis. The unambiguous terminology proposed here aims at uniting researchers to understand the biological relevance of the lack of adult triglyceride accumulation in parasitoids and avoid misleading conclusions (e.g., that adult parasitoids in general can replenish ebbing lipid resources through substantial lipid accumulation (Kaczmarek and Boguś, 2021). Combining chemistry, ecology, and evolution into a truly interdisciplinary and integrative approach is what we now need for understanding the dynamics of fat accumulation in parasitoids, and ultimately the downstream life history consequences of accumulating stored nutrients, or not, from adult dietary intake.

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