Trophic assimilation efficiency markedly increases at higher trophic levels in four-level host–parasitoid food chain

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Trophic assimilation efficiency (conversion of resource biomass into consumer biomass) is thought to be a limiting factor for food chain length in natural communities. In host–parasitoid systems, which account for the majority of terrestrial consumer interactions, a high trophic assimilation efficiency may be expected at higher trophic levels because of the close match of resource composition of host tissue and the consumer’s resource requirements, which would allow for longer food chains. We measured efficiency of biomass transfer along an aphid-primary–secondary–tertiary parasitoid food chain and used stable isotope analysis to confirm trophic levels. We show high efficiency in biomass transfer along the food chain. From the third to the fourth trophic level, the proportion of host biomass transferred was 45%, 65% and 73%, respectively, for three secondary parasitoid species. For two parasitoid species that can act at the fourth and fifth trophic levels, we show markedly increased trophic assimilation efficiencies at the higher trophic level, which increased from 45 to 63% and 73 to 93%, respectively. In common with other food chains, d15N increased with trophic level, with trophic discrimination factors (Δ15N) 1.34 and 1.49‰ from primary parasitoids to endoparasitic and ectoparasitic secondary parasitoids, respectively, and 0.78‰ from secondary to tertiary parasitoids. Owing to the extraordinarily high efficiency of hyperparasitoids, cryptic higher trophic levels may exist in host–parasitoid communities, which could alter our understanding of the dynamics and drivers of community structure of these important systems.

1. Introduction

It has long been recognized [1] that food webs rarely have more than five trophic levels and most often fewer [2], constraining major aspects of food web structure [3]. A number of interacting factors, especially ecosystem size and primary productivity, are found to be related to food chain length [4–7]. An important mechanism behind these relationships is the (in)efficiency of transfer of productivity from one trophic level to the next, so that only large and/or highly productive ecosystems contain sufficient resources to sustain viable populations at high trophic levels [8–11]. A crucial component of this ecological efficiency is the trophic assimilation efficiency: the proportion of consumed resource biomass that is converted into consumer biomass. Theoretical work predicts trophic assimilation efficiency to be in the range of 13–50%, depending on predator–prey mass ratio [12], which is in accordance with the few empirical estimates that exist [13,14] and is generally assumed to be unrelated to trophic level or to decrease with increasing trophic level [15]. Trophic assimilation efficiency of consumer species can be an important factor determining ecosystem stability [16,17], as shown for lakes where during re-oligotrophication an increase in consumer
assimilation efficiency resulted in a destabilizing increase in interaction strengths [18].

Host–parasitoid communities are widely used as model systems in population and multi-trophic community ecology as they arguably represent the majority of trophic interactions in terrestrial ecosystems [19], and a large body of ecological knowledge has been derived from these systems (e.g. [20–22]). Parasitoids are restricted to consuming a single host individual, representing a finite amount of resources with which to complete development from egg to adult, and there is therefore likely to be strong selection for using the resource with high efficiency [23]. Indeed, in host–parasitoid systems, trophic assimilation efficiency seems to be relatively high [23] and may be especially high for high trophic level hyperparasitoids (parasitoids whose hosts are also parasitoids) owing to the close match of resource content of the host and resource requirements of the hyperparasitoid, given their close phylogenetic relationships and similar lifestyles [24]. Therefore, we expect higher trophic assimilation efficiencies for species acting at higher trophic levels. It has been suggested that high competition among hyperparasitoids, and the fact that they are adapted to feeding on fellow Hymenoptera, may lead to frequent facultative tertiary and possibly even higher orders of parasitism [25,26]. However, there is a general assumption that, owing to physiological constraints, such interactions are negligibly rare in the wild. This assumption, and the fact that instances of higher-order parasitism are difficult to identify in the field, means that hyperparasitoids are generally treated as a fixed trophic level [27,28].

