THE ROYAL SOCIETY

Rate of nutrient allocation to egg production in a parasitic wasp

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Recent years have seen a marked increase in our awareness of the need to incorporate greater physiological realism into studies of parasitoid behaviour and population dynamics. Quantification of the number of eggs produced as a result of a host-feeding event, the host-feeding gain, is essential for predicting when a parasitoid should bypass an opportunity for current reproduction (i.e. laying eggs) in order to feed from the host and, thus, increase its chances for future reproduction (i.e. producing further eggs). Using radioactively labelled amino acids, one of the main constituents of insect haemolymph, we followed the incorporation of a known quantity of nutrients into each individual egg laid over a long period of time relative to the average life span of the parasitoid. Although the maximum incorporation of nutrients obtained by the female from a discrete feeding event occurs within a short period of time, a large proportion of nutrients are stored and used gradually for egg production throughout the life of the parasitoid. We therefore provide novel experimental evidence showing that feeding gain is not a discrete event in time occurring shortly after feeding, as has so far been assumed, but is instead spread throughout the parasitoid's lifetime. This has important consequences for calculating the increase in lifetime fitness as a result of a feeding event, a common currency of models that aim to predict feeding and oviposition behaviour in parasitoids.

Keywords: parasitoids; egg production; host feeding; nutrient allocation; reproductive trade-offs; radioactive labelling

1. INTRODUCTION

Behavioural ecologists have long been concerned with the question of when should an animal feed. Feeding not only carries costs in terms of time and energy expenditure while foraging for, handling and consuming food items (Stephens & Krebs 1986), but it may also increase the risk of predation (McNamara & Houston 1987). Furthermore, there are costs associated with storing reserves (Cuthill & Houston 1997). In the large majority of animal species, however, adult feeding is essential for body maintenance, as well as for increasing the storage reserves that could eventually be used in times of food shortage or increased energy expenditure. In many organisms, such as in insects, feeding also has a direct effect on reproduction, since it is strongly linked to the number and quality of eggs produced by the female (Engelmann 1970; Wheeler 1996). Animals have to make behavioural decisions on a regular basis as to whether to invest their time and energy in reproductive or feeding-related activities (Cuthill & Houston 1997). Synovigenic parasitoids offer an ideal opportunity to test such reproductive trade-offs. In these parasitic wasps, not only is adult feeding essential for egg production, but also opportunities for reproduction and for adult feeding are often found in the same resource, the host (Godfray 1994).

On finding a host a female parasitoid is faced with a behavioural decision: whether or not to renounce the opportunity for current reproduction (oviposition) in favour of anticipated chances for future reproduction (host feeding). Host feeding kills the host or reduces its quality as an oviposition site but also yields two major potential benefits to the parasitoid: increased fecundity and longevity (Jervis & Kidd 1986; Heimpel & Collier 1996). The most realistic predictions to date as to when a female parasitoid should feed from the host instead of laying an egg in it have been generated from dynamic state variable modelling (Houston *et al.* 1988; Mangel & Clark 1988; Clark 1993). Without exception, all the models developed so far have placed egg load high on the list of physiological variables determining host-feeding decisions (Houston *et al.* 1992; Chan & Godfray 1993; Collier *et al.* 1994; Heimpel *et al.* 1994; Collier 1995*a*; but see Rosenheim & Rosen 1992). The predictions of these models with respect to what is the 'critical egg load' (*sensu* Collier 1995*a*) at or below which host feeding should occur are, however, drastically different depending on the degree of physiological realism introduced into them. Two key physiological variables that have been the focus of much recent attention are the 'host-feeding gain', i.e. the number of eggs produced as a result of a single feeding bout and the length of the 'egg maturation delay', i.e. the time required for the nutrients ingested through host feeding to be converted into eggs. Models making the simplified assumption that eggs are produced immediately after a feeding bout predict that females should only

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host feed when their egg load has dropped to zero (Chan & Godfray 1993; Collier *et al.* 1994). The incorporation of an egg maturation delay into the models has drastically changed these predictions: host feeding should occur at non-zero egg loads in order to avoid becoming egg limited (Collier *et al.* 1994; Collier 1995*a*; McGregor 1997; Heimpel *et al.* 1998). Furthermore, the critical egg load is expected to depend on the host-feeding gain (Collier 1995*a*).

