IDEAS AND PERSPECTIVES

Mothers reduce egg provisioning with age

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Abstract

Precise and comprehensive data on resource allocation into individual eggs are rare and this empirical void in the literature of life history strategies contrasts with the large number of theoretical studies. We show a marked decrease in reproductive investment in eggs with mother's age for egg size, sugar, protein, lipid and energy contents of eggs for a parasitic wasp. Egg size is a good predictor of offspring fitness, measured as survival of starving neonate larvae, but does not reveal possible biochemical changes. Lipids stabilize quickly at a minimal threshold while proteins and sugars decrease smoothly down to about 30% of the amount invested in the first egg. Because proteins have the highest correlation with egg size, we predict that they should be better predictors of larval fitness than lipids and sugars. Assessing the adaptive value of the observed patterns will require a multidimensional approach to egg provisioning.

Keywords

Egg composition, egg size, life history strategies, nutrient allocation, offspring fitness, parasitoid.

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INTRODUCTION

Reproductive investment is an essential feature in the study of life histories (Smith & Fretwell 1974; Charlesworth 1980; Perrin & Sibly 1993; Einum & Fleming 2000; Roff 2002) and is fundamental to numerous fields of research in behavioural, evolutionary and population ecology (Rivero & Casas 1999; Hochberg & Ives 2000; LaMontagne & McCauley 2001; Roff 2002). Several optimality models have been developed to predict the optimal maternal investment per offspring on the basis that reproductive strategies evolve to maximize the number of viable offspring, and thus parental fitness (Lack 1947; Stearns 1992).

One of the most widely used predictors of reproductive investment is egg size (Winkler & Wallin 1987; Sinervo & Licht 1991; Bernardo 1996). This approach assumes that (1) large eggs produce offspring with higher fitness and (2) large eggs are more costly to produce (Bernardo 1996; McIntyre & Gooding 2000; Roff 2002). These assumptions are however largely contingent on egg nutrient composition being correlated with egg size, an *a priori* that has rarely been tested (see e.g. Einum & Fleming 2000 for an exception). Egg size and composition are however not necessarily correlated and variation in egg composition can be ecologically and evolutionarily more important than variation in egg size (Begon & Parker 1986; Bernardo 1996; Fox & Czesak 2000). Furthermore, numerous studies have demonstrated that

females could vary their investment per egg (e.g. as a function of age), a pattern reflecting physiological constraints on egg production or an adaptive strategy (Begon & Parker 1986; Clutton-Brock & Godfray 1993; Bernardo 1996; Roff 2002).

In this study, we investigated the physiology of maternal reproductive investment in the parasitoid *Eupelmus vuilletti* (Hymenoptera, Eupelmidae). We aimed to (1) quantify the amount of resources allocated to eggs throughout the lifetime of the female using both egg size and biochemical composition as indicators of maternal investment, and (2) explore the relationship between maternal reproductive investment per egg and neonate larval fitness.

MATERIALS AND METHODS

Eupelmus vuilletti (CRW) (Hymenoptera, Eupelmidae) is a solitary ectoparasitoid that feeds and oviposits on the third and fourth larval instars of *Callosobruchus maculatus* (Coleoptera, Bruchidae). These Coleoptera develop during their post-embryonic instar within the pods and seeds of *Vigna unguiculata* (Fabaceae). All experiments were carried out on insects that had been raised in the laboratory at 23 °C, a 13 : 11 light : dark photoperiod, and 75% humidity. Hosts for the experiment were extracted from the seeds and placed individually inside gelatin capsules (for details see Giron *et al.* 2002). This system does not alter the natural oviposition pattern of females nor their life expectancy (Giron *et al.*

2002) and allows for the number and the developmental stage of the hosts to be controlled. Furthermore, it facilitates the collection of eggs laid by the parasitoid. As a female lays several eggs per day, we will use the term 'age' for a female's age and 'oviposition rank' (position in the laying sequence) for an egg's status.