Here, we test this fundamental assumption by measuring assimilation efficiency along food chains in host–parasitoid systems. First, we used nitrogen stable isotope analysis ($\delta^{15}N$) [29] to test whether hyperparasitoids can truly act as tertiary parasitoids, feeding on other hyperparasitoids, as $\delta^{15}N$ systematically increases with trophic level in other systems [30,31]. Then we measured efficiency of biomass transfer from primary parasitoid hosts to three hyperparasitoid species in the laboratory, and for two of these hyperparasitoid species we also measured the efficiency of biomass transfer when they feed on the other hyperparasitoid. We further compared carbon content and the carbon to nitrogen (C/N) ratio for the different trophic levels along the food chain, to test for the nutritional quality of the hosts at different trophic levels for the parasitoids. We use these data to test the hypothesis that trophic assimilation efficiency increases at the higher trophic level, reducing constraints on food chain length in host–parasitoid systems.

2. Material and methods

(a) Study system

All species were collected in the field around Bern, Switzerland. Cultures were kept in climate chambers at 20/18°C with a 16 L:8 D cycle. The primary parasitoid Aphidius megourae (Stary 1965) was reared on the aphid Megasoter vicina (Burkot 1876) feeding on bean plants (Vicia faba L.). The larvae of primary aphid parasitoids first feed on the aphids' haemolymph and later kill the aphid by feeding on other tissues. They then pupate within the mummified skin of the aphid, creating the so-called mummy. They are commonly attacked by a diverse guild of hyperparasitoids belonging to two functional groups: (i) the secondary endophagous koinobiont parasitoids, which lay their eggs in the parasitoid larva within the still-living aphid, where they remain to hatch after mummification of the aphids [25] (from here on called endoparasitoids) and (ii) the so-called mummy parasitoids or secondary ectophagous idiobiont parasitoids, which attack their host at the pupal stage within the aphid mummy by depositing the eggs on the parasitoid host [26] (from here on mummy parasitoids). We used (i) the endoparasitoid Alloxysta sp. (Foerster 1869), and the two mummy parasitoids (ii) Corphus clavata (Walker 1833) and (iii) Dendocerus carpenteri (Curtis 1829). Alloxysta lays an egg in the primary parasitoid larva in the still-living parasitized aphid host, where it remains and hatches only after mummification of the aphid [32]: this means the primary parasitoid larvae has stopped feeding on the aphid host, which allows us to estimate true trophic assimilation efficiencies even from the primary parasitoid to the next level (Alloxysta). All parasitoids used in this experiment had their host inside the aphid mummy as single resource as they were reared in individual gel capsules with no other resources available.

(b) Study design and experimental set-up

One 14-day-old plant with 20 adult aphids was placed in each of 10 cages (24.5 × 24.5 × 24.3 cm, MegaView Science Co., Taiwan). Adult aphids were removed from cages after 3 days to obtain cohorts of 160–200 juveniles, which were parasitized by the parasitoid A. megourae (8–14 individuals added at day 6 and stayed for 48 h; parasitism rate 80–90%). Parasitized aphids were split at day 11: one-third were used to rear primary parasitoids and the other two-thirds were put onto another bean plant in a new cage with 5–10 female Alloxysta and 1–5 male Alloxysta (figure 1). At day 17 (for A. megourae cages) and day 20 (for Alloxysta cages), half of the mummies were transferred to Petri dishes together with three D. carpenteri or C. clavata females. After 48 h, the hyperparasitoids were removed from the Petri dishes to prevent multiple parasitism of hosts. Primary parasitoids started to eclose on day 20 after A. megourae attacked the aphids, secondary and tertiary parasitoids on days 31 and 36, respectively. We created the food chains in two separate runs: one with D. carpenteri and another with C. clavata as mummy parasitoid. Cages were daily checked for the formation of mummies, which were collected separately. After eclosion, individuals were stored in a freezer at −30°C.