While comparisons of the number of eggs laid by host-fed females with respect to starved or sugar-fed females abound in the literature (reviewed by Collier (1995*b*) and Heimpel & Collier (1996)), there are few studies that have tried to quantify the value of a single host-feeding meal in terms of the number of eggs produced (Heimpel *et al.* 1994, 1997; Collier 1995*b*). Studies in which such host-feeding gain was measured after a short, fixed amount of time, however, fail to take into account that part of the nutrients obtained may be stored and used in egg production at a much later stage. Recent indirect indications indeed suggest that parasitoids may benefit from nutrients long after host feeding (Collier 1995*b*; Heimpel *et al.* 1997). This suggests that the host-feeding gain may be a continuous rather than discrete variable and, therefore, cannot be measured in terms of the number of eggs laid or produced per unit time, except for a final count at the end of the life of the parasitoid (as done by Heimpel *et al.* (1997)).

Following the incorporation of nutrients into the egg through time has thus become imperative. How rapidly are different nutrients incorporated into the egg? For how long after the host-feeding meal is the female able to produce eggs from the nutrients ingested? Does this depend on the size of the meal? Here, we use radioactive labelling to follow the incorporation of nutrients into every individual egg laid over a period of two weeks and correlate this with the amount ingested. The possibility of marking parasitoid eggs through feeding was first experimentally demonstrated by Grosch & Sullivan (1953) but has not, to our knowledge, been employed since, although the technique is used in other insect groups (King & Wilson 1955; Gilbert 1972; Kloft 1992; Boggs 1997). We used radioactively labelled amino acids for two important reasons: (i) they are one of the main components of the insects' haemolymph (Florkin & Jeuniaux 1964; Mullins 1985), and (ii) they are essential for building up the vitellogenin, the main protein of the egg. We measured how quickly the marked amino acids are incorporated into the eggs of a host-feeding parasitoid after food ingestion and how long after a feeding event eggs still benefit from these nutrients. The pattern of nutrient allocation to eggs has important consequences for calculation of the increase in fitness as a result of a feeding event and, therefore, for predictions of models that use lifetime fitness as a currency for predicting the host-feeding decisions of parasitoids.

2. MATERIAL AND METHODS

(a) *Experimental protocol*

Dinarmus basalis Rond. (Hymenoptera: Pteromalidae) is a host-feeding synovigenic parasitoid of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). Parasitoids for the experiments were kept in culture on third- to fourth-instar *C. maculatus* larvae infecting *Vigna ungiculata* Walp. (Fabaceae) beans (13 L:11 D photoperiod, 33:23 °C temperature and 75% humidity). In order to obtain females of a known age, the beans containing the parasitized hosts were sieved on the morning of the day of the experiment to eliminate all individuals that had emerged during the previous few days. The beans were then re-sieved 5 h later and 22 females of approximately equal size were isolated in a Petri dish (diameter 9 cm) together with an equal number of males, in order to ensure mating. Females were kept deprived of hosts for a total of four days under the above-mentioned temperature, humidity and photoperiod conditions. Each female was subsequently weighed on a micro-balance and immediately allocated to one of the two feeding treatments as specified below.

The radioactively marked diet consisted of a 10% (w:v) commercial sugar solution to which a ^{3}H-marked amino acid mixture (37 MBq ml^{-1}, ICN Pharmaceuticals) was added (eight parts of sugar solution to two parts of amino acid mixture). The concentration of amino acids in the artificial diet (*ca.* 3.7 μg ml^{-1}) was chosen on the basis of their radioactivity; this had to be sufficiently high to be detectable by the scintillation analyser but not so high as to be lethal to the females. The control diet simply consisted of the 10% sugar solution. After the initial weighing, females were individually placed inside a gelatine capsule (length 2 cm and diameter 0.6 cm) the cap of which had been finely pierced to allow the introduction of one of the extremes of a microcapillary tube (length 10 cm and internal diameter 0.60 mm) containing one of the two nutrient solutions. Females were allowed to feed from the tube for 1 h after which they were reweighed. The amount of diet ingested was calculated from the difference between the parasitoid's initial weight and the weight after feeding. Previous experiments have shown that such a technique provides an accurate way of quantifying the amount of liquid solution ingested by parasitoids (A. Rivero and J. Casas, unpublished data). A total of 15 females were allocated to each of the control and radioactively marked diet treatments.