Egg size and composition

To determine egg composition during a female's life span, we carried out a first experiment whereby 70 newly emerged females were placed individually inside a Petri-dish (diameter 5.5 cm) and given one host per hour for a total of 6 h (between 9 a.m. and 3 p.m., which corresponds to peak daily reproductive activity; D. Giron, personal observation). This was repeated until the death of the female. The gelatin capsules containing the hosts were collected each day. The length (L) and the width (W) of each egg was immediately measured with a micrometer, and the volume (V) of each egg was estimated by the equation $V = (\pi \times L \times W^2)/6$ (Avelar 1993).

Quantification of lipids and sugars in eggs was carried out using a modification of the colorimetric techniques developed by Van Handel (Van Handel 1993; Giron *et al.* 2002). Protein analysis was conducted using the Bradford assay procedure (Giron *et al.* 2002). The techniques used were not sensitive enough for individual analyses of eggs. Therefore, egg composition was determined using batches of five eggs of the same oviposition rank (position in the laying sequence) but from different, arbitrarily chosen, females. Each batch was used for the colorimetric analysis of only one type of nutrient (lipids, carbohydrates or proteins). This means that our results are correlational, and we cannot assess the actual covariation of these three nutrients within eggs. The analyses were carried out on eggs from rank 1 to 40 in the laying sequence, with between one and three batches per oviposition rank and per nutrient class (rank 1–25: three batches; rank 26–37: two batches, rank 38–40: one batch). Beyond rank 40, the number of eggs obtained was too low for analysis. We evaluated the mean energy content of the eggs in each rank by converting the mean nutrient content into Joules (conversion factors: proteins 16.0 J g^{-1}; carbohydrates 16.0 J g^{-1}; lipids 37.5 J g^{-1}; see Rivero & Casas 1999; McIntyre & Gooding 2000).

Correlation among egg size, egg composition and offspring fitness

To determine the effects of egg size on the survival of the resulting offspring, we carried out a second experiment whereby 30 females were allowed to oviposit in the same conditions as described above. The size of each egg laid by each female during its entire life was measured and then each egg was placed individually into a gelatin capsule (without a host) until eclosion. Once emerged, larval status (alive or dead) was checked on an hourly basis by detecting the presence of a heartbeat. For this purpose the heart was observed through the cuticle with the aid of a binocular microscope. The survival of neonate larvae in the absence of a food source has been previously used as an estimate of offspring fitness (see Guisande & Harris 1995; Diss *et al.* 1996) and was chosen here for three reasons. First, many eggs are laid near, rather than on, the host (D. Giron, personal observation). Thus, the first hours before attachment has been secured and host fluids have been obtained are a crucial phase of a neonate's life. Second, the survival criterion does not explicitly include differences in host quality, which can have a critical influence on offspring fitness (Godfray 1994). Finally, most of the studies that have failed to detect fitness advantages from large eggs have reared progeny in high-quality environments. Selection is however expected to be generally stronger in low-quality environments (Fox & Czesak 2000). The effect of egg size on offspring survival was analysed using generalized non-linear regression techniques (SYSTAT, Chicago, IL, USA).

Egg composition was determined using batches of eggs. Therefore, correlation analyses between egg size and absolute egg composition were performed using the values obtained for each batch at each oviposition rank ($n = 120$ batches). For estimating the relative composition as function of egg size, we based our correlation analyses on the mean values obtained at each oviposition rank ($n = 40$ batches).

RESULTS

Theoretical studies predict that reproductive investment per egg either stays constant, increases or decreases with age (Roff 2002). Hence, egg size, lipid, carbohydrate, protein and energy contents were expressed as percentages of the mean values of the first egg laid. Moreover, this makes visual comparison of the slopes in Figs 1(a) and 2(a–d) easier.

