

Fatty acid composition remains stable across trophic levels in a gall wasp community

BERTANNE VISSER[†], COBY VAN DOOREMALEN[‡],
ALBA VÁZQUEZ RUIZ and JACINTHA ELLERS

Section Animal Ecology, Department of Ecological Science, VU University, Amsterdam, The Netherlands

Abstract. Acquiring sufficient nutrients is particularly important for insects that are unable to synthesize certain nutrient types *de novo*, as is the case for numerous parasitoid species that do not synthesize lipids. The lipid reserves of parasitoids are acquired from a single host during larval development. This imposes constraints on the quantity and quality of available lipids. In the present study, the lipid dynamics throughout the trophic cascade are investigated by measuring lipogenic ability, modifications in fatty acid composition and host exploitation efficiency in species at different trophic positions within the community of parasitoids associated with the gall wasp *Diplolepis rosae* L. (Hymenoptera: Cynipidae). The results obtained show that lipid levels remain stable or decline after feeding in all species, indicating that none of the wasps synthesize lipids. Fatty acid composition is highly similar between the gall wasp, parasitoid and hyperparasitoid species, with the exception of the parasitoid *Orthopelma mediator* Thunberg (Hymenoptera: Ichneumonidae). The divergence of fatty acid composition in *O. mediator* suggests that this species is able to modify its fatty acid composition after the consumption of host lipids. The efficiency of exploitation of host resource, in terms of dry body mass acquired, varies among the species (41–70%), although it is high overall compared with the efficiencies reported in other animals. Hence, for parasitoid wasps that lack lipid synthesis capabilities, the efficiency of host exploitation is high and fatty acids are consumed directly from the host without modification, leading to stable fatty acid compositions throughout the trophic cascade.

Key words. *Diplolepis rosae*, Exploitation efficiency, hyperparasitoid, inquiline, lipid content, lipogenesis, nutrient acquisition, parasitoid.

Introduction

In insects, the acquisition of sufficient nutrients during development is critical for fuelling resource allocation later in life, particularly when certain nutritional components cannot be produced *de novo*. For parasitoids that feed and develop

on other arthropods (Godfray, 1994), lipids form an essential resource because parasitoids generally lack the capacity for *de novo* lipid synthesis in the adult life-stage (Visser & Ellers, 2008; Visser *et al.*, 2010). Lipid reserves are an important energy source required to survive periods of food scarcity and, typically, all animals synthesize lipids readily when excess carbohydrates are available in the diet; hence, parasitoids form an exception to this general rule (Visser & Ellers, 2008). Because the diet of free-living adult parasitoids contains no (or only few) lipids, bulk lipid reserves must be acquired during the larval stage (Eijs *et al.*, 1998; Giron *et al.*, 2002; Giron & Casas, 2003). Larval development is restricted, however, to a single host; thus, unlike predacious and herbivorous insects, parasitoids cannot compensate for unfavourable nutrient ratios through selective feeding on several hosts (Anderson *et al.*, 2005; Frost *et al.*, 2005; Behmer, 2009).

Correspondence: Bertanne Visser, Institut de Recherche sur la Biologie de l'Insecte, Université de Tours, IRBI UMR CNRS 7261, Avenue Monge, 37200 Tours, France. Tel.: +33 247367347; e-mail: bertannevisser@gmail.com

[†]Present address: Institut de Recherche sur la Biologie de l'Insecte, Université de Tours, IRBI UMR CNRS 7261, Tours, France.

[‡]Present address: Bio-Interactions and Plant Health, Plant Research International, Droevendaalsesteeg, Wageningen, The Netherlands.

