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Lifetime allocation of juvenile and adult nutritional resources to egg production in a holometabolous insect

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In holometabolous insects reproductive success is strongly determined by the nutritional resources available to the females. In addition to nutrients derived from adult feeding, resources for egg production may come from the limited reserves accumulated during the larval stages. The pattern of allocation of these larval reserves to egg production is expected to be strongly linked to the nutritional ecology of the adult. We investigate the temporal pattern of allocation of larval reserves to reproduction in a host-feeding parasitoid wasp. As predicted by the dynamics of allocation of an adult meal, larval reserves are the main source of nutrients for four or five days after emergence. However, despite the high frequency of host feeding and the high nutrient content of a haemolymph meal, which we predicted would lead to larval reserves being conserved in the event of host deprivation, larval reserves contribute to egg production throughout the lifetime of the female. We propose several mechanistic and adaptive explanations for our results, including the possible existence of a limiting or key nutrient for egg production of exclusively larval origin. We make further predictions concerning the pattern of allocation of larval resources in parasitoids with different adult nutritional requirements.

Keywords: parasitoid; egg production; nutrient allocation; larval reserves; radioactive labelling

1. INTRODUCTION

The pattern of allocation of nutritional resources to reproduction has critical consequences for the fitness of organisms and is fundamental to numerous fields of research in behavioural, evolutionary and population ecology (Roff 1992). Organisms with life cycles consisting of several well-differentiated life stages are particularly interesting from the point of view of resource-allocation decisions (Boggs 1981). Multistage life cycles allow the exploitation of different types of environments by the same individual (Wilbur 1991), with the consequent qualitative and quantitative differences in the types of nutrients available to the juvenile and adult stages. In addition, the allocation of nutritional resources is partitioned between the stages, with juvenile resources being mostly invested in growth, while adult resources are mostly invested in reproduction (Truman & Riddiford 1999). During the complex process of metamorphosis, however, surplus nutrients acquired during the younger stages and not used for larval maintenance are reallocated, and the adult emerges with a certain level of reserves of juvenile origin. The quantity, quality and pattern of allocation of such juvenile reserves to egg production and survival are key aspects of the life history of multistage organisms. Issues such as what proportion of the juvenile resources should be allocated to reproduction and how much should be saved for survival, or whether these resources should be spent at the beginning of the adult life or saved in case of future food shortages, will have key consequences for the fitness of individuals and for the resilience of natural populations to changes in adult food availability (Boggs 1997*a*,*b*).

Two possible scenarios for the allocation of juvenile- and adult-derived resources have been proposed for holometabolous insects, the group of organisms where the transition between life stages, and the ensuing reallocation of resources, is one of the most drastic found in nature (Boggs 1997*a*,*b*; O'Brien *et al*. 2000). In the first scenario, larval- and adult-derived nutrients may constitute a common pool, or pure mix, of resources. The concept of a single pool of nutrients from which resources are allocated to either reproduction or survival was implicit in Van Noordwijk & De Jong's (1986) original Y model of resource allocation, and has been adopted as a convenient conceptual framework in some of the classic trade-off manipulation experiments (Chippindale *et al*. 1993; Tatar & Carey 1995). A second scenario, however, involves compartmentalization of the resources available for egg production and survival. Under this scenario, three different strategies are possible for the differential use of larval and adult food sources (Boggs 1997*a*). These three strategies represent the extremes of a likely continuum. First, eggs could be built entirely from adult food sources. Larval reserves would be conserved and used exclusively for adult maintenance, or for reproductive purposes only in the event of food shortages during the life span of the female. Second, eggs could be built entirely from larval reserves, until these are depleted, at which point, if necessary, they would be replaced with adult-derived nutrients. Finally, both larval and adult food sources could be invested in egg production throughout the lifetime of the female, at a certain rate, which could be constant or could vary over time. The strategy chosen should depend on two factors: the timing of feeding with respect to reproduction (with its two

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extremes represented by autogeny, where eggs can be produced without adult food, and anautogeny, where females need to feed to be able to produce eggs) and the quantity, quality and predictability of adult food sources in the environment (Karlsson 1994; Boggs 1997*b*; Oberhauser 1997).