Females laid an average of 28.5 ± 2.7 eggs over a period of 11.5 ± 0.4 days. Egg size declined with position in the laying sequence and the last eggs laid were about 12% smaller than the first eggs (Fig. 1a). Neonate larval survival, when deprived of food, was higher for larger eggs (Fig. 1b). The sigmoidal shape of the relationship [non-linear regression, $y = 26.32/[1 + 74014.00 \times \text{EXP}(-1.37 \times x)]$ was assessed by checking for systematic linear or curvilinear behaviour of the residuals with respect to size. None was found and only 12 points of 760 had residual values higher than 1.96 (data not shown).

Egg composition decreased during the life of a female (Fig. 2a–d). The pattern of decrease in nutrient content with rank was relatively similar to the pattern observed for egg

Figure 1 Egg size. (a) Egg volume (mean ± SE) as function of oviposition rank (position in the laying sequence). Data are expressed as percentages of the mean egg volume obtained for first eggs laid (first oviposition rank: volume = 9.35 ± 0.16 μm³). (b) Survival of neonate larvae (in hours) when deprived of food as function of egg volume (in μm³).
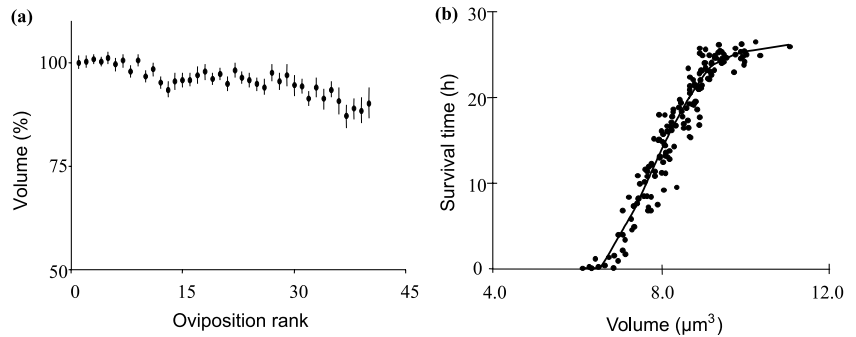
Figure 2 Egg composition relative to the first egg. Mean lipid (a), carbohydrate (b), protein (c) egg content (mean ± SE) as function of oviposition rank (position in the laying sequence). Lipid, carbohydrate and protein contents are expressed as percentages of the mean values obtained for first eggs laid (first oviposition rank: lipids 0.42 ± 0.01 μg, carbohydrates 0.47 ± 0.04 μg and proteins 0.41 ± 0.02 μg). (d) Mean energy egg content and relative contribution of lipids (grey), carbohydrates (white) and proteins (black). Data are expressed as percentages of the mean values obtained for first eggs laid (first oviposition rank: mean energy content = 31.21 ± 0.27 μJ with lipids = 52.3%, carbohydrates = 22.0% and proteins = 25.7% of energy content).
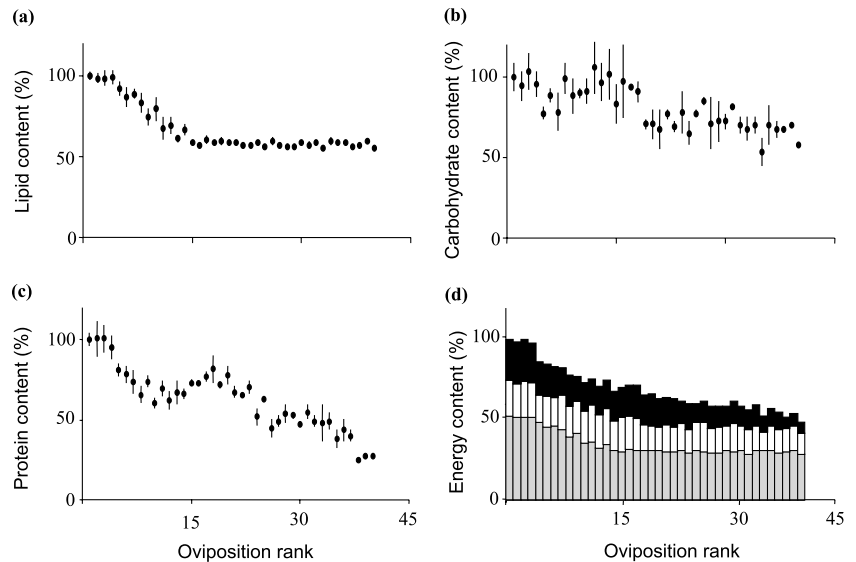
size, with the exception of lipids, which reached a stable lower value after $c.$ the 13th egg ($c.$ 0.24 μg egg^{-1}) (Fig. 2a).

