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

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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). Multi-stage 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 type 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 by the younger stages and not used for larval maintenance are reallocated, and the adult emerges with a certain amount 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 multi-stage organisms. Questions such as how many of the juvenile resources should be allocated for reproduction and how many saved for survival or whether these resources should be spent at the beginning of the adult life or saved in the event of future food shortages will bear key consequences for the fitness of individuals and for the resiliency of natural populations to changes in adult food availability (Boggs 1997a; Boggs 1997b).

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 ones found in nature (Boggs 1997a; Boggs 1997b; O'Brien *et al.* 2000). Firstly, larval and adult-derived nutrients may constitute a common pool, or pure mix, of resources. The concept of a single pool of nutrients from where 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 compartmentalisation of the resources available for egg production and survival. Under this second scenario three different strategies are in turn possible for the differential use of larval and adult food sources (Boggs 1997a). These three strategies

represent extremes of a likely continuum**.** First, eggs could be entirely built from adult food sources. Larval reserves would be conserved and used exclusively in adult maintenance, or resorted to 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 vary with time. The strategy chosen should depend on two factors: the timing of feeding with respect to reproduction (with its two 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 1997b; Oberhauser 1997).

Some of the most comprehensive theoretical models of resource allocation to reproduction and survival have been developed with the purpose of predicting 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; Collier 1995a; Heimpel *et al.* 1998). These models have provided us with an unprecedented understanding of the behavioural trade-offs and physiological constraints faced by foraging insects. The wide range in parasitoid nutritional requirements and on the potential of the different types of adult food for egg production and survival (Rivero & Casas 1999a) 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, so-called *synovigenic* , 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 1999a). *Proovigenic* 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 in terms of 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 1995a; 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 choose a parasitoid whose pattern of allocation of nutrients from adult food has been studied in detail (Rivero & Casas 1999b). *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 4-5days for the nutrients taken with the first meal to be fully invested in the eggs (Rivero & Casas 1999b). Females in the presence of hosts will feed regularly and feeding contributes to egg production, survival and storage (Rivero & Casas 1999b). 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 two make two specific predictions about its pattern of allocation of larval reserves: (a) 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 4-5 days after emergence and (b) 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 1999b), larval reserves should not be invested in egg production at any other point of the female life span but saved to prolong survival in the event of host deprivation. We discuss the use of larval reserves to the specific life history and nutritional requirements of this species and compare our results with those found in butterflies and moths (Boggs 1997a; O'Brien *et al.* 2000). Alternative patterns of allocation of larval reserves in parasitoids with different life histories and nutritional requirements are proposed.

Materials and methods

Dinarmus 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 ^{13}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 pool.

Marking the larval reserves

Third instar hosts were extracted from the beans and injected with either 1µl of a ^{3}H-marked amino acid mixture (37MBq ml^{-1}, ICN Pharmaceuticals) or with 2µl of a ^{14}C-marked amino acid mixture (3.7MBq ml^{-1}, ICN Pharmaceuticals). The amino acid mixtures had been previously diluted with Ringer's solution to a total activity of 7kBq ml^{-1} (for the ^{3}H mixture) or 3.5kBq (for the ^{14}C mixture) so that the total activity injected in each larva was in both cases 7kBq. 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 on the mid-lateral side of the host's body which, our own trials showed, eliminated almost entirely 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 two hours to allow the wound to scar over and the distribution of the radioactivity within the body.

Parasitoid eggs were obtained by individually placing *D. basalis* females in small Petri dishes (diameter 5.5 cm) with two non-radioactive third-instar larvae of *C. maculatus* placed within 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 time one egg of each female was collected and transferred onto a randomly chosen ^{3}H- or ^{14}C-injected larvae. The rest of the eggs were used as a control for background radiation (see below). Although parasitoids will readily oviposit directly on injected larvae, this technique was preferred because it reduced considerably the manipulation of the injected larvae; parasitoids usually lay 3-4 eggs per host in a 24 h period, often on the underside of the larva, which therefore needs to be taken off the gelatine capsule and turned in order to completely eliminate any eggs in excess of one.

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 inside a small Petri dish at 13L:11D photoperiod, 33:23^{o}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, 15-16 days. The adult parasitoid breaks free from the artificial bean and into the Petri dish by biting off the

gelatine capsule. A total of 6 female parasitoids with its larval reserves marked with ^{3}H and 8 female parasitoids with its larval reserves marked with ^{14}C were obtained in this manner.