Some of the most comprehensive theoretical models of resource allocation to reproduction and survival have been developed in order to predict female reproductive behaviour in a group of insects known as parasitoid wasps (Houston *et al*. 1992; Chan & Godfray 1993; Collier *et al*. 1994; Godfray 1994; Heimpel *et al*. 1994, 1998; Collier 1995*a*). These models have provided us with an unprecedented understanding of the behavioural trade-offs and physiological constraints faced by foraging insects. The wide range of parasitoid nutritional requirements and potentials of the different types of adult food to contribute to egg production and survival (Rivero & Casas 1999*a*) makes them ideal models to study the pattern of larval-resource use in insects. Parasitoids search the environment for hosts, usually other insects, in which to lay their eggs. Some parasitoids, the so-called synovigenic parasitoids, need to feed throughout their adult life, in many cases from the host itself, in order to produce eggs (Flanders 1950). While in some synovigenic species host feeding will provide resources for maintenance, in others the female also has to forage for sugar sources, such as nectar or honeydew for survival (Jervis & Kidd 1986; Heimpel & Collier 1996; Rivero & Casas 1999*a*). Pro-ovigenic parasitoids, on the other hand, are born with their entire, or near entire, complement of eggs (Flanders 1950) but may rely on sugar sources for survival. In both types of parasitoids, the decisions of the females regarding when to search for food and when to search for oviposition sites are triggered by physiological parameters. Indeed, in all the mathematical models of parasitoid behaviour developed so far, foraging decisions have been shown to be strongly dependent on the pattern of allocation of nutrients from adult food to egg production and survival (Houston *et al*. 1992; Chan & Godfray 1993; Collier *et al*. 1994; Heimpel *et al*. 1994; Collier 1995*a*; Sirot & Bernstein 1996).

In this paper, we quantify the pattern of allocation of larval reserves to egg production in a synovigenic parasitoid using a double-radiotracer technique. We deliberately chose a parasitoid whose pattern of allocation of nutrients from adult food has been studied in detail (Rivero & Casas 1999*b*). *Dinarmus basalis* is a host-feeding parasitoid of bruchid beetles infecting silos all over the world. Females emerge with no mature eggs in their ovarioles and, although they will feed immediately after emergence, it takes four or five days for the nutrients taken with the first meal to be fully invested in the eggs (Rivero & Casas 1999*b*). Females in the presence of hosts will feed regularly, and feeding contributes to egg production, survival and storage (Rivero & Casas 1999*b*). Although host feeding is rarely concurrent (i.e. females will not usually both feed and lay an egg in the same host), hosts are found in patches (several hosts per infected bean, groups of beans) and thus feeding and reproductive opportunities are likely to be correlated. The nutritional ecology of this species allows us to make two specific predictions about its pattern of allocation of larval reserves: first, given the delay in the appearance of nutrients in the eggs after the initial host meal, larval reserves should be the main source of nutrients for egg production for four or five days after emergence, and second, given the frequent host-feeding events, the high nutrient content of a haemolymph meal (Florkin & Jeuniaux 1964; Mullins 1985) and the ability of the female to store amino acids for future reproductive purposes (Rivero & Casas 1999*b*), larval reserves should not be invested in egg production at any other point of the female life span but should be saved to prolong survival in the event of host deprivation. We discuss the use of larval reserves in the context of the specific life history and nutritional requirements of this species and compare our results with those found in butterflies and moths (Boggs 1997*a*; O'Brien *et al*. 2000). Alternative patterns of allocation of larval reserves in parasitoids with different life histories and nutritional requirements are proposed.

