

The physiological costs of being small in a parasitic wasp

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ABSTRACT

Knowledge of the relationship between body size and fitness is essential in many models of parasitoid evolutionary ecology. One clear point emerging from previous studies is that the relationship between size and fitness can depend strongly (qualitatively and quantitatively) on the environment in which it is measured. Our results show that, in the parasitoid wasp *Nasonia vitripennis*, the relationship between parasitoid size and fitness is strongly dependent on the nutritional status of the female; being small is more costly when food is not available. Lipid availability is the main factor mediating the relationship between size and fitness. Small females suffer disproportionately the costs of not feeding because they emerge with small lipid reserves and thus have to rely more heavily on carbohydrates for survival and reproduction. In other words, feeding is more important for small females. Our results (1) provide a physiological explanation for why laboratory estimates of the size–fitness relationship are not as steep as field estimates and (2) suggest that females are likely to show size-dependent foraging and feeding strategies.

Keywords: lipids, parasitoids, resource allocation, size–fitness relationship.

INTRODUCTION

Parasitoid wasps have proved to be excellent experimental models for testing a variety of theories on the evolution of life-history strategies (Godfray, 1994). Female wasps search the environment for hosts. Upon finding a host, each female must make a series of decisions, such as whether to feed or oviposit on the host, how many eggs to lay (clutch size) and what sex the offspring should be (sex ratio) (Charnov and Skinner, 1984, 1985). In many cases, theory has been shown to be able to predict reasonably well the reproductive strategies (behaviour) of females. This is due to the simple link between reproductive behaviour and fitness in these animals; the fitness consequences of, for example, feeding or laying an egg in a host are immediate and relatively clear.

However, many hypotheses about life-history evolution in parasitoids have been tested qualitatively but not quantitatively (Godfray, 1994). The reason for this is that quantitative

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tests require accurate estimates of the relationship between parasitoid size and fitness. For example, application of clutch size theory provides qualitative explanations for variation in clutch size across and within species, but quantitative predictions require accurate estimates of the size–fitness relationship (Godfray, 1994). Consequently, in recent years there has been a considerable increase in attempts to estimate the size–fitness relationship in parasitoids, both in the laboratory and in the field (Visser, 1994; Kazmer and Luck, 1995; West *et al.*, 1996; Eilers *et al.*, 1998, 2001).

One clear point to emerge from these studies is that the size–fitness relationship can depend strongly (qualitatively and quantitatively) on the environment in which it is measured (e.g. whether a food source or hosts are provided) and the fitness measure used (e.g. egg production or longevity) (King and Hopkins, 1963; Benson, 1973; Tagaki, 1985; Hardy *et al.*, 1992; Bai and Smith, 1993; Visser, 1994; West *et al.*, 1996). A possible explanation for this is that wasps of different sizes are differentially allocating resources to reproduction and survival according to environmental conditions, such as the availability of food. This could involve a number of interactions between environmental conditions and wasp size, mediated by, and depending heavily upon, physiological mechanisms. For example, when food is abundant, can it enable small females to make up for a lack of stored reserves relative to large females? Also, does female size influence how the different energy resources, such as lipids and sugars, are stored and utilized?

Here we examine whether environmental conditions determine the size–fitness relationship in *Nasonia vitripennis*, a parasitoid wasp that matures eggs throughout its lifetime. Specifically, we focus upon the influence and interaction between two factors: (1) the amount of food (honey solution) given to adults (females were either fed *ad libitum* or starved) and (2) body size. We measure the influence (and interaction) of these variables upon two main fitness traits – egg production and longevity – as well as on the underlying physiological resources: ovarian proteins and lipid, glycogen and sugar reserves. We provide a physiological explanation for why field estimates of size–fitness relationships are steeper than laboratory estimates and we discuss the implications for life-history evolution.

MATERIALS AND METHODS

Parasitoid biology

Nasonia vitripennis (Hymenoptera: Pteromalidae) is a gregarious, host-feeding ectoparasitoid of dipteran pupae in bird nests. Females are synovigenic: they are born with a very limited number of mature eggs and egg production and maturation are continuous throughout the life of the female (in contrast to ‘proovigenic’ parasitoids, which are born with their lifetime complement of eggs; Godfray, 1994).

In the field, hosts occur in small isolated patches of between 1 and 39 hosts per nest. The number of females ovipositing in each patch can vary from 1 to 9 (Molbo and Parker, 1996). As a result, the number of adult parasitoids that can be reared successfully from a single host pupa varies enormously (anything between 10 and 50; Werren, 1983; Molbo and Parker, 1996), which leads to a large variation in adult body size (Charnov and Skinner, 1985; Molbo and Parker, 1996; Flanagan *et al.*, 1998). The flightless males emerge before the females and mate with them as they emerge. Females are winged and need to disperse after mating to find new, suitable hosts in which to feed and lay eggs (Werren, 1983; Molbo and Parker, 1996). Until a food source is found, females run ‘on empty’, relying on the resources

accumulated as a larva. Temporal starvation is thus a likely scenario in the field, and its duration will greatly depend on the quality of the environment in which the females forage.

Experimental set-up

Parasitoids were reared by placing 10–15 female parasitoids with about 30 fly pupae (*Calliphora vicina*, Diptera: Calliphoridae) in specimen tubes (7.5 cm long, 2.5 cm in diameter). Under these high-density conditions, the resulting parasitoids show a wide variation in body size (personal observation). The parasitoid cultures and the experiment were carried out at the same temperature (25°C) and under the same light:dark photoperiod (16:8). Experimental females were obtained by dissecting parasitized hosts and isolating 12-day-old female parasitoid pupae individually in glass tubes (7.5 cm long, 8 mm in diameter). Tubes were kept under standard temperature and light conditions and checked every 2 h. On emergence, females were immediately allocated at random to one of the treatment combinations detailed below. Females were kept in the tubes for 24, 48 or 72 h. During this time, the females were deprived of hosts to avoid the confounding effects of ovipositing behaviour, as in many parasitoids egg production, and hence the pattern of resource allocation, depends on the rate of oviposition (e.g. Rivero-Lynch and Godfray, 1997).