To deal with the dietary constraints imposed by developing on a single host, some parasitoid species have evolved intricate mechanisms to increase resource quantity through physiological manipulation of their host's metabolism (Rivers & Denlinger, 1994; Nakamatsu, 2003; Nakamatsu & Tanaka, 2004). Parasitoids are further found to be highly efficient in resource exploitation in terms of carrying over their host's resources. For example, the hyperparasitoid *Gelis agilis* maintains 90% of the body mass of its host *Cotesia glomerata* when parasitizing in the fourth trophic level, and 75% within the fifth trophic level (Harvey *et al.*, 2006, 2009). However, not only the quantitative efficiency of carry-over is important, but also the quality of the resources. Parasitoids are expected to manipulate hosts to increase the availability of essential nutrients; for example, through the injection of venom or other maternal substances during oviposition, which can regulate growth and metabolism (Rivers & Denlinger, 1994; Visser & Ellers, 2008).

Little is known about the efficiency with which specific nutrient classes, such as lipids and essential fatty acids, are transferred to higher trophic levels. Efficient carry-over of bulk lipid reserves from the host is necessary for key life-history traits, although specific components of the lipid fraction can be of further importance for maintaining vital functions. Polyunsaturated fatty acids (PUFAs), for example, are precursors of eicosanoids, which are signalling molecules that can have a critical effect on reproduction and immunological responses (Stanley, 2006). It is therefore expected that the level of PUFAs will be maintained across the trophic cascade. Fatty acid composition also has a vital role in maintaining membrane fluidity in the face of environmental variation, such as temperature or drought (Hazel, 1995). It is not currently known whether the lack of lipogenesis in parasitoids has also rendered fatty acid modification dysfunctional. If the latter were true, almost identical fatty acid compositions would be expected in species occupying different positions within the trophic cascade, reflecting host fatty acid composition. Trophic complexity might therefore be constrained by the specific nutrient composition of the species at the base of the trophic cascade and the efficiency with which these compounds are carried over.

Hymenopteran gall wasps and their associated parasitoids provide excellent systems for studying nutrient flow throughout the trophic cascade because, similar to their close parasitic relatives, gall wasps rely on a single host for their larval nutrition. Moreover, they can alter their host's physiology substantially in terms of nutrient composition by redirecting the host plant's physiology and growth (Shorthouse *et al.*, 2005). For example, Harper *et al.* (2004) show that galls induced by cynipid wasps attacking oak trees form lipid-rich nutritive cells lining the inner gall chamber that are used for larval feeding by the insect. Gall formation is initiated by the gall inducer or its progeny to increase protection from predators and parasitoids (Price *et al.*, 1987; Price & Pschorn-Walcher, 1988; Hartley & Lawton, 1992; Hartley, 1998) and these gall-forming insects support a species-rich community of parasitoids and hyperparasitoids (Stone *et al.*, 2002; Hayward & Stone, 2005).

The present study focuses on the community of insects associated with the cynipid gall wasp *Diplolepis rosae*

L. (Hymenoptera: Cynipidae), which induces gall formation on plants of the Rosaceae family. Its inquiline *Perichlistus brandtii* Ratzeburg (Hymenoptera: Cynipidae) is unable to induce gall formation but oviposits before gall growth and is able to affect growth of the internal gall chambers (Randolph, 2005). *Diplolepis rosae* and *P. brandtii* are the main gall inhabitants, and are attacked by several parasitoids, including *Orthopelma mediator* Thunberg (Hymenoptera: Ichneumonidae), *Pteromalus bedeguaris* Thomson (Hymenoptera: Pteromalidae) and *Torymus bedeguaris* L. (Hymenoptera: Torymidae) (Fig. 1). *Orthopelma mediator* mainly parasitizes the gall-maker and is reported to parasitize only 2.8% of potential *P. brandtii* hosts (Randolph, 2005). *Pteromalus bedeguaris* and *T. bedeguaris* can act as primary parasitoids on *D. rosae* and might occasionally attack *O. mediator* (Randolph, 2005), although exact estimates of hyperparasitism frequency remain unknown. In the present study, these species are used to measure the capacity for lipid synthesis in adults, changes in fatty acid composition over trophic levels, and the efficiency of host resource exploitation in terms of body mass and the amount of lipid reserves utilized. In accordance with previous studies (Giron & Casas, 2003; Visser *et al.*, 2010), it is expected that all wasp species would lack the ability for lipid synthesis, which could constrain the acquisition of sufficient lipid reserves higher in the trophic cascade. Fatty acid composition is predicted to show similarity between trophic levels, which would suggest the direct consumption of host lipid reserves. A further prediction is that exploitation efficiencies of dry mass and lipid content are high, particularly for hyperparasitoids, because resources tend to become more limited higher in the trophic cascade (Harvey *et al.*, 2006, 2009).