2. MATERIAL AND METHODS

D. basalis (Hymenoptera: Pteromalidae) is a host-feeding synovigenic ectoparasitoid of third- to fourth-instar larvae of *Callosobruchus maculatus* (Coleoptera: Bruchidae) infecting *Vigna ungiculata* beans (Fabaceae). The pattern of incorporation of nutrients from larval reserves and adult feeding into the eggs laid by the parasitoid was determined using double-label radiotracer techniques with ^{14}C and ^{3}H. The purpose of this technique was to obtain eggs containing a ^{3}H larval pool and a ^{14}C adult-feeding pool, as well as eggs containing a ^{14}C larval pool and a ^{3}H adult-feeding pool.

(a) *Marking the larval reserves*

Third-instar hosts were extracted from the beans and injected with either 1 µl of a ^{3}H-marked amino-acid mixture (37 MBq ml^{-1}, ICN Pharmaceuticals) or 2 µl of a ^{14}C-marked amino-acid mixture (3.7 MBq ml^{-1}, ICN Pharmaceuticals). The amino-acid mixtures had been previously diluted with Ringer's solution to a total activity of 7 kBq ml^{-1} (for the ^{3}H mixture) or 3.5 kBq ml^{-1} (for the ^{14}C mixture) so that the total activity injected into each larva was, in both cases, 7 kBq. Injections were carried out using a graduated micro capillary connected to a manual pump and with the aid of a binocular microscope. Injections took place midway along the side of the host's body, which, our own trials showed, almost entirely eliminated the loss of bodily fluids through the wound. Larvae that 'bled' profusely after the injection were immediately discarded. Injected larvae were kept at room temperature for a minimum of 2 h to allow the wound to scar over and for the radioactivity to be distributed within the body.

Parasitoid eggs were obtained by individually placing *D. basalis* females in small Petri dishes (diameter of 5.5 cm) with two non-radioactive third-instar larvae of *C. maculatus* positioned in an artificial bean made from a gelatine capsule (for details of how hosts are prepared inside the capsule see Gauthier & Monge 1999). Females were left to oviposit on the hosts for 24 h, after which one egg from each female was collected and transferred onto a randomly chosen ^{3}H- or ^{14}C-injected larva. The rest of the eggs were used as controls for background radiation (see § 2c). Although parasitoids will readily oviposit directly on injected larvae, our technique was preferred because it considerably reduced the manipulation of the injected larvae; parasitoids usually lay three or four eggs per host in a 24 h period, often on the underside of the larvae, which therefore need to be taken off

the gelatine capsules and turned in order to eliminate any multiple eggs.

Once the egg had been transferred onto the surface of the injected larva, the larva was placed inside an artificial bean (as above) and the bean was kept in a small Petri dish at 13 L:11 D photoperiod, 33°C:23°C temperature and 75% humidity until the emergence of the parasitoid. The time taken by *D. basalis* to develop from egg to adult inside the gelatine capsule is the same as the normal developmental time inside a real bean: 15 or 16 days. The adult parasitoid breaks free from the artificial bean and into the Petri dish by biting off the gelatine capsule. A total of six female parasitoids with larval reserves marked with ^{3}H and eight female parasitoids with larval reserves marked with ^{14}C were obtained in this manner.

(b) *Marking the adult reserves*

One day after emergence, the parasitoids with their larval reserves marked were individually weighed inside a gelatine capsule and then placed in a Petri dish (diameter of 5.5 cm) with an artificial bean containing a third-instar host under the temperature, humidity and photoperiod mentioned in § 2(a). The hosts had been previously injected with either ^{3}H or ^{14}C amino-acid mixture (see § 2a). Females that had had their larval reserves marked with ^{3}H were provided with ^{14}C-injected hosts and vice versa. Preliminary trials had shown that females both feed and oviposit on these injected hosts. Egg laying and host feeding cannot be decoupled in this species, and, although females seem to make feeding tubes at least some of the time, these feeding tubes are easily broken and thus the frequency of host feeding cannot be estimated. Each day the host was removed, the eggs were extracted and the female was provided with a new injected host. This process continued until the death of the parasitoid. The gelatine capsules containing the eggs were stored on a daily basis at −80°C for later analysis. At the end of the experiment the female was also stored at −80°C to analyse the radioactivity remaining in the body.