Since host-feeding and egg-laying cannot be decoupled, females in this experiment had no access to a host meal. However, an important alternative source of nutrients for many parasitoids in the field is sugar-rich sources such as nectar or honeydew (Leius, 1960; Jervis *et al.*, 1993; Gilbert and Jervis, 1998; Jervis, 1998). To determine the effect of food on resource allocation, half of the females in each treatment were fed *ad libitum* on a rich honey solution. For this purpose, a piece of filter paper (~30 mm²) soaked in a solution of honey in water (~30% honey by volume) was placed inside the tube on a daily basis (where appropriate). The other half were given a similar piece of paper soaked in water (starved). After the specified experimental period had elapsed, females were killed and stored by deep freezing them at –80°C for subsequent dissection and biochemical analysis (see below).

In addition to these six treatment combinations (replication as follows: 24 h unfed, $n = 21$; 24 h fed, $n = 19$; 48 h unfed, $n = 15$; 48 h fed, $n = 15$; 72 h unfed, $n = 15$; 72 h fed, $n = 16$), three other treatments were set up. To determine the reserves at emergence (teneral reserves), a randomly chosen group of females was killed and stored at –80°C immediately after emergence (0 h) ($n = 14$). Finally, to determine longevity and the resources at death (assumed to be the minimum resources possible in an organism), two additional groups of females, one fed on honey solution as above ($n = 14$) and one starved ($n = 17$), were kept in the tubes until they died. Longevity was recorded and female bodies were then frozen as above for subsequent biochemical analysis.

Dissections and sample preparations

Females of all treatments were removed from the freezer and allowed to thaw for about 1 h. One of the legs was pulled out and the length of the hind tibia (a standard measurement of size in parasitoids; Godfray, 1994) was measured with a micrometer. Females were then dissected under a binocular microscope in 50 μ l 2% sodium sulphate to extract the ovarioles. The ovarioles were then placed on a slide and the number of mature eggs

determined under the optical microscope. Egg maturity was assessed following the method of King and Richards (1969). Ovarioles were placed in a 1.5 ml Eppendorf tube, crushed with a pin and 100 μl of a solution of physiologic water (0.15 M NaCl) and 0.001% Triton X-100 (Sigma-Aldrich) was added. Controls consisted of the physiologic water and Triton solution but no ovaries. Tubes were left for 5 days at 4°C for the Triton to dissolve the proteins in the ovaries.

To prepare the dissected body of the female for biochemical analysis (van Handel, 1988), it was placed in a Pyrex glass tube (7.3 cm long, 1 cm in diameter) and crushed with a glass rod. The sodium sulphate in which it was dissected was recovered with a pipette and added to the tube. An extra 50 μl sodium sulphate and 750 μl of a chloroform–methanol solution (1:2, v:v) were added to the tube, which was then left at room temperature for 24 h. The same procedure was followed for the controls but without the female's body.

Biochemical analyses

Protein analysis of the ovaries was carried out using the Bradford dye-binding microassay procedure (Bradford, 1976). For this purpose, 80 μl of the sample were extracted into a plastic tube (7.5 cm long, 1 cm in diameter), to which 720 μl physiologic water and 200 μl Bradford reagent (Bio-Rad Laboratories, Munich, Germany) were added. After 15 min, samples were read in a spectrophotometer (Jenway-6300) at OD₅₉₅. Protein concentrations were obtained from a standard curve based on bovine serum albumin (Sigma-Aldrich).

Quantification of the body reserves of the females was carried out using a modification of the colorimetric technique developed by van Handel (1988) for mosquito analysis. This technique allows the determination of lipids, glycogen and sugars (glucose) on the same specimen. Tubes containing the sample with the sodium sulphate and chloroform–methanol mixture (see above) were centrifuged at 1500 rev·min⁻¹ for 15 min. Two 350 μl fractions of the supernatant were extracted into separate Pyrex glass tubes, one for lipid analysis and the other for sugar analysis. The solvent in all tubes was evaporated completely in a heating block at 95°C.

For lipid determination, 40 μl of sulphuric acid were added to the tubes and heated at 95°C for 2 min; then, 960 μl of vanillin reagent were added (for details of reagent preparation, see van Handel, 1985b), left for 10 min to allow the colour to develop and read in the spectrophotometer at OD₅₂₅ against the control. Lipid concentrations were obtained from a standard curve done with commercial vegetable oil (1 mg·ml⁻¹ in chloroform; van Handel, 1985b). For sugar determination, 1000 μl anthrone reagent (for details, see van Handel, 1985a) were added to the tubes, heated at 95°C for 15 min and read at OD₆₂₅ against the control. Sugar concentrations were obtained from a standard curve done with glucose (1 mg·ml⁻¹ in 25% ethanol; van Handel, 1985a).

The precipitate in the original tube containing all the glycogen was washed with 2000 μl 80% methanol to get rid of any remaining sugars. Tubes were recentrifuged at 1500 rev·min⁻¹ and the supernatant was disposed of. Glycogen concentrations were determined by adding 1000 μl anthrone reagent to the washed precipitate and heating at 95°C for 15 min. Since the glycogen fraction contains all the fractured fragments of the female exoskeleton, the samples had to be transferred into 1.5 ml Eppendorf tubes and recentrifuged at 14,000 rev·min⁻¹ for 10 min to precipitate the fragments before reading at OD₆₂₅. As for the sugars, concentrations were obtained from a standard glucose curve.