Materials and methods

Insects

Galls containing *D. rosae*, its inquiline *P. brandtii* and parasitoids were collected near Lyon, France, in September 2008. Until wasp emergence, galls were subsequently placed at 20, 10, 5, 10 and 20 °C to approximate to temperature changes throughout autumn, winter and spring. Before emergence, galls were placed individually in glass jars sealed with foam stoppers at a temperature of 20 °C, a relative humidity of 75% and an LD 12 : 12 h photoperiod.

Lipogenic ability

Upon emergence, wasps were either frozen at –20 °C directly or allowed to feed on honey and water provided on cotton wool for 14 days to test for lipogenic ability. After the treatment ended, lipid content was determined. Lipid content was measured for nine to 37 females per species for *D. rosae*, *P. brandtii*, *O. mediator* and *P. bedeguaris* using ether extraction as described by Visser *et al.* (2010). The lipid content of each individual was estimated by subtracting the dry weight after ether extraction from the dry weight before

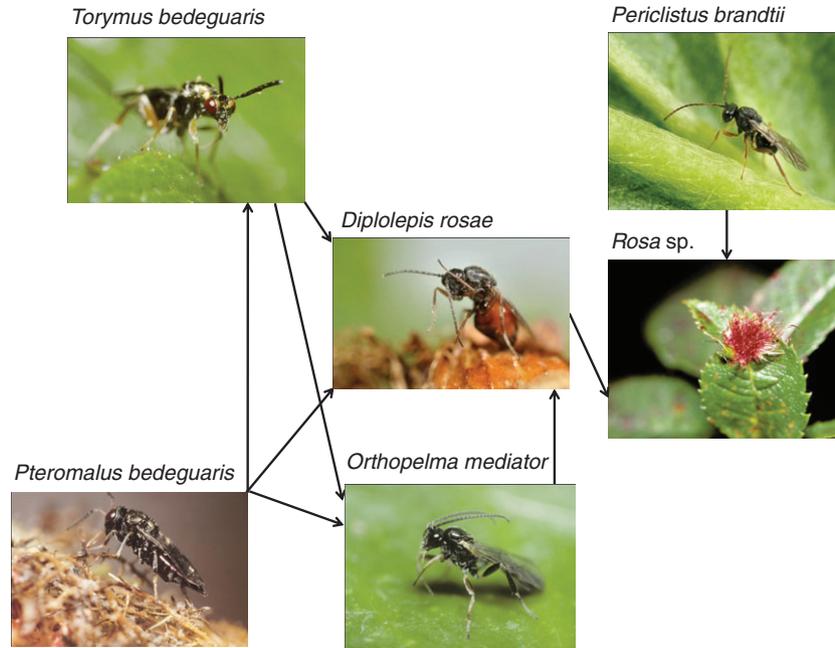


Fig. 1. Overview of main interactions within the gall community: the gall wasp *Diplolepis rosae*, its inquiline *Periclistus brandtii* and the parasitoids *Orthopelma mediator*, *Pteromalus bedeguaris* and *Tormus bedeguaris*. Photography courtesy of Robin Williams.

ether extraction (with a precision of 0.1 µg). An increase in lipid content of females in the honey-feeding treatment compared with lipid content at emergence indicates an ability for lipogenesis, whereas declining or constant lipid levels were taken as evidence for lack of lipogenesis (Visser *et al.*, 2010). Low rates of parasitism by *T. bedeguaris* led to insufficient sample sizes to test for lipogenic ability.