(c) *Sample preparation and radioactivity quantification*

Quantification of the amount of each isotope incorporated into the eggs was carried out using a liquid-scintillation analyser (TriCarb1900, Packard Instruments). The batch of eggs laid by each female on each day was prepared by crushing them together in a liquid-scintillation tube and immediately adding 100 µl of a tissue solvent (Soluene®-350, Packard Instruments). After 30 min, 1 ml of the liquid-scintillation cocktail (Hionic-Fluor™, Packard Instruments) was added. In order to control for background radiation in the environment, one extra tube for each batch of eggs prepared was used, containing the tissue solvent and the liquid-scintillation cocktail but no egg. After 1 h, all tubes were read in the liquid-scintillation analyser for 15 min and the number of disintegrations per minute (DPM) on each side of the energy spectrum (0–15 keV for ^{3}H and 16–156 keV for ^{14}C) was recorded. An external standard was used as a quench-indicating parameter (the transformed spectral index of the external standard, tSIE). In addition, an automatic efficiency control function that makes use of the tSIE values to adjust the lower and upper energy boundaries of each isotope to compensate for differences in quench levels between the isotopes was selected. Since larval and adult resources are marked in each female, with a different isotope, each with its own specific activity and metabolic pathway, it is not possible to calculate the relative investment of larval and adult food sources into eggs in terms of proportions. DPM for ^{3}H and ^{14}C are, thus, reported separately.

Further, in order to control for the presence of natural radiation in the eggs, a series of control tubes was prepared by crushing unmarked eggs either singly or in batches ranging from two to seven eggs, and analysing them separately in the same way as the experimental eggs. The mean radioactivity in these unmarked eggs was very low (1.52 ± 0.69 DPM for ^{3}H and 5.56 ± 1.06 DPM for ^{14}C after correction for environmental radioactivity, $n = 7$ in both cases) and was independent of the number of eggs in the batch (Kruskal–Wallis test, $\chi^2_6 = 7.0$, non-significant, for ^{3}H and $\chi^2_6 = 2.3$, non-significant, for ^{14}C). This lack of correlation between DPM and the number of unmarked eggs in the batch suggests that, in fact, the DPM readings are precision errors in the radioactivity measurements of the liquid-scintillation analyser rather than natural radiation associated with parasitoid eggs. However, in order to obtain a stringent measure of the radioactivity incorporated into the eggs as a result of our experiment, as opposed to background noise or error detection in the liquid-scintillation analyser, these measurements were taken into account. For each isotope, the radioactivity incorporated per egg as a result of our experimental treatment was calculated by dividing the DPM of the egg batch, as calculated by the liquid-scintillation analyser, by the number of eggs in the batch and then subtracting the mean DPM of unmarked eggs (7.0 DPM for ^{3}H or 2.3 DPM for ^{14}C).

The females were analysed to determine the quantity of resources of larval and adult signature not invested in eggs and remaining in the body at the end of the experiment. For this purpose, each female was separated into two fragments, the abdomen and the rest of the body, which were crushed, prepared and read separately in the liquid-scintillation analyser following the same procedure as for the eggs. In order to avoid losing radioactivity from the body as a result of dissection, the mature eggs remaining in the ovaries were not dissected out of the abdomen before it was crushed and read in the liquid-scintillation analyser. The values for the quantities of nutrients remaining in the abdominal tissue and fat body may thus be slightly overestimated. In addition, in order to estimate the quantity of larval reserves present in the body of the female at emergence, an additional group of females with the larval reserves marked with either ^{3}H ($n = 10$) or ^{14}C ($n = 10$) was obtained as described in § 2a. These females were killed on the day of emergence and the radioactivity present in the two body fragments (head–thorax and abdomen) was quantified using the same procedure. The hind-tibia lengths of all females involved in the experiment were measured in order to control for size-related differences in resource acquisition or allocation. Non-parametric tests reported were carried out using SPSS (SPSS Inc., USA).