Statistical analyses

The effects of size and food availability (starved or fed *ad libitum*) were analysed with generalized linear modelling techniques available in the GLMStat (v.5.3, 2000) statistical package. The significance of each explanatory variable and their interactions was assessed by removing it from the model and analysing the resulting change in deviance (see Crawley, 1993). After the minimal model (the model including only significant terms and interactions) was obtained, its appropriateness was tested by inspecting a plot of the residuals against the fitted values. Bonferroni-corrected contrasts were carried out using the SPSS statistical package (SPSS Inc., 1995). The significance of Bonferroni-corrected contrasts was determined by establishing whether the 95% confidence intervals included 0 (Norušis, 1995).

RESULTS

Egg production and longevity

All females, irrespective of their size, emerged with no mature eggs in their ovarioles. To determine the pattern of egg production with time, an initial analysis was carried out using time and feeding status as factors and size as a covariate. In this analysis, the three-way interaction was significant ($F_{2,99} = 8.25$, $P < 0.01$; Fig. 1), indicating that the slope of the size–eggs relationship is dependent on both feeding status and time since emergence (Fig. 1). Subsequent analyses were carried out separately for each of the time intervals. The number of mature eggs present 24 h after emergence was highly dependent on size ($F_{1,38} = 27.14$, $r^2 = 0.43$, $P < 0.001$), but independent of whether the females were fed or not ($F_{1,38} = 0.00$, n.s.) (Fig. 1a). The next two time intervals showed a similar pattern, whereby egg load has highly size-dependent. Here, however, the slope of the size–egg relationship was significantly steeper for unfed than for fed females ($F_{3,29} = 34.52$, $r^2 = 0.71$, $P < 0.001$ and $F_{3,30} = 18.15$, $r^2 = 0.67$, $P < 0.001$ for the minimal model containing the interaction between size and feeding status at 48 h and 72 h respectively) (Fig. 1b,c).

Fed females lived about five times longer than unfed ones (15.71 ± 2.51 days and 3.94 ± 0.23 days, respectively; mean \pm standard error). The interaction between size and feeding status was significant ($F_{1,30} = 13.60$, $P < 0.001$). Female longevity when not fed was very highly significantly dependent on size (t -test for the significance of the slope, $t = 3.90$, $P < 0.005$) (Fig. 2a). However, although there was a trend in the same direction for fed females, the slope of the line was not statistically significantly different from zero ($t = 1.91$) (Fig. 2b).

Protein reserves

Protein is one of the main components of the egg yolk of insects (Nijhout, 1994) and parental nutritional experience has been shown to influence egg protein levels in some species (Rossiter *et al.*, 1993). Size had a significant effect on the total protein concentration in ovaries ($F_{1,112} = 17.86$, $r^2 = 0.13$, $P < 0.001$), and females fed on honey solution for 72 h had significantly higher protein concentrations in their ovaries than females in the other treatment combinations ($F_{5,112} = 3.32$, $r^2 = 0.14$, $P < 0.001$). The interaction between size and the time or sugar regime was, however, not significant ($F_{5,112} = 1.10$, n.s.). The amount

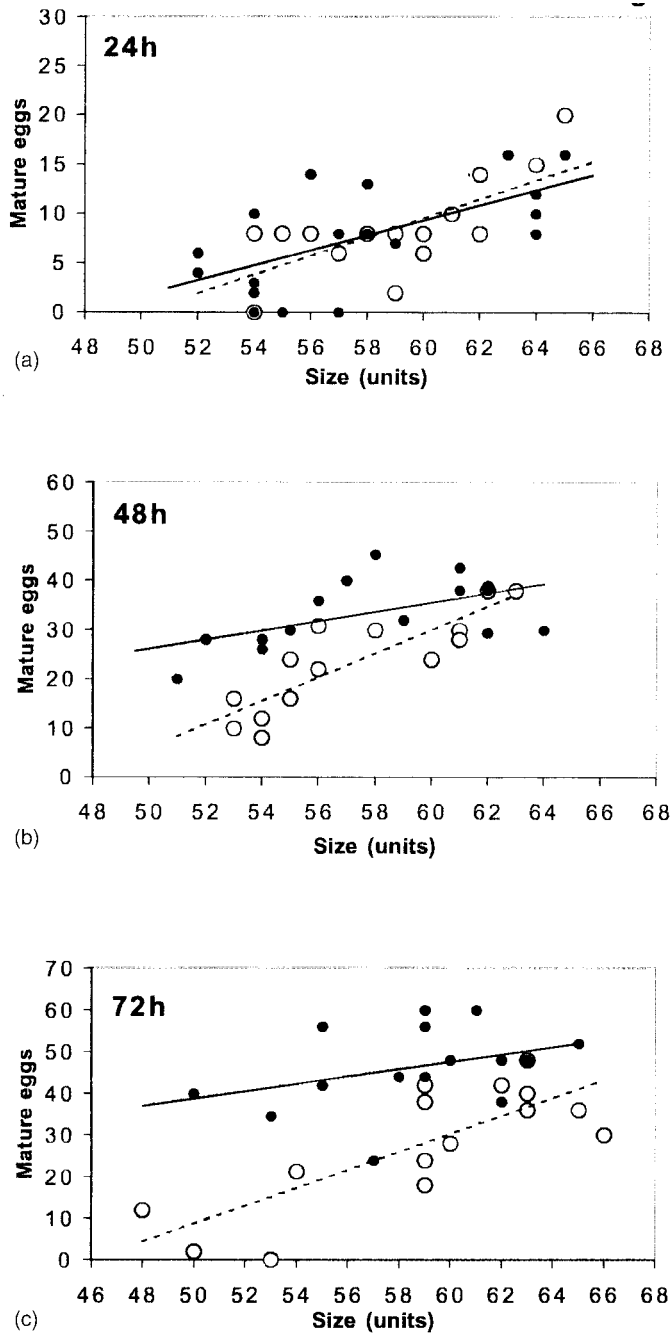


Fig. 1. Regression of number of mature eggs against female size at different time intervals. Solid line and solid circles are fed females; dashed line and empty circles are unfed females.