Fatty acid composition

To assess fatty acid composition, newly-emerged individuals were frozen at -20°C . Each sample contained three pooled individuals, or a single small rose gall. The number of replicates for each species was: *D. rosae* ($n=6$); *P. brandtii* ($n=3$); *O. mediator* ($n=5$); *P. bedeguaris* ($n=3$); *T. bedeguaris* ($n=2$); and rose galls ($n=3$). Samples were prepared according to van Dooremalen *et al.* (2009). Fatty acids were extracted using dichloromethane/methanol (2:1, v/v). After the vial headspace was flushed with nitrogen gas, 0.3 µg of C19:0 internal standard (Fluka, Switzerland) was added to each sample. Samples were saponified in a methanolized sodium hydroxide solution (45 g of NaOH, 150 mL of CH_3OH , 150 mL of milli-Q H_2O ; Millipore, Billerica, Massachusetts) at 70°C for 90 min (Chamberlain *et al.*, 2004). Saponification was followed by acid methanolysis in methanolized HCl (325 mL of 6.0 N HCl, 275 mL of CH_3OH) at $80 \pm 1^{\circ}\text{C}$ for 10 min. Methylated fatty acids were extracted using hexane/methyl tertiary butyl ether (1:1 v/v). This solution was dried with nitrogen gas and methylated fatty acids were dissolved in hexane and stored at -80°C until further processing. Fatty acid composition analysis was performed by gas-chromatography, using an

instrument equipped with a standard split/splitless injector and flame-ionization detector (Agilent Technologies, Santa Clara, California) and a polar BPX70 column (60 m \times 0.25 mm i.d., df 0.25 µm; SGE International, Australia). Samples were injected (1-µL aliquot) in the pulsed splitless mode. The temperature-programmed oven was set to 70°C for 2 min, which then increased by $20^{\circ}\text{C min}^{-1}$ to 150°C , continuing with a gradient of $15^{\circ}\text{C min}^{-1}$ to 250°C over 10 min.

Statistical analysis

Data for lipogenic ability testing were inspected for potential outliers before analysis as described by Visser *et al.* (2010). Normality of error structures was inspected and heterogeneity of variances determined using Levene's test. Non-normal data were cube root-transformed to normality. Datasets assuming normal distributions were compared using *t*-tests if variances were equal and Welch's *t*-test if variances were unequal. Statistical analyses were performed using R PROJECT 2.12.1 (R Development Core Team, 2010).

Data handling and analysis of fatty acid composition were carried out as described by van Dooremalen & Ellers (2010). Fatty acids were identified based on comparison of retention times between samples and a standard (37-Component FAME Mix; Supelco, Bellefonte, Pennsylvania). Fatty acid concentrations below the detection limit were excluded from further analysis (i.e. C_{20:0}) (22% missing values). All peak areas were log-ratio transformed (Nash *et al.*, 2008) and multivariate statistics performed to explore changes in fatty acid composition. Principle component analysis was used to obtain two principle components (PC1, PC2) for each sample

using PAST (Hammer *et al.*, 2001). PC1 and PC2 scores were correlated with individual fatty acids using Pearson's correlation coefficient to determine the contribution of each fatty acid to differences between species. Subsequently, analysis of variance was used with post-hoc Bonferroni correction to assess the effect of species on fatty acid composition.