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 (5.5 cm) with an artificial bean containing a third instar host under the above-mentioned temperature, humidity and photoperiod. The hosts had been previously injected with either ^{3}H or ^{14}C amino acid mixture (as above). Females that 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 times, these feeding tubes are easily broken and thus frequency of host feeding cannot be estimated. Each day the host was removed, the eggs were extracted and the female provided with a new injected host. This process was repeated daily until the death of the parasitoid. The gelatine capsules containing the eggs were stored on a daily basis at -80^{o}C for later analysis. At the end of the experiment the female was also stored at -80^{o}C to analyse the radioactivity remaining in the body.

Sample preparation and radioactivity quantification

Quantification of the amount of each isotope incorporated into the eggs was carried out using a liquid scintillation analyser (LSA, TriCarb1900, Packard Instruments). The batch of eggs laid by each female on each day were prepared by crushing them together in a liquid scintillation tube and immediately adding 100µl of a tissue solvent (Soluene®-350, Packard). After 30 min, 1ml of the liquid scintillation cocktail (Hionic-Fluor™, Packard) was added. In order to control for background radiation in the environment, for each batch of eggs prepared one extra tube containing the tissue solvent and the liquid scintillation cocktail but no egg was used. After one hour, all tubes were read in the LSA for 15 min each and the number of desintegrations per minute (DPMs) on each side of the energy spectrum (0-15 KeV for ^{3}H and 16-156 KeV for ^{14}C) obtained. An external standard was used as a quench-indicating parameter (tSIE: the transformed Spectral Index of the External standard). In addition, an automatic efficiency control function (AEC) 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 relative investment of larval and adult food sources into eggs in terms of proportions. DPM's 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 were prepared by crushing unmarked eggs either singly, or in batches ranging from 2 to 7 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 DPMs for ^{3}H and 5.56 ± 1.06 DPMs 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 $\chi^2_6 = 7.0$, NS for ^{3}H and $\chi^2_6 = 2.3$, NS 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 measures of the LSA rather than natural radiation associated to parasitoid eggs. However, in order to obtain a stringent measure of what was the radioactivity incorporated into the eggs as a result of our experiment, as opposed to background noise or simply error detection in the LSA, these measures were taken into account. For each isotope, the radioactivity incorporated *per egg* as a result of our experimental treatments was calculated by dividing the DPM of the egg batch, as calculated by the LSA, by the number of eggs in the batch, and subtracting the mean DPM of unmarked eggs (either 7.0 DPMs or 2.3 DPMs depending on the isotope).

The females were analysed to determine the amount of resources from 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 in the LSA separately following the same exact procedure as for the eggs. In order to avoid loosing radioactivity from the body as a result of a dissection, the mature eggs remaining in the ovaries were not dissected out of the abdomen before this was crushed and read in the LSA. The values for the amount of nutrients remaining in the abdomen tissue and fat body may thus be slightly overestimated. In addition, in order to estimate the amount of larval reserves present in the body of the females at emergence, an additional group of females with the larval reserves marked with ^{3}H (n=10) and ^{14}C (n=10) was obtained as above. These females were killed on the day of emergence and the radioactivity present in the two body fragments (head-thorax and abdomen) quantified using the same procedure. The hind tibia length of all females involved in the experiment was

measured in order to control for size-related differences in resource acquisition or allocation. Non-parametric tests reported were carried out using SPSS.

Results

The mean weight and tibia length of females emerging from hosts injected with ^{3}H and ^{14}C did not differ significantly (mean ± s.e. 1.27 ± 0.07 mg and 1.25 ± 0.06 mg weight respectively, Mann-Whitney U=15.0, NS and 0.74 ± 0.014 mm and 0.76 ± 0.004 mm respectively, Mann-Whitney U=10.0, NS). Females also laid a similar mean total number of eggs in both treatments (mean ± s.e. 64.38 ± 6.62 and 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, NS). The mean number of eggs laid per day varied from 4.80 ± 0.37 and 3.89 ± 0.35 (mean ± s.e. 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 (8.20 ± 0.37 and 8.25 ± 1.03 respectively). Egg laying decreased monotonically from day 5 onwards so that towards the end of the experiment (day 14) the mean number of eggs laid was 4.67 ± 0.67 and 3.50 ± 0.50 (for females laying in ^{3}H and ^{14}C larvae).