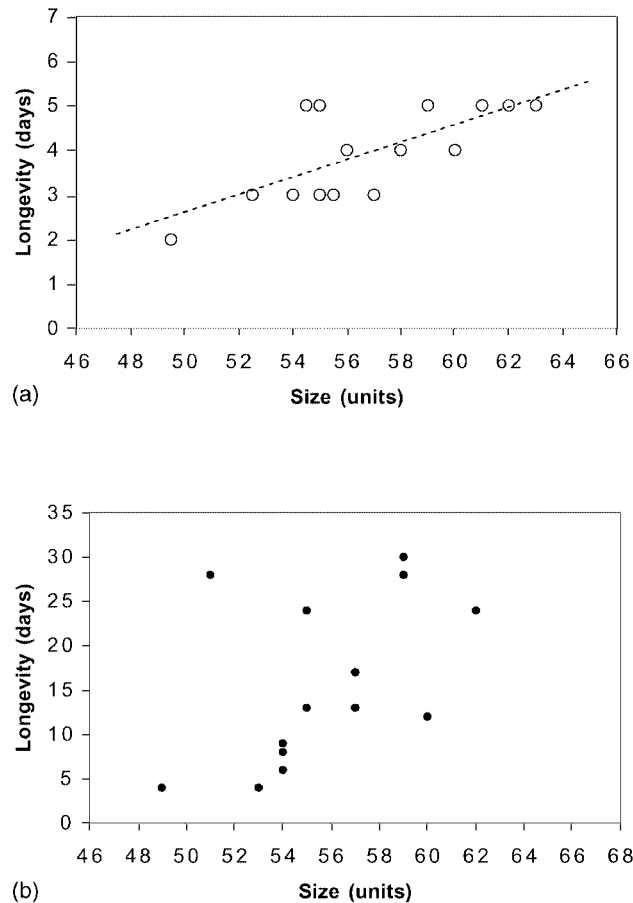


Fig. 2. Regression of female longevity against size in (a) unfed and (b) fed females.

of protein invested per mature egg was estimated by dividing the total amount of proteins by the number of mature eggs. This, however, was not correlated with female size in either fed ($F_{2,46} = 0.48$, N.S.) or unfed ($F_{2,47} = 0.20$, N.S.) females.

Lipid reserves

Females were born with a high amount of lipid deposits (45.37 ± 3.33 vs 15.03 ± 2.83 μg of sugars and 17.43 ± 1.58 μg of glycogen). The amount of teneral lipids was strongly correlated with female size ($F_{1,12} = 36.02$, $r^2 = 0.75$, $P < 0.001$), with the largest females having twice as many teneral lipids as the smallest ones (Fig. 3a,b). Lipid reserves decreased rapidly with time so that by 72 h only half of the original reserves remained (21.37 ± 2.03 μg for fed females and 21.34 ± 3.18 μg for unfed females).

To establish the pattern of lipid use with time, we carried out analyses of covariance for the effects of size and time (0, 24, 48 and 72 h) separately for fed and unfed females. In both cases, the slope of the lipid–size relationship changed significantly with time (fed:

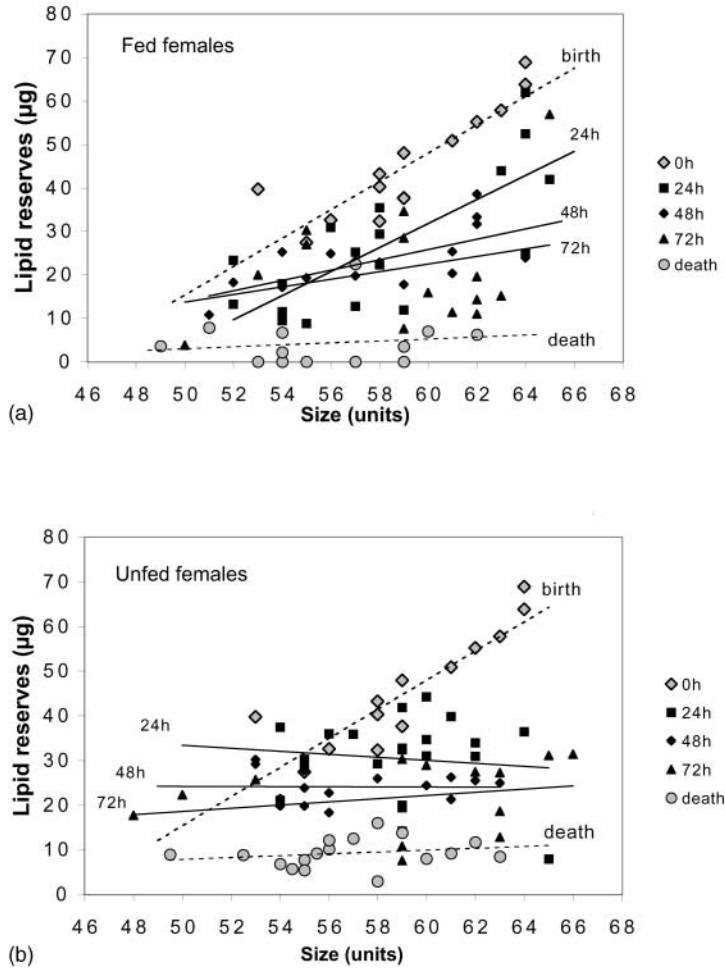


Fig. 3. Regression of lipid reserves against female size at different time intervals for fed females (a) and unfed females (b). Line for lipid reserves at birth is given as reference and is the same for (a) and (b).