Parasitoids were dried for 3 days at 65°C and then weighed (Sartorius Genius, ME ±0.01 mg). Individual weights of parasitoids were used to calculate the biomass transfer along the food chain and estimated as ‘individual biomass at higher trophic

![Figure 1. Experimental protocol for creating the food chains. Mummy parasitoids that were added to the food chain as secondary or tertiary parasitoids were either D. carpenteri or C. clavata.](image-url)
level/individual biomass at lower trophic level × 100’ (see statistical analysis for exact method). For stable isotope analysis, approximately 0.3–0.5 mg of dried insect material (two to eight individuals) was transferred into tin capsules (5 × 9 mm, HEKA-tech GmbH, Germany). Then samples were combusted with an ECS 4010 elemental analyser (Costech, Milan, Italy) and analysed using a Delta V Plus isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). For each sample, carbon content, nitrogen content and C/N ratio were measured alongside isotope ratios (δ¹⁵N and δ¹³C).

(c) Statistical analyses

All statistical analyses were performed using R v. 3.1.0[33]. Species- and trophic-level specific differences in dry weights and δ¹⁵N values were tested using linear models based on generalized least squares (errors are allowed to have unequal variances) provided by the nlme package [34]. We used VarIdent to account for variance heterogeneity in effect sizes between groups of parasitoids. For differences in hyperparasitoid weights according to the trophic levels and species, we specified the following six a priori contrasts [35], (i) the mummy parasitoid C. clavata secondary versus tertiary level, (ii) the mummy parasitoid D. carpenteri secondary versus tertiary level, (iii) the endoparasitoid versus mummy parasitoids as secondary parasitoids, (iv) the endoparasitoid versus mummy parasitoids as tertiary parasitoids, (v) C. clavata versus D. carpenteri as secondary parasitoids, and (vi) C. clavata versus D. carpenteri as tertiary parasitoids.

Biomass transfer efficiencies for all hyperparasitoid species were estimated from dry weight data. We used the function ‘sim’ from the R-package ‘arm’ [36] to simulate values from the posterior distribution of the species means, which were then used to estimate the proportions as derived parameters. A random sample of 5000 values from the posterior distribution of the model parameters (model: parasitoid dry weight ~ parasitoid trophic group) was drawn for each trophic group (e.g. for C. clavata as secondary parasitoid). From these we estimated the 5000 values for the posterior distribution of the proportions ‘species A higher trophic level / species B lower trophic level’ for the pairs D. carpenteri and C. clavata acting at the different trophic levels versus their food base (A. megourae or Alloxysta sp.). We then tested for the posterior probability of the hypothesis that proportions (i) D. carpenteri tertiary/Alloxysta sp. > D. carpenteri secondary/A. megourae and (ii) C. clavata tertiary/Alloxysta sp. > C. clavata secondary/A. megourae. The Bayesian p-values presented in the results indicate the proportion of simulated values for which the hypothesis was true. Nitrogen content and C/N ratios in the proportion of simulated values for which the hypothesis was true. Nitrogen content and C/N ratios in the proportion of simulated values for which the hypothesis was true.

For the stable isotope analysis, we tested for enrichment in δ¹⁵N from (i) primary parasitoids versus secondary endoparasitoids and mummy parasitoids and (ii) the endoparasitoid Alloxysta sp. versus secondary mummy parasitoids.

The response variable, δ¹⁵N values for hyperparasitoids, was corrected against the base of the parasitoid food web (the mean for primary parasitoids for each experimental run), because primary parasitoid δ¹⁵N differed significantly by 1.1 ± 0.31‰ (t₁₁₄ = −3.293, p = 0.0064) between the two experimental runs with D. carpenteri and C. clavata.

3. Results

(a) Stable isotope analysis

We found a significant increase in δ¹⁵N along the food chain with Δδ¹⁵N = 1.34 (±0.11) and 1.49 (±0.25)% from primary parasitoids to endoparasitic and ectoparasitic secondary parasitoids, respectively, and 0.78 (±0.15)% from secondary to tertiary parasitoids (for details, see electronic supplementary material, Appendix S1). Both groups of secondary parasitoids, the endoparasitoid Alloxysta sp. and the mummy parasitoids, were similarly enriched in δ¹⁵N (t₂₃₅ = −0.54, p = 0.5864) but clearly separated from primary parasitoids (figure 2; t₁₃₅ = 5.86, p < 0.001). δ¹⁵N values significantly separated secondary mummy parasitoids from tertiary mummy parasitoids (figure 2; t₂₃₅ = 2.15, p = 0.0381).