Table 1. Mean (± s.e.) DPMs from larval and adult origin recovered from the laid eggs, and from the head-thorax and abdomen fractions of females of the two treatments at the end of the experiment ('End', ^{3}H larval / ^{14}C adult: n=6; ^{14}C larval / ^{3}H adult: n=8) and on the day of emergence ('Start', n=10 for both treatments). Total radioactivity is calculated as the sum of eggs, head-thorax and abdomen fractions. Numbers in parenthesis are the mean (± s.e.) proportion of the total radioactivity found in each fraction calculated over the total recovered at the start and end of the experiment.

Source	Eggs		Head-thorax		Abdomen		Total	
	Start	End	Start	End	Start	End	Start	End
Larval (^{3}H)	-	5393.7 ± 751.5 (23.6 ± 1.5 %)	17024.1 ± 615.6 (71.1 ± 2.9 %)	13902.3 ± 1833.1 (60.8 ± 1.4 %)	6928.6 ± 1239.9 (28.9 ± 2.9 %)	3565.3 ± 320.3 (15.6 ± 0.8 %)	23952.7 ± 1316.4	22861.3 ± 2754.1
Adult (^{14}C)	-	6338.1±875.2 (61.7 ± 4.5 %)	-	1898.1 ± 139.9 (18.5 ± 1.3 %)	-	2039.7 ± 599.2 (19.8 ± 5.0 %)	-	10275.9 ± 1254.3
Larval (^{14}C)	-	555.6 ± 35.5 (15.0 ± 1.4 %)	4198.2 ± 438.8 (87.3 ± 1.7 %)	2600.0 ± 316.4 (70.4 ± 3.1 %)	611.5 ± 53.6 (12.7 ± 1.7 %)	537.4 ± 90.9 (14.6 ± 2.6 %)	4809.7 ± 462.1	3693.0 ± 369.4
Adult (^{3}H)	-	7352.5±1027.7 (45.8 ± 3.6 %)	-	5086.2 ± 811.8 (31.7 ± 4.6 %)	-	3611.6 ± 517.0 (22.5 ± 1.6 %)	-	16050.3 ± 1779.1

Larval nutrients incorporated into the egg marked with ^{3}H rendered higher total DPMs than nutrients marked with ^{14}C (Table 1). This is likely to be due to the ten-fold difference in the specific activity of the two isotopes. Although the low DPMs in the ^{14}C treatment resulted in higher errors, the incorporation of larval nutrients marked with both isotopes followed a similar pattern. The maximal incorporation of resources with a larval signature occurred on day 1 (mean ± s.e. 300.60 ±40.56 and 24.66 ± 1.67 DPMs for ^{3}H and ^{14}C respectively), decreasing thereafter and levelling off from ca. day 6 onwards (at around 20-30 DPMs for reserves marked with ^{3}H and 1-5 DPMs for ^{14}C respectively) (Figs 1a and 2a).