$F_{3,63} = 3.31$, $r^2 = 0.25$, $P < 0.05$; unfed: $F_{3,64} = 7.52$, $r^2 = 0.13$, $P < 0.001$ (Fig. 3a,b). Contrast analysis, however, revealed differences between fed and unfed females. Unfed females show a drastic decrease in the slope of lipids between birth (0 h) and the first 24 h (99% joint Bonferroni confidence intervals: lower = -6.15 , upper = -1.03) (Fig. 3b). When starved, large females thus use disproportionately more lipids in the first 24 h than smaller females. In contrast, in fed females, the decrease in lipid reserves in the first 24 h was proportional to female size (95% joint Bonferroni confidence intervals between 0 and 24 h: lower = -1.82 , upper = 2.82 , n.s.) (Fig. 3a). Thereafter, however, we found no drastic, statistically significant, changes in the slope of successive time intervals in either fed (between 24 and 48 h: lower = -0.39 , upper = 3.55 , n.s.; between 48 and 72 h: lower = -1.83 , upper = 2.44 , n.s.) or unfed females (between 24 and 48 h: lower = -1.92 , upper = 1.27 , n.s.; between 48 and 72 h: lower = -1.74 , upper = 1.00 , n.s.).

Glycogen and sugar reserves

Neither glycogen nor sugar reserves at birth was significantly correlated with female size ($F_{1,11} = 0.03$ and $F_{1,12} = 0.03$, respectively; n.s.). Although glycogen reserves at birth were comparatively low when compared with the lipid reserves (see above), they were significantly higher than the resources at death in both fed ($1.32 \pm 0.53 \mu\text{g}$; $F_{1,27} = 64.73$, $r = 0.54$, $P < 0.001$) and unfed ($4.37 \pm 0.59 \mu\text{g}$; $F_{1,30} = 56.49$, $r^2 = 0.58$, $P < 0.001$) females (Fig. 4a,b).

While in fed females glycogen reserves did not change with time ($F_{3,62} = 1.45$, n.s.) (Fig. 4a), a significant interaction between size and time was seen in unfed females ($F_{3,64} = 5.3$, $r^2 = 0.15$, $P < 0.005$). Contrast analysis revealed that this significant interaction was due to the relationship between glycogen stores and size being significantly steeper at 24 h than at 0 h (95% joint Bonferroni confidence intervals: lower = 0.93, upper = 3.35). Despite being

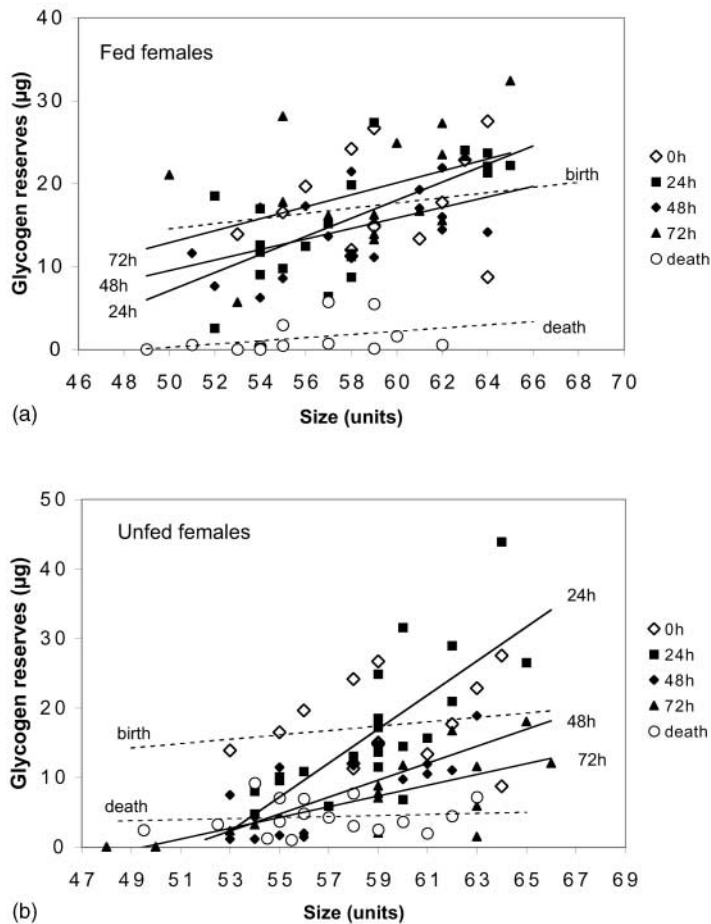


Fig. 4. Regression of glycogen reserves against female size at different time intervals for fed females (a) and unfed females (b). Line for glycogen reserves at birth is given as reference and is the same for (a) and (b).

unfed, large females thus appear to accumulate glycogen reserves in the first 24 h after birth (Fig. 4b).