(b) Biomass transfer between trophic levels

Body mass of D. carpenteri decreased significantly when acting as tertiary parasitoid compared with its mass when acting as secondary parasitoid (electronic supplementary material, Appendix S2; table 1). By contrast, we did not find a significant difference in mass between the two trophic levels of C. clavata (electronic supplementary material, Appendix S2; table 1). The mass of secondary C. clavata was significantly lower than the mass of secondary D. carpenteri with the same pattern when both were acting as tertiary parasitoids (electronic supplementary material, Appendix S2; table 1).

D. carpenteri (acting as secondary or tertiary parasitoid) was more efficient than Alloxysta or C. clavata (figure 3, table 1). D. carpenteri converted 73% of the host’s body mass as secondary parasitoids and remarkably, 93% of the host’s body mass when acting as tertiary parasitoid (figure 3, posterior probability of 0.999 that the efficiency is higher for D. carpenteri at the tertiary level). C. clavata also showed higher efficiency when acting as tertiary parasitoid, with 45% as secondary and 63% as tertiary (figure 3, posterior probability of 0.999 for higher efficiency at tertiary level) but with less efficiency than D. carpenteri (figure 3, posterior probability of 1 for higher efficiency in D. carpenteri for both trophic levels). The nitrogen content was 1.19 times higher in Alloxysta than in A. megourae (electronic supplementary material, Appendix S3, posterior
probability of 1 for higher content in Alloxysta) and the C/N ratio dropped from 5.28 in A. megourae to 4.21 in Alloxysta (posterior probability of 0.999 for lower ratio in Alloxysta).

4. Discussion

We found extraordinarily high trophic assimilation efficiency for hyperparasitoids, which markedly increased along the trophic chain for both mummy parasitoids D. carpenteri and C. clavata. Both species can act at the secondary and tertiary parasitism level but were far more efficient at the tertiary level in converting host biomass. Differences in $^{15}$N enrichment allowed us to confirm that both species of mummy parasitoids were capable of acting as true tertiary parasitoids. The high efficiency of biomass transfer indicates there is no physiological barrier to these intraguild interactions between hyperparasitoids at higher trophic levels, thereby falsifying the assumption that there are strong constraints on food chain length in host–parasitoid food webs.

These results have significant implications for our understanding of these important systems. In addition to predicted effects of assimilation efficiency on community stability [16–18], constraints on food chain length have been shown to explain many of the universal properties found in network structure among food webs [3]. The possibility of cryptic higher trophic levels, owing to relaxation of these constraints, therefore, also means that host–parasitoid networks may contain a hidden structure that is fundamentally different from other food webs, with implications for community dynamics and stability [27].

Our results suggest that the higher up in the trophic chain a hyperparasitoid acts, the more easily it can convert the host tissue. A possible reason for this is that unprofitable food components have already been removed earlier from the food source and plant allelochemicals diluted, benefiting insect predators and parasitoids [37,38]. Plant defensive chemicals may be assimilated at the first parasitism level [39], but not passed on to the higher trophic levels. Towards the top end of the food chain, nitrogen tends to be concentrated leading to a closer match between the nutritional content of host and the nutritional requirements of consumer [23]. And indeed, the nitrogen content was higher with the C/N ratio consequently being lower in the body of Alloxysta, the host for the tertiary parasitoids, than in A. megourae, the host for the secondary parasitoids. Interestingly, the C/N ratios of the mummy parasitoids were very similar to that of Alloxysta (electronic supplementary material, Appendix S3). For the mummy parasitoids Alloxysta as host can be more efficiently exploited than the primary parasitoid, leading to the higher trophic assimilation efficiency at the higher trophic level. Therefore, D. carpenteri and C. clavata were far more efficient as tertiary parasitoids than as secondary parasitoids.