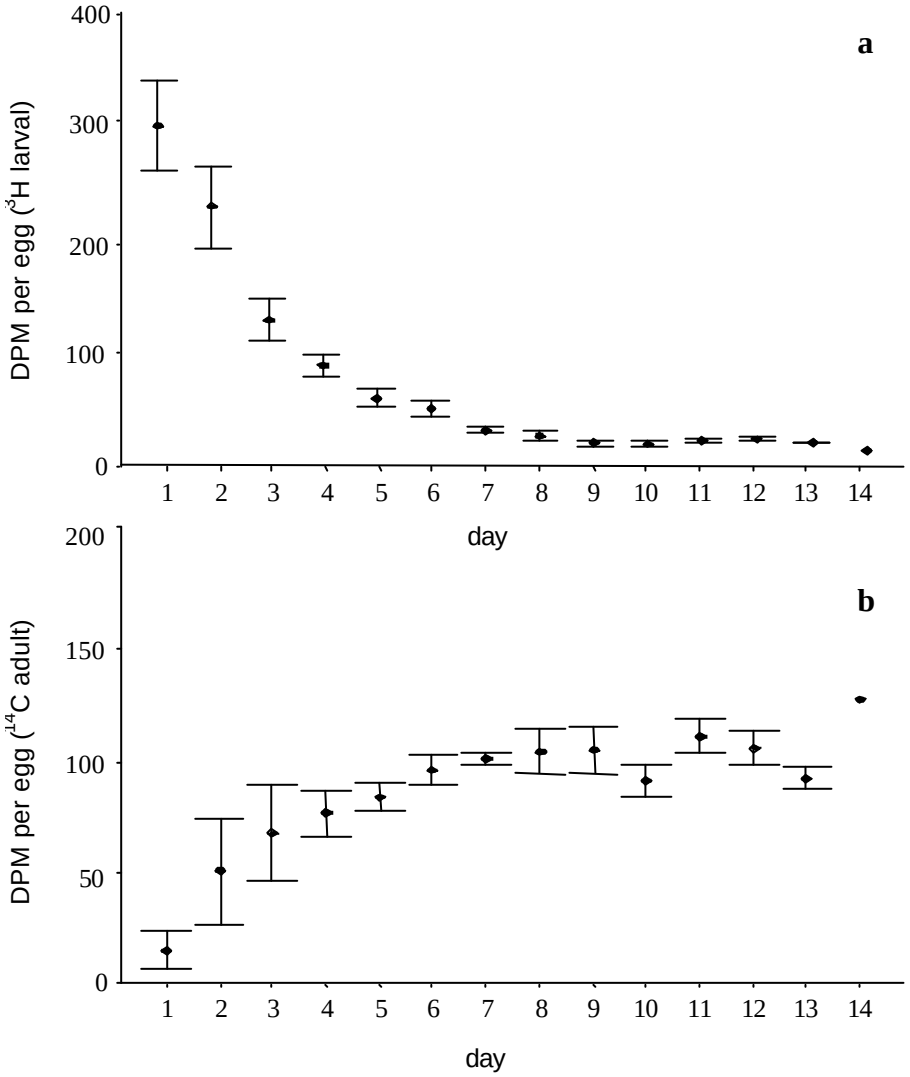

Figure 1. Time course of appearance of radioactivity in the eggs produced by females with their larval reserves marked with ^{3}H (a) and their adult reserves marked with ^{14}C (b). Values shown are mean radioactivity per egg (see text for details).