Immediately after the feeding period, females were placed in a Petri dish (diameter 5.5 cm) with a pierced gelatine capsule (as above) containing a third-instar host (for details of how hosts are prepared inside the capsule see Gauthier & Monge (1999)). Every hour for the remaining 4 h of the first feeding day (day 1), the host was replaced by a fresh one. At the end of the day (18.00) and in order to reduce the heterogeneity of the conditions imposed by periods in which the female was allowed to produce and lay eggs (during the experiment) and periods in which otherwise the female would only have been producing eggs (the night), the females were stored at 10 °C. The following morning parasitoids were placed at room temperature for 30 min before new hosts were provided. Each day for the next 12 days (days 2–13) each female was provided with one host per hour for a total of 8 h a day (09.00 to 17.00). During the experiment the females were not provided with a sugar source but could feed from the hosts provided, although the frequency of host feeding could not be estimated. All females were stored in the cold between 18.00 and 08.30 the following morning. Capsules containing the hosts and the parasitoid eggs were stored at −80 °C for later analysis. At the end of the experiment the females were dissected under a binocular microscope to extract the ovarioles and the number of mature and immature eggs inside them were counted.

(b) *Sample preparation and radioactivity quantification*

Quantification of the amount of ingested isotope incorporated into the eggs was carried out using a liquid scintillator analyser (LSA, TriCarb1900, Packard Instruments). Parasitoid eggs from both the control and marked diet treatments were individually prepared by crushing them in a liquid scintillation tube and immediately adding 500 µl of a tissue solvent (Soluene®-350, Packard). After 30 min, 5 ml of the liquid scintillation cocktail (Hionic-Fluor™, Packard) was added. In order to control for background radiation, for each batch of eggs prepared one extra tube containing the tissue solvent and the liquid scintillation cocktail but no egg was also prepared. After 1 h, all tubes were read in the LSA for 10 min each and the number of disintegrations per minute (DPM) calculated (using the transformed spectral index of external spectrum (tSIE) as a quenching-indicating parameter). More than one egg was sometimes laid in the same hour, making it impossible to know which one was laid first. In such cases, each egg was analysed separately and allocated a DPM which was the average of the DPMs of the eggs laid in that time interval by the female. Out of the 779 hosts in which oviposition occurred, two eggs were found in 53 cases (6.8%) and three to four eggs in only three cases (0.4%). A total of 839 laid eggs were individually analysed.

The bodies of the females and the ovaries, which had been dissected out of the body cavity, were crushed separately and each fraction was prepared and read in the LSA in exactly the same way as the eggs that had been laid. Out of a total of 15 females allocated to the radioactive treatment, six exhibited a difference in weight before and after the feeding experiment that was below 0.05 µg and no radioactivity was found in either the eggs that had been laid or the body. These females were thus considered not to have fed and were eliminated from the analysis. Similarly, six out of the 15 control females showed a change in weight which was below 0.05 µg and were thus also eliminated from the analysis. For the purpose of the analyses the final number of replicates was therefore $n = 9$ for both the marked and control treatments. Statistical analyses used SPSS for Windows v. 6.1.3 (Norusis 1993).

3. RESULTS

The marking treatment did not adversely influence female behaviour. Females in both treatments consumed similar amounts of their diet (mean ± s.e. 0.165 ± 0.046 µg and 0.218 ± 0.030 µg for control and marked treatments, respectively; $t = 0.95$ and $p > 0.05$) and laid approximately the same number of eggs (41.13 ± 3.11 and 40.50 ± 4.11, respectively; $t = 0.12$ and $p > 0.05$). A strong significant linear relationship was found between the amount of their diet ingested and the total radioactivity recovered from the marked females, calculated by adding the DPMs for the body, ovaries and eggs that had been laid (slope = 154 007.26, intercept = −659.388, $r^2 = 0.68$, $F_{1,7} = 14.88$ and $p < 0.01$). On average 37.16 ± 4.16% of the total radioactivity ingested was recovered at the end of the experiment. Most of these marked elements were recovered from the eggs that had been laid (46.52 ± 3.38% compared to 4.16 ± 0.48% found in the ovaries and 49.31 ± 3.46 in the rest of the body). The radioactivity found in the ovaries at the end of the experiment was uncorrelated with either the number of mature eggs (Pearson's correlation coefficient = 0.06 and $p > 0.05$) or the total number of eggs (0.1428 and $p > 0.05$) found inside the ovarioles.