3. RESULTS

The mean weights and tibial lengths of females emerging from hosts injected with ^{3}H and ^{14}C did not differ significantly (for weight: mean ± s.e.m. = 1.27 ± 0.07 mg and mean ± s.e.m. = 1.25 ± 0.06 mg, respectively, Mann–Whitney $U = 15.0$, non-significant; and for tibial length: mean ± s.e.m. = 0.74 ± 0.014 mm and mean ± s.e.m. = 0.76 ± 0.004 mm, respectively, Mann–Whitney $U = 10.0$, non-significant). Females also laid a similar mean total number of eggs in both treatments (mean ± s.e.m. = 64.38

Table 1. *Mean ± s.e.m. disintegrations per minute of larval and adult origin recovered from the laid eggs and from the head–thorax and abdominal fractions of females at the end of the experiment* (*'end': ^3H larval/^{14}C adult, n = 6; ^{14}C larval/^3H adult, n = 8*) *and on the day of emergence* (*'start': n = 10 for both treatments*)

(Total radioactivity is calculated as the sum of the radioactivities of the eggs, head–thorax and abdominal fractions. Numbers in parentheses are the mean ± s.e.m. percentages of the total radioactivity recovered at the start and end of the experiment.)

	eggs		head–thorax		abdomen		total	
source	start	end	start	end	start	end	start	end
larval (^{3}H)	—	5393.7 ± 751.5 (23.6 ± 1.5)	17 024.1 ± 615.6 (72.9 ± 2.9)	13 902.3 ± 1833.1 (60.1 ± 1.4)	6928.6 ± 1239.9 (27.1 ± 2.9)	3565.3 ± 320.3 (15.9 ± 0.8)	23 952.7 ± 1316.4	22 861.3 ± 2754.1
adult (^{14}C)	—	6338.1 ± 875.2 (62.4 ± 4.5)	—	1898.1 ± 139.9 (18.4 ± 1.3)	—	2039.7 ± 599.2 (19.1 ± 5.0)	—	10 575.8 ± 1254.3
larval (^{14}C)	—	555.6 ± 35.5 (15.7 ± 1.4)	4198.2 ± 438.8 (85.9 ± 1.7)	2600.0 ± 316.4 (69.6 ± 3.1)	611.5 ± 53.6 (14.1 ± 1.7)	537.4 ± 90.9 (14.7 ± 2.6)	4809.6 ± 462.1	3692.9 ± 369.4
adult (^{3}H)	—	7352.5 ± 1027.7 (46.0 ± 3.6)	—	5086.2 ± 811.8 (32.0 ± 4.6)	—	3611.6 ± 517.0 (22.1 ± 1.6)	—	1650.3 ± 1779.1

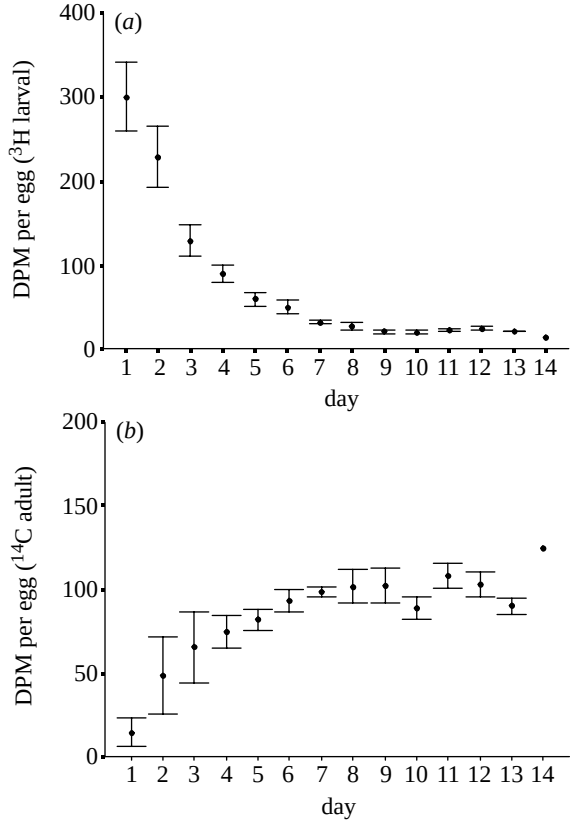

Figure 1. Time course of radioactivity in the eggs produced by females with (*a*) their larval reserves marked with ^{3}H and (*b*) their adult reserves marked with ^{14}C. Values shown are the mean radioactivities per egg (see § 2c for details).