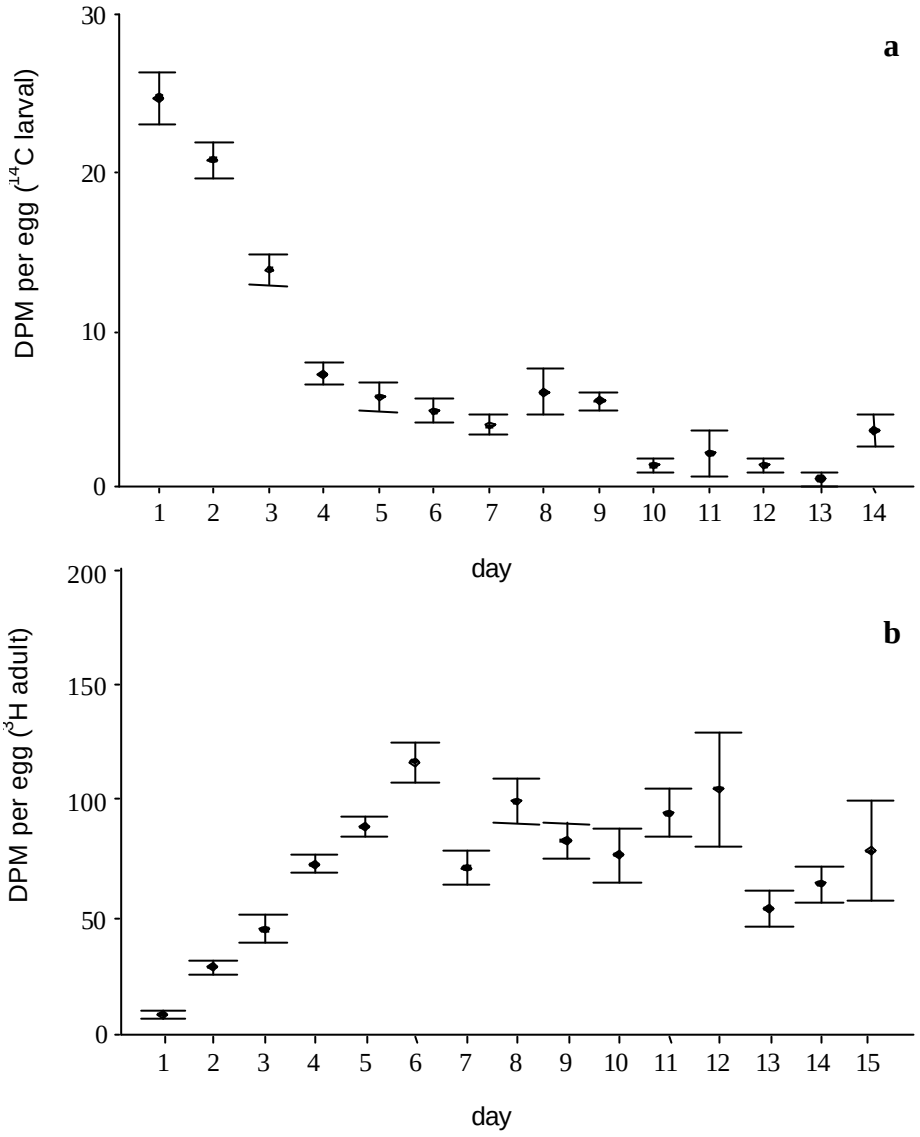

Figure 2. Time course of appearance of radioactivity in the eggs produced by females with their larval reserves marked with ^{14}C (a) and their adult reserves marked with ^{3}H (b). Values shown are mean radioactivity per egg (see text for details).

The total radioactivity from adult food recovered in eggs was however independent of the isotope used (Table 1, Mann-Whitney U= 11.0, NS)). The reasons for why the ten-fold 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 associated to differences in the metabolism of ingested C and H in the adult. In both treatments the incorporation of nutrients from adult food in to the eggs was minimal at the beginning of the experiment and increased with time, levelling off at around 100 DPMs from day 6 onwards (Figs. 1b and 2b)

The radioactivity recovered from the head-thorax and abdomen fractions of females at the end of the experiment and of 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 thorax fraction. By the end of the experiment larval reserves in the thorax have decreased by an average of 18.33% (^{3}H) and 38.1% (^{14}C) while those in the abdomen have decreased by 48.5% (^{3}H) and 12.1% (^{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 when the isotope used was ^{14}C.

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 and 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 abdomen fraction and from the eggs, confirming the predominant role of host feeding for 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 (Figs 1a and 2a). As predicted, this result is in agreement with the temporal pattern of allocation of adult food into eggs found in this species (Rivero & Casas 1999b). Female *Dinarmus basalis* require 4-5 days to make full use of its first host-meal for egg production after emergence. During this time egg production requirements are subsidised with 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 it reaches a baseline level from ca. day 6 onwards. This baseline level represented a as much as 10-20% of the maximum larval-derived egg DPMs, which where 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 1997a; O'Brien *et al.*

2000). Here it has been suggested that the prolonged contribution of larval reserves to egg production stems from a compartmentalisation of larval and adult food sources into two separate pools (O'Brien *et al.* 2000). In addition to a pure mix pool where nutrients from larval and adult origin mix and from where allocation of nutrients to egg production depends simply on the relative concentration of each of the two types of nutrient in the pool, there would be a non-mix pool of larval origin. This pool would provide nutrients to the eggs independently and constantly throughout the life 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 that cannot be found by the foraging adult. The existence of such a limiting or "key" nutrient would arise either through differences in the foraging habits and type of nutrients obtained by the larval and foraging stages, or through differences in the metabolic paths available to each of the stages. 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, and such is the case in *D. basalis*, the adult parasitoid only feeds from small amounts of haemolymph that exude from punctures in the host cuticle (Jervis & Kidd 1986) while the larval stages consume 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 eggs may be the result of an anatomical compartmentalisation 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, mobilisation 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 abdomen radioactivity of larval origin between the beginning and the end of the experiment was lower than the amount

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

Further studies are required in order to determine whether the observed pattern of larval resource allocation has a simple mechanistic explanation, or whether it has an adaptive basis. If adaptive, similar studies in parasitoids exhibiting different life histories and nutritional requirements should be expected 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 expected longevities, due to for instance intense predation pressure, (e.g. *A. 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 de-couple host feeding and egg laying (Collier 1995b) are particularly interesting to test the plasticity of larval reserves allocation 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 of the importance of adult feeding among behavioural ecologists and theoretical population biologists, the pattern of utilisation of stored versus incoming sources in insects has been largely overlooked (Rivero & Casas 1999a). Our results call for further studies on the role and adaptive nature of larval reserve use in parasitoids. The suggestion that the body of the females may not behave as a simple pool of nutrients that gets replenished every time the insect eats, but that resources may be metabolically or anatomically compartmentalised (Boggs 1997a; Boggs 1997b; 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 1999a; Rosenheim *et al.* 2000) and, more generally, for our understanding of the factors limiting lifetime reproduction in insects.