Females emerged with a very small amount of sugar reserves relative to lipids (see above). These sugar reserves at birth were indeed statistically indistinguishable from the resources at death ($8.35 \pm 3.18 \mu\text{g}$; $F_{1,28} = 1.00$, n.s.). Sugar reserves were independent of size in both fed ($F_{1,61} = 0.03$, n.s.) and unfed females ($F_{1,63} = 2.55$, n.s.). Fed females, however, accumulated significant amounts of sugars with time ($F_{3,61} = 14.38$, $r^2 = 0.43$, $P < 0.0001$).

DISCUSSION

Our results show that the relationship between parasitoid size and fitness is strongly dependent on the nutritional status of the female; females pay a greater cost for being small when they are starved. Specifically: (a) from 48 h onwards, the slope of the fecundity–size relationship is significantly steeper when the female is unfed (Fig. 1b,c) and (b) the effect of size on longevity is only statistically significant in unfed females (Fig. 2a). In the remainder of this discussion, we examine the pattern of resource allocation that leads to these relationships and its implications for our understanding of life-history strategies.

To understand the role of feeding in small and large females, we carried out a detailed physiological analysis of resource allocation. For this purpose, we quantified the three main energetic resources in the insect body: sugars, glycogen and lipids (Rivero and Casas, 1999). Of these, sugars are largely due to feeding, while glycogen and lipids are storage compounds and are therefore regulated by physiological mechanisms (van Handel, 1988). All three resources can be burned to fulfil the various energetic requirements of the insect body but, so far as is known, only lipids are directly involved in egg production (Nijhout, 1994). From an adaptive point of view, lipids are a key physiological currency in parasitoids, as they appear to be the common resource underlying the trade-off between reproduction and survival (Ellers, 1996; Ellers and van Alphen, 1997).

In *N. vitripennis*, the largest females emerge with twice as much lipid as the smallest females (Fig. 3a,b). Lipid reserves appear irreplaceable through feeding, as they decrease with time even in females fed *ad libitum*. Interestingly, these results are in line with those of two previous studies, where, in contrast with other insect taxa (Nayar and van Handel, 1971; Brown and Chippendale, 1974; van Handel, 1984; Warburg and Yuval, 1997), parasitoids also seemed unable to synthesize lipids *de novo* (Ellers, 1996; Olson *et al.*, 2000). Further detailed studies need to be carried out to determine how widespread this characteristic is in parasitoids. The adaptive consequences of this lack of lipogenesis are immediately obvious: teneral lipids would represent a non-replaceable resource and thus are potentially limiting, particularly for small females.

The pattern of use of lipid reserves in the first few days after birth indeed suggests that large females rely more heavily on lipid reserves than small females (Fig. 3a,b). The pattern of lipid use is, however, dependent on the nutritional status of the females. While in fed females lipid reserves decrease progressively with time (Fig. 3a), large unfed females appear to make use of a disproportionate amount of their lipid reserves in their first 24 h of life (Fig. 3b). The largest females, when starved, lose on average over $30 \mu\text{g}$ of fat (roughly 50% of their reserves) during the first day. There is no straightforward explanation for this observation, given that the number of eggs produced in the first 24 h interval is similar for fed and unfed females (Fig. 1a) and that there is no evidence of differences in egg quality between females of different sizes. Yet, the drastic decrease in lipids concomitant with a

drastic increase in egg production suggests that these two phenomena are likely to be associated. Moreover, a higher metabolic rate associated with increasing body size, a well-known phenomenon in animals (West *et al.*, 1997), cannot explain why the disproportionate use of lipids only occurs in the first 24 h. One potential explanation is that the energetic costs associated with egg production are partly met by sugars in fed females, but exclusively by lipids in unfed ones, as is thought to be the case in some insects (Engelmann, 1970; Clements, 1992), although the energy costs incurred by egg production require further study (Wheeler, 1996). Large unfed females would thus have to burn more lipids to fulfil the energetic requirements associated with producing up to 15 eggs in the first 24 h of life (Fig. 1a).

The decrease in lipid reserves in starved females appears to be associated with an increase in glycogen reserves taking place in the same period of time. Despite being unfed, the largest females gained on average 10 μg of glycogen over the first 24 h, increasing their overall reserves by more than 50%. In insects, glycogen is an important resource for flight (Nayar and van Handel, 1971; Mason *et al.*, 1989; Wheeler, 1996; Zera *et al.*, 1999). An attractive explanation would be that, faced with a harsh local environment, large females can afford to transform some of the lipids into fuel for flight. This explanation is tentative and requires further investigation. Despite the importance of dispersal in models of parasitoid behaviour and population dynamics, there have so far been no studies on the physiology of flight in parasitoids.

Irrespective of the destiny of the lipids burned in the first 24 h, thereafter all unfed females, irrespective of their size, start their lives with a similar amount of lipid reserves. How can we explain, then, why the largest females live longer? Starved females may rely on the other source of stored energy, glycogen, which from 24 h on is correlated with size (Fig. 4a). *N. vitripennis*, however, has at its disposal another important source of nutrients: the eggs themselves (King, 1963; Hopkins and King, 1964). Although egg resorption is widely thought to provide nutrients for metabolic maintenance in parasitoids (Leius, 1961a,b; King and Hopkins, 1963; van Lenteren *et al.*, 1987), we are unaware of any formal studies that quantify its role in extending female longevity.

In summary, we suggest that lipid availability is largely responsible for the nutrition-dependent relationship between size and fitness. Periods of temporal starvation are likely to occur in the field, where newly emerged females need to disperse from their original patches to find new hosts in which to feed and lay eggs (Werren, 1983; Molbo and Parker, 1996). Small females suffer disproportionately the costs of not feeding because they are born with small lipid reserves and thus need to rely more heavily on carbohydrates for survival and reproduction. Large unfed females, on the other hand, rely heavily on their large lipid reserves, which provide roughly twice the amount of calories per unit as carbohydrates (Nijhout, 1994).