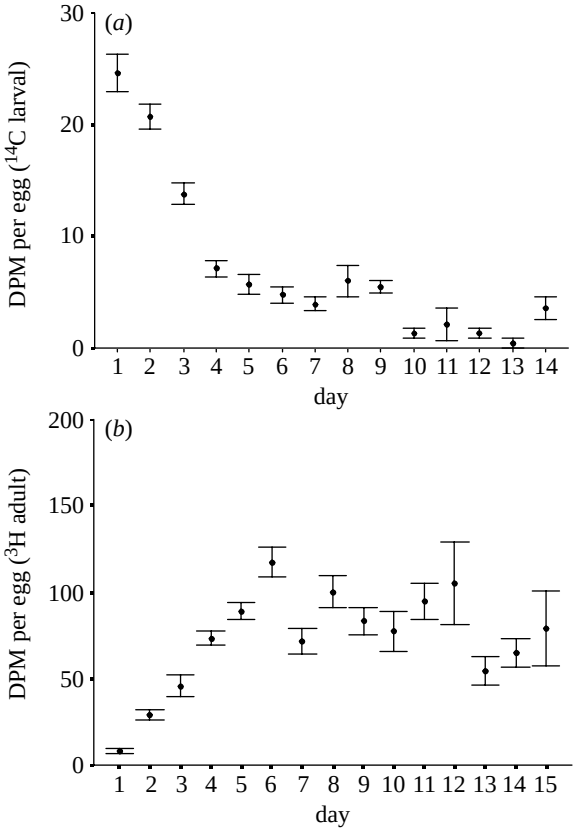

Figure 2. Time course of radioactivity in the eggs produced by females with (*a*) their larval reserves marked with ^{14}C and (*b*) their adult reserves marked with ^{3}H. Values shown are the mean radioactivities per egg (see § 2c for details).

± 6.62 and mean ± s.e.m. = 75.00 ± 6.08 for females laying eggs and host feeding in ^{3}H- and ^{14}C-injected hosts, respectively, Mann–Whitney $U = 13.5$, non-significant). The number of eggs laid per day varied from mean ± s.e.m. = 4.80 ± 0.37 and mean ± s.e.m. = 3.89 ± 0.35 (for females laying eggs and host feeding in ^{3}H- and ^{14}C-injected hosts, respectively) on the first day, to a maximum on day 5 of mean ± s.e.m. = 8.20 ± 0.37 and mean ± s.e.m. = 8.25 ± 1.03, respectively. Egg laying decreased monotonically from day 5 onwards so that towards the end of the experiment (day 14) the number of eggs laid was mean ± s.e.m. = 4.67 ± 0.67 and mean ± s.e.m. = 3.50 ± 0.50, respectively.

Larval nutrients incorporated into the eggs marked with ^{3}H rendered higher total DPM than eggs marked with ^{14}C (table 1). This is probably due to the ten-fold difference in the specific activities of the two isotopes. Although the low DPM in the ^{14}C treatment resulted in higher errors, the incorporation of larval nutrients marked with each isotope followed a similar pattern. The maximal incorporation of resources with a larval signature occurred on day 1 (mean ± s.e.m. = 300.60 ± 40.56 DPM and mean ± s.e.m. = 24.66

± 1.67 DPM for reserves marked with ^{3}H and ^{14}C, respectively), decreasing thereafter and levelling off from *ca.* day 6 onwards (at around 20–30 DPM for reserves marked with ^{3}H and 1–5 DPM for reserves marked with ^{14}C, respectively) (figures 1*a* and 2*a*).