Results

Lipogenic ability

Fourteen days of feeding on honey did not lead to significantly elevated lipid levels compared with emergence in any of the species. *Diplolepis rosae* had the highest lipid content at adult emergence (0.280 ± 0.029 mg; 1 SE), whereas *P. bedeguaris* emerged with the lowest lipid reserves (0.099 ± 0.026 mg; 1 SE). Two species showed a significant decrease in lipid reserves after 14 days of feeding: *D. rosae* ($t = 8.8$, $P < 0.001$, $n = 35$; Fig. 2) and *O. mediator* (Welch's $t = 4.166$, $P < 0.001$, $n = 37$; Fig. 2). In the other two species, lipid reserves remained stable after feeding: for *P. brandtii* and *P. bedeguaris*, there was no significant difference between lipid reserves at emergence and after feeding (*P. brandtii*: Welch's $t = 0.280$, $P = 0.787$, $n = 9$; *P. bedeguaris*: $t = 0.151$, $P = 0.884$, $n = 9$; Fig. 2). At emergence, lipid levels in percentages (taking into account dry body mass) were 26%, 25%, 16% and 22% for *D. rosae*, *P. brandtii*, *O. mediator* and *P. bedeguaris*, respectively (Table 1). These numbers correspond to previous findings and are consistent with the high capital lipid reserve levels that are observed typically in parasitoids lacking lipid synthesis (Visser *et al.*, 2010). No evidence is thus found of active adult lipid synthesis in any of the wasp species within this community.

Fatty acid composition

The lipid fraction contained six saturated fatty acids (SFAs) ranging in carbon length between 12 and 20 carbon atoms, two mono-unsaturated fatty acids (MUFAs) and three PUFAs. The most abundant fatty acids were the SFA palmitic acid ($C_{16:0}$), the MUFA oleic acid ($C_{18:1n9c}$) and the two PUFAs linoleic and linolenic acid ($C_{18:2n6c}$, $C_{18:3n3}$) (Table 2).

To compare the proportional abundance of these 11 fatty acids among trophic levels, principal component analysis of the variation in fatty acid composition was used. Two principal components were extracted. PC1 explained 46.4% of the variation, with high positive loadings of $C_{12:0}$ and $C_{18:0}$ and high negative loadings of $C_{18:1n9c}$, $C_{18:3n3}$ and $C_{20:4n6}$. The PC1 scores were significantly higher for rose galls than for all of the wasp species ($F_{5,16} = 21.68$, $P < 0.001$; Fig. 3). The largest difference was observed between the rose galls and the parasitoid *O. mediator* and this species also had significantly lower PC1 scores than the other wasp species. On average, the rose galls showed higher levels of $C_{18:0}$ and $C_{20:4n6}$, as well as lower levels of $C_{18:1n9c}$ and $C_{18:3n3}$ than the wasp species (Table 2).

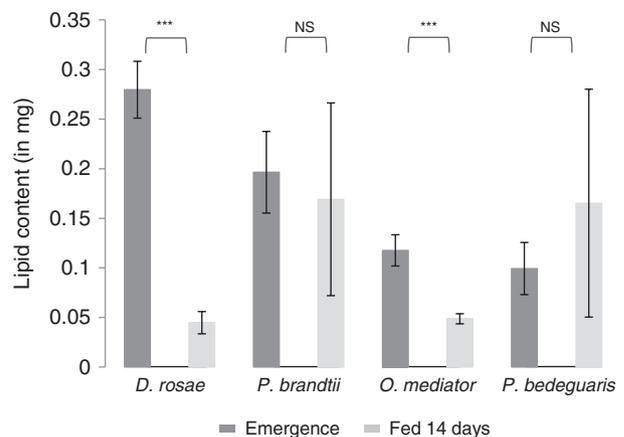


Fig. 2. Mean \pm SE lipid content (mg) for females at emergence (dark grey bars) and after 14 days of feeding (light grey bars). NS, not significant. *** $P < 0.001$.

The second principal component explained 30.3% of the variation. PC2 was positively correlated with the PUFA $C_{18:2n6c}$, whereas it had negative loadings of the SFAs $C_{14:0}$, $C_{15:0}$ and $C_{16:0}$. The rose galls differed significantly in PC2 score from all wasp species, with the exception of the parasitoid *O. mediator* ($F_{5,16} = 12$, $P < 0.001$; Fig. 3). The rose galls and *O. mediator* contained up to 2.5- and 4.2-fold more $C_{16:0}$ than the other wasps, respectively. In addition, the rose galls contained the highest level of $C_{18:2n6c}$ compared with the wasp species, whereas *O. mediator* contained the lowest levels of this fatty acid (Table 2).