The consequences of these observations for adaptive behaviour should be explored. In particular, because the fitness cost of not feeding is greater for small females, we might expect size-dependent foraging and feeding strategies. In the field, parasitoids can obtain their adult nutritional resources either from the host itself (host feeding) or from non-host sugary sources such as nectar or honeydew. Feeding incurs a cost for the female parasitoid, both in terms of time and energy investment (when hosts and food are found in different parts of the environment; Sirot and Bernstein, 1996) and in terms of lost reproductive opportunities (when an egg cannot be laid in the same host where host-feeding took place; Godfray, 1994). Since the fitness costs of starvation are higher for small females, they may

be predicted to spend more time foraging for food and be more likely to host-feed (possibly increasing further the slope of the size–fitness relationship under field conditions). This suggestion could be examined theoretically and tested empirically.

Many hypotheses about parasitoid ecology and life-history evolution require reliable estimates about the relationship between female size and fitness (Charnov and Skinner, 1984, 1985; Godfray, 1994). Our results provide a physiological explanation for why size–fitness relationships are steeper in the field (Hardy *et al.*, 1992; Godfray, 1994; West *et al.*, 1996, 1999), although other mechanisms may also be at work (West *et al.*, 1996). This study adds to the growing number of areas in evolutionary ecology in which an understanding of the physiological basis of resource allocation is required for further progress (Jervis and Kidd, 1995; Rivero and Casas, 1999).

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REFERENCES

- Bai, B. and Smith, S.M. 1993. Effect of host availability on reproduction and survival of the parasitoid wasp *Trichogramma minutum*. *Ecol. Entomol.*, **18**: 279–286.
- Benson, J. 1973. Intraspecific competition in the population dynamics of *Bracon hebetor* Say (Hymenoptera: Braconidae). *J. Anim. Ecol.*, **42**: 105–124.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**: 248–254.
- Brown, J.H. and Chippendale, G.M. 1974. Migration of the monarch butterfly, *Danaus plexippus*: energy sources. *J. Insect Physiol.*, **20**: 1117–1130.
- Charnov, E.L. and Skinner, S.W. 1984. Evolution of host selection and clutch size in parasitoid wasps. *FLA Entomol.*, **67**: 5–21.
- Charnov, E.L. and Skinner, S.W. 1985. Complementary approaches to the understanding of parasitoid oviposition decisions. *Environ. Entomol.*, **14**: 383–391.
- Clements, A.N. 1992. *The Biology of Mosquitoes*. London: Chapman & Hall.
- Crawley, M.J. 1993. *GLIM for Ecologists*. Oxford: Blackwell.
- Ellers, J. 1996. Fat and eggs: an alternative method to measure the trade-off between survival and reproduction in insect parasitoids. *Neth. J. Zool.*, **46**: 227–235.
- Ellers, J. and van Alphen, J.J.M. 1997. Life history evolution in *Asobara tabida*: plasticity in allocation of fat reserves to survival and reproduction. *J. Evol. Biol.*, **10**: 771–785.
- Ellers, J., van Alphen, J.J.M. and Sevenster, J.G. 1998. A field study of size–fitness relationships in the parasitoid *Asobara tabida*. *J. Anim. Ecol.*, **67**: 318–324.
- Ellers, J., Bax, M. and van Alphen, J.J.M. 2001. Seasonal changes in female size and its relation to reproduction in the parasitoid *Asobara tabida*. *Oikos*, **92**: 309–314.
- Engelmann, F. 1970. *The Physiology of Insect Reproduction*. Oxford: Pergamon Press.
- Flanagan, K.E., West, S.A. and Godfray, H.C.J. 1998. Local mate competition, variable fecundity and information use in a parasitoid. *Anim. Behav.*, **56**: 191–198.
- Gilbert, F. and Jervis, M. 1998. Functional, evolutionary and ecological aspects of feeding-related mouthpart specializations in parasitoid flies. *Biol. J. Linn. Soc.*, **63**: 495–535.
- Godfray, H.C.J. 1994. *Parasitoids: Behavioural and Evolutionary Ecology*. Monographs in Behavior and Ecology. Princeton, NJ: Princeton University Press.