The total radioactivity from adult food recovered in eggs was independent of the isotope used (table 1, Mann–Whitney $U=11.0$, non-significant). The explanation for the finding that the tenfold difference in specific activity between the two isotopes is detected in the larval reserves but not in the reserves obtained from adult food is not clear, but may be related to differences in the metabolism of ingested C and H in the adult. In both treatments, the incorporation of nutrients from adult food into the eggs was minimal at the beginning of the experiment and increased with time, levelling off at around 100 DPM from day 6 onwards (figures 1*b* and 2*b*).

The radioactivities recovered from the head–thorax and abdomen fractions of the females at the end of the experiment and from the females killed on the day of emergence are also shown in table 1. On emergence, over 70% (80% for ^{14}C) of the larval reserves are located in the head–thorax fraction. By the end of the experiment, larval reserves in the head–thorax had decreased by an average of 18.33% for ^{3}H and 38.1% for ^{14}C, while those in the abdomen had decreased by 48.5% for ^{3}H and 12.1% for ^{14}C. Adult food resources, on the other hand, were recovered mostly from the eggs (table 1), although the relative investment in eggs was higher for ^{14}C than for ^{3}H.

4. DISCUSSION

The pattern of allocation of larval- and adult-derived nutrients found in *D. basalis* confirms the existence of priority rules in the allocation of nutrients obtained in different stages of the life cycle of holometabolous insects. Larval reserves were largely found in the head–thorax fraction, which can be roughly approximated to the resources allocated to growth and somatic maintenance on emergence (Boggs 1981; Karlsson 1994; Stevens *et al.* 1999), while only a small percentage was recovered from the abdomen. In contrast, most of the adult food ingested was recovered from the abdominal fraction and from the eggs, confirming the predominant role of host feeding in egg production in this species. Larval reserves were, nevertheless, the most important source of nutrients for egg production in the first few days after female emergence (figures 1*a* and 2*a*). As predicted, this result is in agreement with the temporal pattern of allocation of adult food to eggs found in this species (Rivero & Casas 1999*b*). Female *D. basalis* require four or five days to make full use of their first host meal for egg production. During this time, egg-production requirements are subsidized by the resources accumulated as a larva. The investment of larval reserves in egg production decreases in subsequent days as the female accumulates nutrients from host feeding, until a baseline level is reached from *ca.* day 6 onwards. This baseline level represented as much as 10%–20% of the maximum larval-derived egg DPM, which were found on day 1. Therefore, contrary to our second prediction, larval reserves contribute to egg production throughout the lifetime of the female. Interestingly, this result is similar to that found in Lepidoptera, the only other system where, to our knowledge, the temporal pattern of larval-resource allocation has been quantified (Boggs 1997*a*; O'Brien *et al.* 2000). In the Lepidoptera it has been suggested that the prolonged contribution of larval reserves to egg production stems from a compartmentalization of larval and adult food sources into two separate pools (O'Brien *et al.* 2000). In addition to a pure-mix pool, where nutrients of larval and adult origin mix and from which allocation of nutrients to egg production depends simply on the relative concentrations of each type of nutrient, there would be a non-mix pool of larval origin. This pool would provide nutrients to the eggs independently and constantly throughout the lifetime of the female.