In all of the females fed the radioactive amino acids, the very first egg laid was found to be marked (mean ± s.e. = 217.97 ± 38.26 DPMs; in all cases the DPM for first eggs of control females was zero). Except for one female where the first egg was laid by hour eight of day 2, all other females laid their first egg either 2 h ($n = 6$ females), 3 h ($n = 1$) or 4 h ($n = 1$) after the diet was supplied. The mean DPMs per egg laid and per day are shown in figure 1*a* and 1*b*. The maximum DPMs were reached quickly: eggs 7–10 on days 3–4. There is a long tail extending over 40 eggs and 13 days. The maximal DPM attained strongly depended on the amount of their diet ingested (figure 2). The cumulative proportions of nutrients ingested and incorporated into the eggs are shown in figure 3. The mean amount of marked elements ingested and incorporated into all the eggs that had been laid was 17.17 ± 2.10%. At the point of maximal incorporation, egg 10 (figure 1*a*), 7.43 ± 0.79% of the ingested nutrients had been used, corresponding to 42.81 ± 4.08% of the total allocated to eggs, with the rest being spread over the remaining 30 or more eggs.

4. DISCUSSION

Models aiming to predict when a parasitoid should bypass an opportunity for current reproduction (i.e. laying eggs) in order to feed from a host and, thus, increase its chances for future reproduction (i.e. producing eggs) need to quantify the lifetime fitness advantages of each alternative behaviour. Measuring the proximate increase in fitness from laying an egg is, at least *a priori*, a relatively easy task. Measuring the increase in fitness from feeding is, however, not so simple, as it involves a set of complex physiological processes. Furthermore, where resources can be stored for later use, the fitness advantages from a particular feeding event are likely to be spread over the lifetime of the parasitoid and, thus, may not be quantifiable in the short-term.

There are few studies that have attempted to quantify the long-term feeding gains from a single host meal. Collier (1995*b*) determined that the host-feeding gain of *Aphytis melinus* females feeding on small (second instar) hosts was similar to that of females feeding on large (third instar) hosts: approximately 1.5–2 eggs in two days. Provided that the uptake of nutrients is higher when feeding from larger hosts, it is reasonable to assume that the expected difference in host-feeding gain is obscured by differential storage of nutrients. When host-feeding gain was calculated on the basis of lifetime fecundity, Heimpel *et al.* (1997) found the lifetime host-feeding gain to be twice as high: four eggs. These results strongly suggested that some of the nutrients are not immediately used for egg production but are stored for later use. Our results provide conclusive evidence to this effect.

In the present experiment a discrete feeding event was mimicked by providing female *D. basalis* with a known quantity of radioactively labelled amino acids for a short period of time (1 h). The rate of incorporation of the marked elements into the eggs was then measured for a long period of time (13 days) relative to the average life span of a female under laboratory conditions (two to