Host exploitation efficiency

The inquiline *P. brandtii* develops on the same host species as the gall wasp *D. rosae*, although dry body mass and lipid amount of *P. brandtii* were 70% of that found for *D. rosae* (Table 1); hence, *P. brandtii* was less proficient than *D. rosae* in attaining resources from the gall. *Orthopelma mediator* and *P. bedeguaris* both use *D. rosae* as their main host for development. The primary parasitoid *O. mediator* obtained a total dry mass of 66% and lipid reserves of 42% of those of the host *D. rosae*. *Pteromalus bedeguaris* was least efficient in exploiting the gall wasp's resources with a total dry body mass at 41% and 35% of the lipid reserves of *D. rosae*. However, *P. bedeguaris* can develop potentially on *O. mediator*. Depending on the relative proportion of *P. bedeguaris* that had used *O. mediator* as a host species, the exploitation efficiency could increase to 61% for dry body mass and 84% for lipid reserves.

Discussion

The nutrient flow across trophic levels and the efficiency with which essential resources are carried over are among the factors that determine food web structure. In the present study, lipid dynamics and exploitation efficiencies are investigated in a galling community likely to be compromised

Table 1. Dry weight and mean lipid amount at emergence for all species and efficiency of host exploitation on different hosts.

Species	Dry weight, mean \pm SE (mg)	Amount of fat, mean \pm SE (mg)	Percentage fat, mean \pm SE	Exploitation efficiency on <i>Diplolepis rosae</i>		Exploitation efficiency on <i>Orthopelma mediator</i>	
				Dry weight (%)	Amount of fat (%)	Dry weight (%)	Amount of fat (%)
<i>Diplolepis rosae</i>	1.077 \pm 0.099	0.280 \pm 0.029	25.858 \pm 1.302	–	–	–	–
<i>Periclistus brandtii</i>	0.754 \pm 0.110	0.196 \pm 0.041	25.340 \pm 2.711	70	70	–	–
<i>Orthopelma mediator</i>	0.712 \pm 0.048	0.118 \pm 0.016	15.575 \pm 1.556	66	42	–	–
<i>Pteromalus bedeguaris</i>	0.437 \pm 0.106	0.099 \pm 0.026	21.952 \pm 1.259	41	35	61	84

Table 2. Pearson correlation coefficients on principle component (PC)1 and PC2 with associated *P*-values of the principal component analysis of single fatty acids from members of the gall wasp community.

Fatty acid	PC1 scores		PC2 scores		Species Rose gall	<i>Diplolepis rosae</i>	<i>Periclistus brandtii</i>	<i>Orthopelma mediator</i>	<i>Pteromalus bedeguaris</i>	<i>Torymus bedeguaris</i>
	Pearson	<i>P</i> -value	Pearson	<i>P</i> -value						
C12:0	0.741	0.000	–0.399	0.066	0.607	0.126	0.055	0.017	0.062	0.022
C14:0	–0.285	0.198	–0.860	0.000	1.010	0.522	0.195	0.429	0.170	0.187
C15:0	0.147	0.514	–0.816	0.000	0.379	0.041	0.044	0.080	0.053	0.044
C16:0	–0.546	0.009	–0.590	0.004	19.510	6.422	4.949	16.624	4.652	6.503
C16:1	–0.425	0.048	0.170	0.450	0.684	0.363	0.385	0.373	0.474	0.386
C17:0	0.641	0.001	0.426	0.048	0.607	0.162	0.281	0.134	0.518	0.247
C18:0	0.799	0.000	0.521	0.013	8.340	2.000	4.027	0.828	4.347	4.164
C18:1n9c	–0.782	0.000	0.437	0.042	25.830	36.593	42.749	45.040	38.300	39.253
C18:2n6c	0.295	0.182	0.680	0.000	31.378	22.720	23.155	11.001	27.341	23.015
C18:3n3	–0.840	0.000	0.449	0.036	10.610	30.698	23.850	25.271	23.712	25.759
C20:4n6	–0.777	0.000	0.408	0.059	0.206	0.082	0.147	0.103	0.172	0.172
SFA	–	–	–	–	30.453	9.273	9.550	18.111	9.801	11.168
MUFA	–	–	–	–	26.514	36.955	43.133	45.412	38.774	39.639
PUFA	–	–	–	–	42.194	53.499	47.152	36.375	51.226	48.946