It has been suggested that higher trophic levels in arthropod communities contain progressively fewer lipids and more protein in their bodies, which makes carbohydrate and fat less available for higher-order consumers and potentially limiting the number of trophic levels [40,41]. However, it appears that in host–parasitoid systems 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 food chains [42]. C/N ratios were very similar for all hyperparasitoids in our study, suggesting stable carbon availability even at higher trophic levels.

D. carpenteri was more efficient in converting host biomass than C. clavata. C. clavata shows host-feeding prior to oviposition to accumulate enough protein to produce eggs owing to lack of energy uptake as a larva [43]. Therefore, selection pressure for high efficiency should be more pronounced for D. carpenteri. Parasitoids are further capable of taking up sugar in the wild from sources such as honeydew, nectar and extra floral nectar [44].

Owing to the extraordinary efficiency of parasitoids at high trophic levels, cryptic higher trophic levels may exist

### Table 1. Results for six a priori contrasts comparing the weights (in milligrams) of different parasitoid species and for C. clavata and D. carpenteri feeding at both the second and third level of parasitism.

| species compared | value | s.e. | t-value | p-value |
|------------------|-------|------|---------|---------|
| C. clavata 2nd to C. clavata 3rd | 0.0058 | 0.0055 | 1.044 | 0.297 |
| D. carpenteri 2nd to D. carpenteri 3rd | 0.0124 | 0.0048 | 2.596 | 0.001 |
| Alloxysta sp. to C. clavata 2nd and D. carpenteri 2nd | −0.0046 | 0.0040 | −1.141 | 0.254 |
| Alloxysta sp. to C. clavata 3rd and D. carpenteri 3rd | 0.0135 | 0.0037 | 3.610 | <0.0001 |
| C. clavata 2nd to D. carpenteri 2nd | −0.0309 | 0.0056 | −5.474 | <0.0001 |
| C. clavata 3rd to D. carpenteri 3rd | −0.0243 | 0.0046 | −5.276 | <0.0001 |

Figure 3. Biomass transfer (% of dry weight) from one trophic level to the next higher level along the primary parasitoid–secondary parasitoid–tertiary parasitoid trophic chain for the mummy parasitoids (a) D. carpenteri and (b) C. clavata. The sample size is given in brackets.
in host–parasitoid communities, which could alter our understanding of the dynamics and drivers of community structure of these important systems. Stable isotope analysis can be used to study the vertical trophic structure of parasitoids in the field in order to reveal this hidden aspect of the food webs.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material, Appendix S4.

