Biological relevance unites, semantics divides: a comment on Ruther et al (2021)

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Ruther et al (2021) evaluated fatty acid synthesis in several parasitic wasp species to test if the general finding that lipogenesis in parasitoids is lacking is upheld (Visser et al 2010 PNAS). As proposed by Visser & Ellers (2008), parasitoids can readily assimilate the triglyceride stores produced by their host. When large triglyceride stores are carried over from larval feeding into adulthood (i.e., up to 30 to 40% of the parasitoid's dry body weight; Visser et al., 2018, 2021), *de novo* lipid synthesis from adult feeding is either unnecessary or too costly to maintain, leading to trait loss (Ellers et al., 2012). To test the hypothesis that many parasitoids do not synthesize substantial quantities of fat stores as adults, a previous study used feeding experiments on a wide taxonomic range of insects, including parasitoid wasps, parasitoid flies, a parasitoid beetle, and 65 non-parasitoid species (Visser et al., 2010 and references therein). What is striking is that when compared to non-parasitoid insects, 24 out of 29 evolutionarily distinct parasitoid lineages (Coleoptera, Diptera and Hymenoptera; Visser et al., 2010) did not accumulate significant lipid quantities in adulthood even when fed surplus carbohydrates. When little to no lipids are synthesized *de novo* by adult parasitoid wasps, this can lead to significant constraints on energy allocation toward key adult functions, such as maintenance, dispersal, and reproduction (Jervis et al., 2008). To our minds, the most important question is 'why don't parasitoids accumulate substantial quantities of fat as adults like other insects do, and what does this mean for their life histories?'

In their recent paper, Ruther et al (2021) used a sensitive quantitative method for stable isotope tracing of carbon from glucose into fatty acids followed by mass spectrometry to show that all 13 tested parasitoid species (2 of which were also used by Visser et al., 2010) incorporated very low amounts of stable carbon isotopes into fatty acids. This result led the authors to state that parasitoids do not lack lipogenesis and that their findings are novel, because they present their work as contradicting previous studies (Giron & Casas, 2003; Visser et al., 2010, 2012, 2018). However, previous experiments already showed that some parasitoid species synthesize lipids (Visser et al., 2010, 2017, 2018, 2021). What we argue here is that quantity matters; very low amounts of synthesized fatty acids cannot lead to a biologically sufficient increase of energy stores for allocation towards key life history traits. The apparent controversy between research groups is thus due largely to semantics and not critically important to the main scientific problem with regard to the phenomenon of lack of lipogenesis in parasitoids.

Explicit and clear definitions are very important in biology, and in previous publications, including our own, the precision of language used deserved more attention. The definition of lipogenesis is not unambiguous, and the term is frequently used for more than one process. Lipogenesis can be narrowly defined as the conversion of glucose or other catabolic substrates into fatty acids, or it can be defined more broadly as the accumulation of substantial quantities of triglycerides. Observing lack of lipid accumulation does not show absence of fatty acid synthesis (as clearly illustrated in foundational work by Giron & Casas 2003). This distinction is also important from a methodological point of view, because the gravimetric and colorimetric methods (based on weight, as in Visser et al., 2010, Visser et al., 2012, Visser et al., 2018, Visser et al., 2021, or conversion of chromophores measured by light absorbance, as in Muller et al., 2017, respectively) focus on bulk lipid accumulation, while the isotope method measures the synthesis of fatty acids (Giron & Casas, 2003; Prager et al., 2019; Ruther et al., 2021; Visser et al., 2012, 2017, 2021). Although the isotope tracing method provides valuable insights into fatty acid synthesis, some less sophisticated, but cost-effective methods, including gravimetry and colorimetry, still provide important biological insights about bulk lipid quantity, accumulation, and storage. To avoid further confusion in the scientific literature, we propose to use the more accurate term 'lack of lipid accumulation' when referring to low or absent fat accumulation in adult parasitoids. This term 'lipid accumulation' is widely used to describe the process of bulk synthesis of lipids for energy storage across a broad range of taxa (e.g., Guo et al., 2008; Schmid et al., 2005; Teixeira et al., 2003). We suggest that moving forward with this term will more accurately represent the biological phenomenon of importance, the fact that many parasitoids do no accumulate substantial fat reserves as adults and the downstream consequences of this lack of lipid accumulation for their life histories.

With regard to the focus of studies on lack of lipid accumulation and parasitoid life histories, a key result of the study by Ruther et al (2021) is that their labeling experiments show very low isotope traces in the fatty acid fraction, suggesting that the Fatty Acid Synthase enzyme is functional in several parasitoids. This finding is not novel, however, because previous studies already showed 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 parasitoid's biology. The real conundrum, however, is the fact that while parasitoids may be capable of synthesizing fatty acids, the accumulation of storage lipids is not induced by sugar-feeding as it is in non-parasitoid insects. Thus, we posit that it is important to consider the overall impact of lipid synthesis and accumulation. The very low amounts of fatty acid synthesis detected by Ruther et al. (2021) are unlikely to play a critical role as an energy source or as an anabolic substrate for egg production, compared to the wasps' physiological requirements. In fact, a similar finding was proposed almost two decades ago by Giron and Casas (2003) who also found trace amounts of incorporation of glucose into fatty acids using radioactive labels, and concluded that there was no substantial lipogenesis occurring. Is lipogenesis then lacking in most parasitoids? In a strictly chemical sense, the answer is no, but from the perspective of the accumulation of lipid stores for adult energetics and reproduction, we believe the answer is yes. Rather than a core conceptual disagreement between Ruther et al (2021) and us, this seems to be a semantic problem that we hope will be ameliorated by the use of term “lack of lipid accumulation” in future work.