- Hardy, I.C.W., Griffiths, N.T. and Godfray, H.C.J. 1992. Clutch size in a parasitoid wasp: a manipulation experiment. *J. Anim. Ecol.*, **61**: 121–129.
- Hopkins, C.R. and King, P.E. 1964. Egg resorption in *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). *Proc. R. Entomol. Soc. Lond. A*, **39**: 101–107.
- Jervis, M. 1998. Functional and evolutionary aspects of mouthpart structure in parasitoid wasps. *Biol. J. Linn. Soc.*, **63**: 461–493.
- Jervis, M. and Kidd, N. 1995. Incorporating physiological realism into models of parasitoid feeding behaviour. *Trends Ecol. Evol.*, **10**: 434–436.
- Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T. and Dawah, H.A. 1993. Flower-visiting by hymenopteran parasitoids. *J. Nat. Hist.*, **27**: 67–105.
- Kazmer, D. and Luck, R. 1995. Field tests of the size–fitness hypothesis in the egg parasitoid *Trichogramma pretiosum*. *Ecology*, **76**: 412–425.
- King, P. and Hopkins, C. 1963. Length of life of the sexes in *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) under conditions of starvation. *J. Exp. Biol.*, **40**: 751–761.
- King, P.E. 1963. The rate of egg resorption in *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) deprived of hosts. *Proc. R. Entomol. Soc. Lond. A*, **38**: 98–100.
- King, P.E. and Richards, J.G. 1969. Oogenesis in *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). *Proc. R. Entomol. Soc. Lond. A*, **44**: 10–12.
- Leius, K. 1960. Attractiveness of different foods and flowers to the adults of some hymenopterous parasites. *Can. Zool.*, **92**: 235–274.
- Leius, K. 1961a. Influence of food on fecundity and longevity of adults of *Ictoplectis conquisitor* (Say) (Hymenoptera: Ichneumonidae). *Can. Entomol.*, **93**: 771–780.
- Leius, K. 1961b. Influence of various foods on fecundity and longevity of adults of *Scambus buolianae* (Htg.) (Hymenoptera: Ichneumonidae). *Can. Entomol.*, **93**: 1079–1084.
- Mason, L.J., Johnson, S.J. and Woodring, J.P. 1989. Influence of carbohydrate deprivation and tethered flight on stored lipid, fecundity and survivorship of the soybean looper (Lepidoptera: Noctuidae). *Environ. Entomol.*, **18**: 1090–1094.
- Molbo, D. and Parker, E.D. 1996. Mating structure and sex ratio variation in a natural population of *Nasonia vitripennis*. *Proc. R. Soc. Lond. B*, **263**: 1703–1709.
- Nayar, J.K. and van Handel, E. 1971. The fuel for sustained mosquito flight. *J. Insect Physiol.*, **17**: 471–481.
- Nijhout, H.F. 1994. *Insect Hormones*. Princeton, NJ: Princeton University Press.
- Norušis, M. 1995. *SPSS for Windows Advanced Statistics, Release 5*. Chicago, IL: SPSS Inc.
- Olson, D.M., Fadamiro, H., Lundgren, J.G. and Heimpel, G.E. 2000. Effects of sugar-feeding on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiol. Entomol.*, **25**: 17–26.
- Rivero, A. and Casas, J. 1999. Incorporating physiology into parasitoid behavioral ecology: the allocation of nutritional resources. *Res. Popul. Ecol.*, **41**: 39–45.
- Rivero-Lynch, A.P. and Godfray, H.C.J. 1997. The dynamics of egg production, oviposition and resorption in a parasitoid wasp. *Funct. Ecol.*, **11**: 184–188.
- Rossiter, M.C., Coxforster, D.L. and Briggs, M.A. 1993. Initiation of maternal effects in *Lymantria dispar*: genetic and ecological components of egg provisioning. *J. Evol. Biol.*, **6**: 577–589.
- Siro, E. and Bernstein, C. 1996. Time sharing between host searching and food searching in parasitoids: state-dependent optimal strategies. *Behav. Ecol.*, **7**: 189–194.
- Tagaki, M. 1985. The reproductive strategy of the gregarious parasitoid, *Pteromalus puparium* (Hymenoptera: Pteromalidae) 1. Optimal number of eggs in a single host. *Oecologia*, **70**: 321–325.
- van Handel, E. 1984. Metabolism of nutrients in the adult mosquito. *Mosq. News*, **44**: 573–579.
- van Handel, E. 1985a. Rapid determination of glycogen and sugars in mosquitoes. *J. Am. Mosq. Control Assoc.*, **1**: 299–301.
- van Handel, E. 1985b. Rapid determination of total lipids in mosquitoes. *J. Am. Mosq. Control Assoc.*, **1**: 302–304.

- van Handel, E. 1988. Assay of lipids, glycogen and sugars in individual mosquitoes: correlations with wing length in field-collected *Aedes vexans*. *J. Am. Mosq. Control Assoc.*, **4**: 549–550.
- van Lenteren, J.C., van Vianen, A., Gast, H.F. and Kortenhoff, A. 1987. The parasite–host relationship between *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). XVI. Food effects on oogenesis, oviposition, life-span and fecundity of *Encarsia formosa* and other hymenopterous parasites. *J. Appl. Entomol.*, **103**: 69–84.
- Visser, M.E. 1994. The importance of being large: the relationship between size and fitness in females of the parasitoid *Aphaereta minuta* (Hymenoptera: Braconidae). *J. Anim. Ecol.*, **63**: 963–978.
- Warburg, M.S. and Yuval, B. 1997. Effects of energetic reserves on behavioural patterns of Mediterranean fruit flies (Diptera: Tephritidae). *Oecologia*, **112**: 314–319.
- Werren, J.H. 1983. Sex ratio evolution under local mate competition in a parasitic wasp. *Evolution*, **37**: 116–124.
- West, G.B., Brown, J.H. and Enquist, B.J. 1997. A general model for the origin of allometric scaling laws in biology. *Science*, **276**: 122–126.
- West, S.A., Flanagan, K.E. and Godfray, H.C.J. 1996. The relationship between parasitoid size and fitness in the field, a study of *Achrysocharoides zwoelferi* (Hymenoptera: Eulophidae). *J. Anim. Ecol.*, **65**: 631–639.
- West, S.A., Flanagan, K.E. and Godfray, H.C.J. 1999. Sex allocation and clutch size in parasitoid wasps that produce single-sex broods. *Anim. Behav.*, **57**: 265–275.
- Wheeler, D. 1996. The role of nourishment in oogenesis. *Annu. Rev. Entomol.*, **41**: 407–431.
- Zera, A.J., Sall, J. and Otto, K. 1999. Biochemical aspects of flight and flightlessness in *Gryllus*: flight fuels, enzyme activities and electrophoretic profiles of flight muscles from flight-capable and flightless morphs. *J. Insect Physiol.*, **45**: 275–285.