References

1. Elton CS. 1927 Animal ecology. Chicago, IL: University of Chicago Press.
2. Sugihara G, Syonony K, Tromba A. 1989 Scale invariance in food web properties. Science 245, 48 – 52. (doi:10.1126/science.2740915)
3. van Veen FfJ, Murrell DJ. 2005 A simple explanation for universal scaling relations in food webs. Ecology 86, 3258 – 3263. (doi:10.1890/05-0943)
4. Zanden MV, Shuter BJ, Lester N, Rasmussen JB. 1999 Patterns of food chain length in lakes: a stable isotope study. Am. Nat. 154, 406 – 416. (doi:10.1086/303250)
5. Post DM, Pace MJ, Hairston NG. 2000 Ecosystem size determines food-chain length in lakes. Nature 405, 1047 – 1049. (doi:10.1038/35016655)
6. Takimoto G, Post D. 2013 Environmental determinants of food-chain length: a meta-analysis. Ecol. Res. 28, 675 – 681. (doi:10.1007/s11284-012-0493-7)
7. Post DM. 2002 The long and short of food-chain length. Trends Ecol. Evol. 17, 269 – 277. (doi:10.1016/S0169-5347(02)02455-2)
8. Hairston NGr, Hairston NGS. 1993 Cause–effect relationships in energy flow, trophic structure, and interspecific interactions. Am. Nat. 142, 379 – 411. (doi:10.1086/285546)
9. Brose U et al. 2006 Consumer–resource body-size relationships in natural food webs. Ecology 87, 2411 – 2417. (doi:10.1890/0012-9658(2006)087[2411:CRBSSR]2.0.CO;2)
10. Slobodkin LB. 1960 Ecological energy relationships at the population level. Am. Nat. 94, 213 – 236. (doi:10.2307/2458764)
11. Lindeman RL. 1942 The trophic-dynamic aspect of ecology. Ecology 23, 399 – 417. (doi:10.2307/1930126)
12. Andersen KH, Beyer JE, Lundberg P. 2009 Trophic and individual efficiencies of size-structured communities. Proc. R. Soc. B 276, 109 – 114. (doi:10.1098/rspb.2008.0951)
13. Humphreys WF. 1979 Production and respiration in animal populations. J. Anim. Ecol. 48, 427 – 453. (doi:10.2307/1938928)
14. Strayer D. 1991 Notes on Lindeman’s progressive efficiency. Ecology 72, 348 – 350. (doi:10.2307/1938928)
15. Barnes C, Maxwell D, Reuman DC, Jennings S. 2010 Global patterns in predator–prey size relationships reveal size dependency of trophic transfer efficiency. Ecology 91, 222 – 232. (doi:10.1890/08-2061.1)
16. De Angelis DL. 1975 Stability and connectance in food web models. Ecology 56, 238 – 243. (doi:10.2307/393318)
17. Crump R, Gabriel A. 2002 Ecosystem adaptation: do ecosystems maximize resilience? Ecology 83, 2019 – 2026. (doi:10.1890/0012-9658(2002)083[2019:DAEMMR]2.0.CO;2)
18. Kuiper JJ, van Altena C, de Ruiter PC, van Gerven LPA, Janse JH, Mooij WM. 2015 Food-web stability signals critical transitions in temperate shallow lakes. Nat. Commun. 6, 7727. (doi:10.1038/ncomms8727)
19. Hassell MP. 2000 Host–parasitoid population dynamics. J. Anim. Ecol. 69, 543 – 566. (doi:10.1046/j.1365-2656.2000.00445.x)
20. Bukovinszky T, van Veen FfJ, Jongema Y, Dicke M. 2008 Direct and indirect effects of resource quality on food web structure. Science 319, 804 – 807. (doi:10.1126/science.1148310)
21. Hassell MP, Comins HN, May RM. 1991 Spatial structure and chaos in insect population dynamics. Nature 353, 255 – 258. (doi:10.1038/353252a0)
22. Sanders D, Kehoe R, van Veen FfJ. 2015 Experimental evidence for the population-dynamic mechanisms underlying extinction cascades of carnivores. Curr. Biol. 25, 3106 – 3109. (doi:10.1016/j.cub.2015.10.017)
23. Harvey JA, Wagenar R, Bezemer TM. 2009 Interactions to the fifth trophic level: secondary and tertiary parasitoid wasps show extraordinary efficiency in utilizing host resources. J. Anim. Ecol. 78, 686 – 692. (doi:10.1111/j.1365-2656.2008.01516.x)
24. Harvey JA, Vet LE, Witjes L, Bezemer TM. 2006 Remarkable similarity in body mass of a secondary parasitoid Lysibia nana and its primary parasitoid host Cotesia glomerata emerging from cocoons of comparable size. Arch. Insect Biochem. Physiol. 61, 170 – 183. (doi:10.1002/arch.20080)
25. Sullivan SJ, Daniel J. 1972 Comparative behavior and competition between two aphid hyperparasites: Alloxysta victrix and Asaphes californicus (Hymenoptera: Cynipidae; Pteromalidae). Environ. Entomol. 1, 234 – 244. (doi:10.1093/ee/1.2.234)
26. Matejko I, Sullivan DD. 1984 Interspecific tertiary parasitoidism between two aphid hyperparasitoids: Dendrocerus carpenteri and Alloxysta megaruea (Hymenoptera: Megascolidae and Cynipidae). J. Wash. Acad. Sci. 74, 31 – 38.
27. Ings TC et al. 2009 Ecological networks—beyond food webs. J. Anim. Ecol. 78, 253 – 269. (doi:10.1111/j.1365-2656.2008.01460.x)
28. van Veen FfJ, Morris RJ, Godfray HCJ. 2006 Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. Annu. Rev. Entomol. 51, 187 – 208. (doi:10.1146/annurev.ento.51.110104.151120)
29. Vanderkift M, Ponsard S. 2003 Sources of variation in consumer-diet stoichiometry: a meta-analysis. Oecologia 136, 169 – 182. (doi:10.1007/s00442-003-1270-z)
30. Minagawa M, Wada E. 1984 Stepwise enrichment of 15N along food chains: further evidence and the relation between δ15N and animal age. Geochim. Cosmochim. Acta 48, 1135 – 1140. (doi:10.1016/0016-7037(84)90204-7)
31. Perkins MJ, McDonald RA, van Veen FfJ, Kelly SD, Rees C, Bearhop S. 2014 Application of nitrogen and carbon stable isotopes to quantify food chain length and trophic structure. PLoS ONE 9, 1 – 10. (doi:10.1371/journal.pone.0093281)
32. Buitenhuys R, Vet L, Boivin G, Brodeur J. 2005 Foraging behaviour at the fourth trophic level: a comparative study of host location in aphid hyperparasitoids. Entomol. Exp. Appl. 114, 107 – 117. (doi:10.1111/j.1570-7458.2005.00234.x)
33. R Core Team. 2014 R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. See http://www.R-project.org/ (accessed 10 April 2014).
34. Pinheiro J, Bates D, DebRoy S, Sarkar D, orpR Development Core Team. 2013 nlme: Linear and nonlinear mixed effects models. (R package version 3.1-109).
35. Crawley M. 2007 Statistical modelling. In The R book, ch. 9, pp. 323 – 386. New York, NY: John Wiley & Sons, Ltd.
36. Gelman A, Su Y-S. 2014 arm: Data analysis using regression and multilevel/hierarchical models. (R package version 1.7-03).
37. Barnett M, Schmidt J. 1991 A comparison between the amino acid composition of an egg parasitoid wasp and some of its hosts. Entomol. Exp. Appl. 59, 29 – 41. (doi:10.1111/j.1570-7458.1991.tb01463.x)
38. Hartley SE, Jones CG. 1997 Plant chemistry and herbivory, or why the world is green. Oxford, UK: Blackwell Science Ltd.