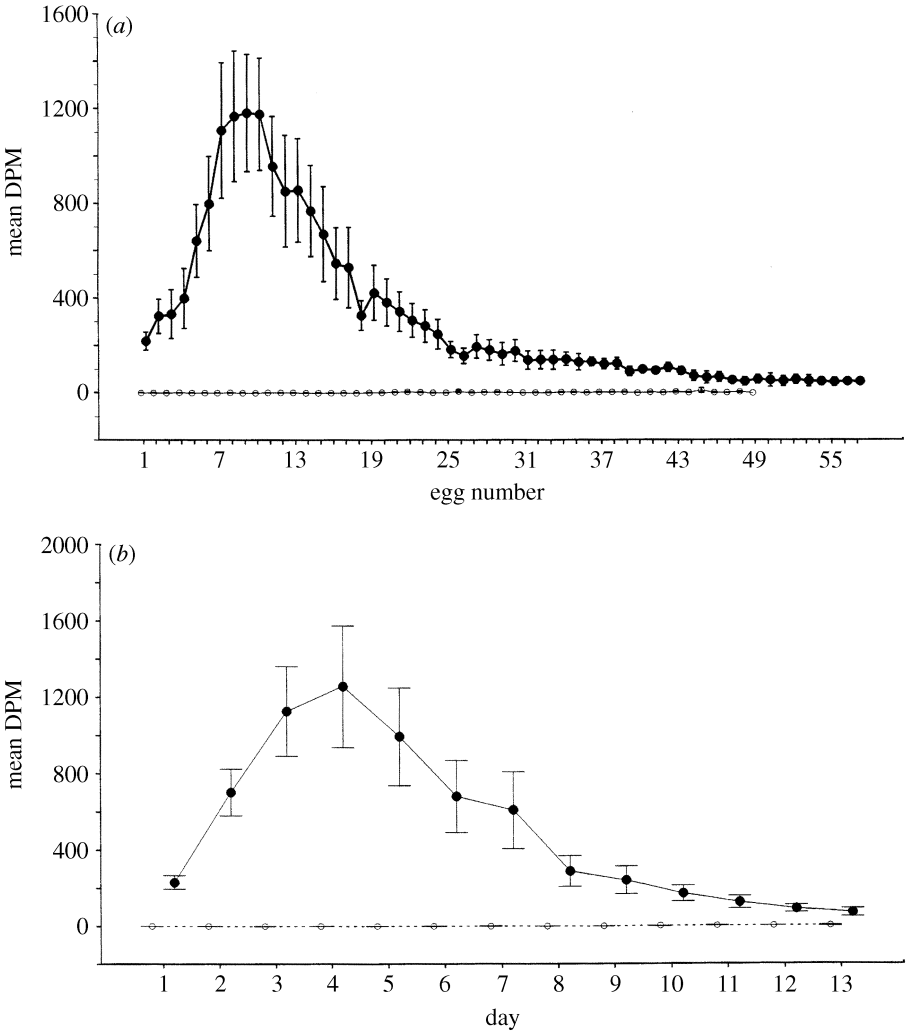

Figure 1. Mean (±s.e.) number of DPMs found in the eggs laid by marked (full circles) and control (open circles) females (*a*) on an egg by egg basis and (*b*) on a daily basis. The daily calculations were carried out by first calculating the mean DPM of the eggs laid per female per day.

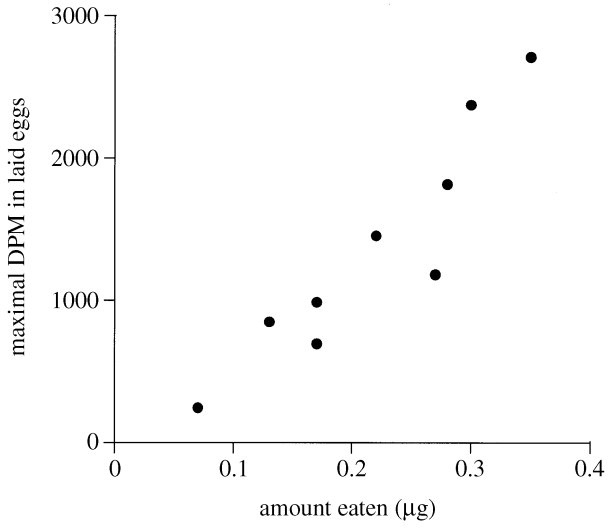

Figure 2. Relationship between the estimated amount of the artificial diet eaten by the females and the maximal DPM found in the eggs which had been laid.

three weeks, A. Rivero, personal observation). The time-dependent rates of nutrient incorporation into eggs (figure 1*a* and 1*b*) clearly show that, although the maximum incorporation of marked elements obtained by the female from a specific feeding event occurs within a short period of time (eggs 7–10 on days 3–4), nutrients are stored and used gradually throughout the life of the parasitoid. At the point of maximal incorporation (egg 10 on day 4) only 42.81 ±4.08% of the elements eventually allocated to eggs had been used (figure 3). Hence, more than half of the nutrients were incorporated into the remaining 30 or so eggs, for a period spanning as long as 13 days after the feeding treatment. Feeding gain is therefore not a discrete event occurring shortly after feeding, as has been assumed in most models of parasitoid behaviour (but see Heimpel *et al.* 1998), but is spread throughout the lifetime of the parasitoid. Further experiments are needed in order to determine the different conditions under which the rate of incorporation of nutrients may vary. In particular, the incorporation of nutrients into eggs may be dependent on the state of maturation of the eggs (Rosenheim & Rosen 1992) and, thus, the rate of incorporation could critically depend on the state of the ovaries at the time of feeding. Feeding gains are predicted to be different in parasitoids having egg loads that are close to the ovaries' maximum carrying capacity (as in the study by Heimpel *et al.* (1994)) compared with parasitoids starved of hosts for long periods. Feeding gains may also depend on previous and/or subsequent feeding meals. In the present experiment, females could not be prevented from host feeding while ovipositing. With each subsequent feeding event the labelled diet is thus potentially 'diluted' within the body of the female. However, it is still too early to determine