The existence and nature of the prolonged contribution of larval reserves to egg production can be explained in different ways. The explanation favoured by O'Brien *et al.* (2000) in their moth system is the existence of one or several nutrients essential for egg production that are found in larval reserves but cannot be found by the foraging adult. The existence of a limiting or 'key' nutrient would arise either through differences in the foraging habits and type of nutrients obtained during the larval and foraging stages or through differences in the metabolic paths available to each stage. Unlike moths and most other holometabolous insects, where the diet and feeding habits of the juvenile and adult stages are drastically different, parasitoids are unique in that adults and larvae feed from the same resource: the host. However, in many species of parasitoid, such as *D. basalis*, the adult feeds only from small amounts of haemolymph that exude from punctures in the host cuticle (Jervis & Kidd 1986), while the larva consumes the entire host, including not only the haemolymph but also the fat body, digestive tract, etc. Comparison of the pattern of allocation of larval resources in strictly haemolymph feeders and in parasitoids that consume the entire host (Jervis & Kidd 1986) would be useful in determining to what extent differences in juvenile and adult diets give rise to the results obtained. If proven, the existence of a key nutrient would have critical consequences for our understanding of the significance of the holometabolous lifestyle. In moths, the use of stable isotopes is likely to go a long way towards answering this question (O'Brien *et al.* 2000).

Alternatively, the pool of nutrients providing a prolonged contribution of larval reserves to egg production may be the result of an anatomical compartmentalization of stored nutrients. The functional significance of the clear regional and structural differentiation within the fat body, the main storage and metabolic organ in insects, has been little explored (Haunerland & Shirk 1995). In addition, the mobilization of other tissues, notably muscles, for egg production and maintenance is a well-known phenomenon in insects (Usherwood 1975; Stjernholm & Karlsson 2000). In this experiment, the mean reduction in abdominal radioactivity of larval origin between the beginning and the end of the experiment was lower than the amount invested in egg production (table 1). This shows conclusively that some of the teneral resources for egg production came not from the abdominal fat body but from the head and/or thorax, commonly known to be composed mostly

of flight muscles (Stevens *et al*. 1999). The results obtained could, thus, be due to differences in the rates of mobilization of nutrients stored in different types of tissues. Alternatively, it is possible that organs or tissues in the reproductive tract and closely associated with egg production (nurse cells or accessory glands) could have simply retained a larval radioactive signature that would automatically 'mark' all the eggs.

Further studies are required to determine whether the observed pattern of larval-resource allocation has a simple mechanistic explanation or whether it has an adaptive basis. If it is adaptive then similar studies in parasitoids exhibiting different life histories and nutritional requirements should be expected to conform to a series of predictions. In non-host-feeding parasitoids, particularly those that produce yolk-rich eggs (e.g. *Leptomastix dactylopii*; Zinna 1959), larval reserves constitute the main source of protein and are, therefore, logically expected to play a high and uniform role in egg production throughout the lifetime of the female. Parasitoids in which host feeding provides nutrients for egg production but not survival (e.g. *Aphytis melinus*; Heimpel & Collier 1996) may, on the other hand, be expected to conserve larval resources for maintenance in order to reduce the need to invest in costly sugar-foraging trips (Sirot & Bernstein 1996). Similarly, parasitoids with very short life expectancies, due to, for instance, intense predation pressure (e.g. *Aphytis aonididae* Rosenheim *et al*. 2000), should be expected to rely entirely on their larval reserves for egg production. In addition, systems where one can experimentally decouple host feeding and egg laying (Collier 1995*b*) are particularly interesting for testing the plasticity of the allocation of larval reserves to egg production in parasitoids faced with environmental variation such as changes in nutrient or reproductive opportunities. The host-injection technique developed in this paper should provide a straightforward way of marking larval reserves in most parasitoid species attacking sizeable hosts.

Although there is a growing awareness among behavioural ecologists and theoretical population biologists of the importance of adult feeding, the pattern of use of stored versus incoming resources in insects has been largely overlooked (Rivero & Casas 1999*a*). Our results call for further studies on the role and adaptive nature of larval-reserve use in parasitoids. The suggestion that the female's body may not behave as a simple pool of nutrients that is replenished every time the insect eats but that resources may be metabolically or anatomically compartmentalized (Boggs 1997*a*,*b*; O'Brien *et al*. 2000) could have important consequences for models aiming to predict parasitoid behaviour on the basis of physiological allocation rules (Rivero & Casas 1999*a*; Rosenheim *et al*. 2000) and, more generally, for our understanding of the factors limiting lifetime reproduction in insects.

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