For each species, the mean proportion of each single fatty acid of the total extracted fatty acids is given, SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acid; PUFA, polyunsaturated fatty acid.

in the ability for lipid synthesis, modification of fatty acid compositions and resource use. The results show that lipids are essential resources for all of the species within the gall wasp community because none of the species increased lipid levels after feeding, a pattern indicative for a lack of lipogenic ability (Visser *et al.*, 2010). A lack of adult lipid synthesis predominates in parasitic Hymenoptera, although there are some exceptions in which adult parasitoids do synthesize lipids (Visser *et al.*, 2010). Variation in lipogenic ability can also be found between populations of the same species (Moiroux *et al.*, 2010). The present findings do not suggest that such variability exists at the population level for species within this gall wasp community. The specimens of *D. rosae*, *P. brandtii* and *O. mediator* sampled in the present study lack lipogenesis and originate from populations in the South of France. Previous findings for the same species obtained from populations in the Netherlands reveal similar patterns (Visser *et al.*, 2010). Thus, a consistent pattern of a lack of lipid synthesis is found amongst members of the rose gall community, which means that each species is dependent on its host's lipid content with respect to obtaining sufficient lipids.

Fatty acid composition of the parasitoid species differs substantially from that of the rose gall; in particular, levels of oleic (C_{18:1n9c}) and linolenic acid (C_{18:3n3}) are higher in

all of the wasp species. The difference in fatty acid levels could be a result of the present analysis reflecting the fatty acid composition in the entire galling tissue instead of solely the nutritive tissue of the gall that is consumed by the larvae (Tooker & De Moraes, 2009). These nutritive tissues line the inner part of the gall chamber in which the larvae develop; thus, nutrient levels tend to vary throughout the gall, in which only nutritive cells are consumed by gall-former and inquiline (Harper *et al.*, 2004). However, analyzing the nutritive cells of the gall separately is difficult to accomplish because galls in the early process of development are harder to detect and, at the time of sampling, nutritive tissues are mostly consumed.

Alternatively, parasitoids may be able to alter their fatty acid composition compared with the host; for example, through elongation and desaturation of fatty acids or through selective uptake of fatty acids. The increased levels of linolenic acid (C_{18:3n3}) in particular would require biosynthesis of an essential fatty acid, a feature only observed in a small number of insect species (Cripps *et al.*, 1986), not including any hymenopteran. It is therefore more likely that fatty acids are carried over from the host but are subsequently metabolized selectively. The results indicate further that the relative abundance of arachidonic acid (C_{20:4n6}) increases at higher trophic levels. This fatty acid is important for egg laying and immunity

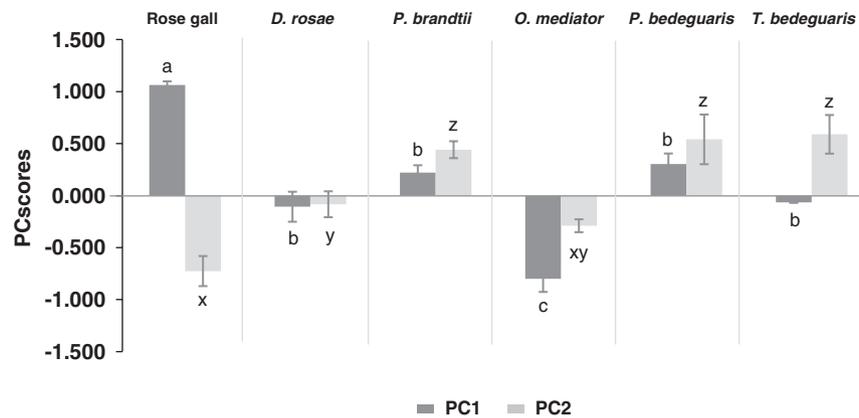


Fig. 3. Principle component (PC) scores of PC1 (mean \pm SE; dark grey bars) and PC2 (mean \pm SE; light grey bars) for fatty acid proportions of all species in the studied gall wasp community. Different letters represent significant differences at $\alpha = 0.05$.