39. van Veen FJF. 2015 Plant-modified trophic interactions. Curr. Opin. Insect Sci. 8, 29 – 33. (doi:10.1016/j.cois.2015.02.009)

40. Wilder SM, Norris M, Lee RW, Raubenheimer D, Simpson SJ. 2013 Arthropod food webs become increasingly lipid-limited at higher trophic levels. Ecol. Lett. 16, 895 – 902. (doi:10.1111/ele.12116)

41. Raubenheimer D, Simpson SJ, Mayntz D. 2009 Nutrition, ecology and nutritional ecology: toward an integrated framework. Funct. Ecol. 23, 4 – 16. (doi:10.1111/j.1365-2435.2009.01522.x)

42. Visser B, Van Dooremalen C, Vázquez Ruiz A, Ellers J. 2013 Fatty acid composition remains stable across trophic levels in a gall wasp community. Physiol. Entomol. 38, 306 – 312. (doi:10.1111/phen.12035)

43. Le Ralec A. 1995 Egg contents in relation to host-feeding in some parasitic hymenoptera. Entomophaga 40, 87 – 93. (doi:10.1007/BF02372684)

44. Casas J et al. 2003 Energy dynamics in a parasitoid foraging in the wild. J. Anim. Ecol. 72, 691 – 697. (doi:10.1046/j.1365-2656.2003.00740.x)