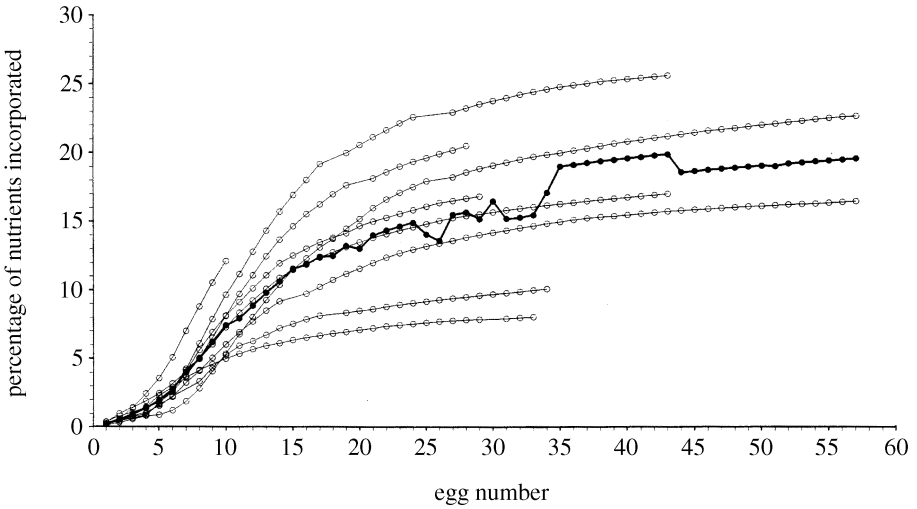

Figure 3. Cumulative percentage of ingested nutrients incorporated into the eggs of each of the nine marked females (open circles) and mean for all females (full circles).

exactly how this influences the shape of the gain curve as the precise temporal pattern of allocation of subsequent meals is unknown.

While our results provide insights into the pattern of nutrient allocation in relation to adult feeding in parasitoids, care should be taken when extrapolating our results to a real host-feeding meal. The insect's haemolymph is a complex, dynamic, mixture of biochemical constituents including inorganic cations and anions, organic acids and phosphates, pigments and many different types of sugars and proteins (Florkin & Jeuniaux 1964; Mullins 1985), which are all likely to follow different metabolic paths once ingested. Furthermore, host feeding probably involves the consumption of a variety of substances extending to loose fat body cells, haemocytes or gut contents. The little that is known about the physiology of egg production in parasitoids, however, suggests that the main role of host feeding is to allow the female to meet the high amino acid demands associated with building up the main constituent of the egg: the vitellogenin (Rivero & Casas 1999). Amino acids are not only one of the main components of the haemolymph but have also been shown to be essential for egg production (Englemann 1970). The yolk of insect eggs is also largely constituted of lipids (Chapman 1982), which may come either from stored reserves in the fat body or through metabolic transformation of sugars obtained with the meal. Further experiments are needed in order to compare the pattern of incorporation of sugar and other components of the host meal into the eggs to the one found for amino acids

Our study focuses on one specific aspect of feeding gains: the direct incorporation of the ingested nutrients into the eggs. The ingested nutrients may, however, participate indirectly in the egg production process by facilitating the mobilization of or simply replacing the reserves stored in the fat body. Thus, the time-course of the appearance of the label in the eggs is not necessarily equivalent to the time-course of benefits reaped by the female. This raises another aspect of the allocation of nutrients into egg production that requires further investigation: that of the temporal pattern of allocation of stored versus incoming resources into egg production. Mobilization of nutrients for egg production which are stored in the fat body is bound to incur metabolic costs, although these costs have not yet been quantified in insects (Rivero & Casas 1999). The detection of significant amounts of radiation in eggs laid only 2 h after the feeding event indeed shows that free amino acids or their metabolic derivates are made available for egg production within a very short period of time and suggests that these nutrients come directly from the haemolymph rather than from stored fat reserves. In other insects, isotopes provided with food can be found in the haemolymph as little as 70 s after ingestion (Kloft 1992) and amino acids are known to pass quickly from the haemolymph to the ovaries to be converted to proteins within the nurse cells (King & Cassidy 1973). Elucidation of the way in which females allocate their incoming and stored resources with respect to the resources stored in the fat body to egg production is essential, as it is expected to have a key effect on the female's fitness and on the resiliency of populations to environmental variation (Boggs 1997; Rivero & Casas 1999). Further experiments using double marking are under way in order to address these issues.

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