because it forms the precursor of eicosanoids (Stanley, 2006). Its relative increase suggests that there is selective attainment or preservation of this fatty acid compared with other fatty acids. Modification of the fatty acid composition higher in the trophic cascade is also observed in other organisms (Cakmak *et al.*, 2007), although the fatty acid composition can also reflect the dietary composition (Fukuda & Naganuma, 2001).

Fatty acid composition is similar for all wasp species in the present study, with the exception of *O. mediator*. *Orthopelma mediator* has a distinct fatty acid composition because of the high level of palmitic acid ($C_{16:0}$) and low levels of linoleic acid ($C_{18:2n6c}$). In the case of palmitic acid, selective uptake from the host is not possible because the levels of palmitic acid are significantly lower in *D. rosae* and *P. brandtii*. The higher levels of palmitic acid in *O. mediator* are suggestive of an active lipogenesis pathway because palmitic acid is the end product of *de novo* fatty acid synthesis. If lipogenesis did occur, the feeding experiment suggests that the rate of lipid synthesis is lower than the rate of lipid metabolism because no accumulation of lipids occurs. Testing whether the decrease in lipid levels is a result of enhanced lipid breakdown requires the tracking of intermediary metabolites into the fatty acid fraction. Such experiments have been performed in other species, in which species lacking lipid synthesis do not incorporate labelled metabolites when lipid levels remain stable (Giron & Casas, 2003) or decrease significantly (Visser *et al.*, 2012), similar to the findings of the present study regarding *O. mediator*. Hence, the results obtained in labelling studies and feeding experiments show consistent patterns, whereby decreasing or stable lipid reserves result from a lack of fatty acid synthesis rather than enhanced lipid breakdown. For *O. mediator*, a more likely explanation is that the high level of palmitic acid results from modification of other fatty acids, which is consistent with the lower levels of linoleic acid compared with the other species. Regarding the fatty acid composition of the hyperparasitoid wasps, it is important to note that it has been assumed that *P. bedeguaris* and *T. bedeguaris* have parasitized *D. rosae* and not *O. mediator*. An exact estimation of the host species used was not possible but, given the similarity found in fatty acid composition between all

wasps, except *O. mediator*, the most parsimonious explanation for the present results is that *D. rosae* is the predominant host for *P. bedeguaris* and *T. bedeguaris*.

The gall wasp *D. rosae* obtains the highest dry body mass compared with all other wasp species. The efficiency with which resources are carried over from the host varies among the other wasp species, in terms of dry body mass (41–70%) and fat reserves (35–83%), although there are no clear differences among trophic levels. In parasitoid species, exploitation efficiencies of host resources are relatively high; for example, 60% in the parasitoid *Venturia canescens* (Howell & Fisher, 1977) and even higher (i.e. up to 75%) for parasitoids up to the fifth trophic level (Harvey *et al.*, 2006, 2009, 2011). Exploitation efficiencies are typically much lower in other animals, ranging between 20% and 40% (Howell & Fisher, 1977). High resource exploitation efficiency might increase food web complexity, particularly the length of food chains, because high efficiency in resource exploitation could reduce net energetic losses and thus enhance nutrient levels sufficiently to allow for additional positions higher in the food chain. It remains unknown, however, how efficiency and attainment of specific nutrient pools are of importance with respect to determining the length of trophic cascades. In the gall wasp system investigated in the present study, the majority of fatty acids are carried over from the host, although lipid reserves decrease at higher trophic levels, suggesting that the amount of lipids acquired from the host rather than fatty acid composition determines exploitation potential higher in the trophic cascade.

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