What are the methodological considerations for future studies? First, to show that fatty acid synthesis is occurring, it is crucial not only to measure the incorporation levels of tracers into fatty acids in different parasitoid species, but also to include a positive control of incorporation into an insect that has high fatty acid synthesis and high adult lipid accumulation (as in Visser et al 2012; 2017). In this case that could be an insect species with typical, established lipid synthesis, such as *Drosophila melanogaster,* honeybees, or any other non-parasitoid insect. We believe that if an insect that does synthesize and accumulate substantial fat stores was used as a positive control it would become apparent that the levels reported in Ruther et al (2021) are very low when considering fatty acid synthesis of typical lipogenic species. Second, there is a need for appropriate negative controls. Ruther et al (2021) compared incorporation rates of wasps that were fed with labeled glucose to wasps that were starved, meaning that two different and potentially confounding factors were varied at the same time: isotope labelling (yes or no) and feeding status (fed vs starved vs host-fed). An important control for incorporation of ^{13}C label from glucose into fatty acids in their study would be to measure wasps that were fed unlabeled glucose (as a negative control for the labeled glucose fed wasps). Another important approach could be to use labeled precursors other than glucose (e.g., acetate; Wallace et al., 2018) that could be traced in both starved and fed wasps (with or without host-feeding). Third, previous studies using isotope-based measurements of fatty acid synthesis should not be unjustly discarded for using the stable isotope of hydrogen, deuterium (^{2}H)(Visser et al., 2012, 2017, 2021). 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 can depend on dietary ^{13}C concentration (Wessels & Hahn, 2010). The advantage of using deuterium is that it measures fatty acid synthesis from acetate, glucose or other precursors, and can provide insights also into other metabolic processes (Murphy, 2006). Therefore, Visser et al (2012, 2017, 2021) preferred to use deuterium over the fatty acid precursor (^{13}C labeled) glucose (Ruther et al 2021), and showed that tracing deuterium can detect a biologically relevant variation in fatty acid incorporation across treatments (Visser et al., 2021). Finally, when considering the synthesis of very small quantities of fatty acids within an insect, an alternative hypothesis to the insect synthesizing these fatty acids themselves is that microbes are involved. The (gut) microbiome could have contributed to the production of some fatty acids that are subsequently absorbed by the wasps' digestive tract or produced in other tissues (e.g., by insect endosymbionts like *Wolbachia*). Bacteria use type II dissociate fatty acid synthesis and possess all the necessary *fatty acid biosynthetic* genes (Cronan & Thomas, 2009; Jiménez et al., 2019; Scholz et al., 2020). Conclusive evidence of the role played by microbiota in producing fatty acids *de novo* would require repeating the experiments using germ-free (Shropshire et al., 2016) and endosymbiont-free parasitoids (Werren & Loehlin, 2009).

The question still stands as to why such a deviating metabolic phenotype of very low lipid accumulation has evolved in parasitoids and how parasitoids can cope with such a low amount of newly synthesized lipids. When little to no fat is synthesized, the parasitoid still relies heavily on fatty acids externally acquired during development, essentially rendering them fatty acid auxotrophs, limiting parasitoids in their lipid allocation toward main fitness functions as adults, including egg production, dispersal, and other metabolically intensive activities (Ellers, 1996; Ellers et al., 2012; Giron et al., 2002; Giron & Casas, 2003; Jervis et al., 2008). Only when insufficient fat amounts are carried over into adulthood from the larval host, for example in hyperparasitoids (containing 4-8% of fat at emergence; Visser et al., 2010, 2017) or when development occurs on lean hosts, such as dietary-restricted *Drosophila* for the wasp *Leptopilina heterotoma* (Visser et al., 2021), *de novo* lipid synthesis occurs in parasitoids in significant quantities when carbohydrates are consumed in excess by adults. Moreover, there appears to be genetic variation in how much fat is synthesized among wasp lines (Visser et al., 2021). Future studies with a range of different parasitoid species should take variation in host lipid content, wasp genotype, and plasticity of lipid accumulation into account. Furthermore, the enzymatic machinery underlying fatty acid synthesis can be involved in other functions than lipid storage, such as the production of cuticular hydrocarbons (Holze et al., 2021). A way to increase our understanding of the mechanisms underlying lack of lipid accumulation is to use inhibitors for key enzymes in the lipid synthesis pathway (e.g., Svensson et al., 2016; Ventura et al., 2015). In conclusion, combining chemistry, ecology, evolution, and biology into a truly interdisciplinary and integrative approach is what we now need for understanding the dynamics of lipid accumulation in parasitoids, and ultimately the downstream life history consequences of accumulating stored nutrients or not from adult dietary intake.

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