Lipid, carbohydrate and protein contents were correlated with egg size (lipid content $r = 0.67$, $n = 120$ batches and $P < 0.005$; carbohydrate content $r = 0.35$, $n = 120$ batches and $P < 0.005$; protein content $r = 0.75$, $n = 120$ batches and $P < 0.005$). Moreover, the relative proportion of carbohydrate (mean ± SE, 26.05 ± 0.46%; $n = 40$) and protein (23.19 ± 0.66%; $n = 40$) varied significantly with egg size (carbohydrate percentage $r = -0.49$, $n = 40$ and $P < 0.005$; protein percentage $r = 0.64$, $n = 40$ and $P < 0.005$). However, the lipid percentage (50.77 ± 0.67%; $n = 40$) did not vary significantly with egg size (lipid percentage $r = -0.28$, $n = 40$ and $P = 0.080$). These results suggest differences in the relative chemical compositions of small and large eggs.

DISCUSSION

Females decreased both the size and nutrient content of eggs over the course of their lives. Based on our results, we

cautiously suggest that a varying investment per egg could have important consequences for offspring fitness. Indeed, we observed the existence of a minimal egg size below which offspring cannot survive, and above which their fitness increases as size increases. Fitness subsequently stabilizes around a maximum.

Different alternative explanations could provide a mechanism for the reproductive pattern observed. Indeed, a reduction in reproductive maternal investment per egg throughout the lifetime of a female may be the result of some essential resource necessary for egg production gradually becoming depleted over time (i.e. resource depletion, Begon & Parker 1986; Bernardo 1996; Roff 2002). Alternatively, Begon and Parker (1986) studied how parental age could influence maternal investment per egg in terms as an adaptive strategy. Assuming that maternal fitness depends not only on the number of offspring and their individual fitness but also on a mother's survival probability, they predict that the optimal maternal strategy would be declining reproductive output with offspring with age (for details see Begon & Parker 1986). Finally, the observed

decline of maternal investment per egg could be due to the intrinsic degeneration of physiological activity with age (i.e. senescence, Charlesworth 1980; Stearns 1992; Tatar 2001).

In theory, therefore, a reduction of reproductive investment per egg could be explained by physiological constraints or adaptive strategies. Our study, in examining changes in nutrient content only, cannot ascribe an adaptive or a non-adaptive explanation for the observed reduction of nutrient allocation per egg with maternal age. Moreover, we could not quantify the total amount of resources available to the females, a critical aspect of many optimality models that aim to determine how mothers should distribute a fixed amount of resources amongst offspring (Clutton-Brock & Godfray 1993; Roff 2002). A definitive test of the adaptive significance of a decrease in reproductive investment per egg with age will require some form of genetic selection or direct manipulative approaches such as physical and physiological manipulations (for specific examples see Sinervo & Licht 1991; Stearns 1992 and Fox & Czesak 2000). This includes for example, manipulation of egg size or partial removal of egg yolk. However the very small size of parasitoid eggs studied here precludes such manipulations.

Egg size is generally regarded as a good predictor of offspring fitness and the low residual variance in our regression shows that this assumption is met. However, egg size alone may hide the complex ways in which different nutrients are actually allocated by mothers to their offspring. Insect eggs contain a large amount of yolk consisting largely of lipids and proteins (Chapman 1998). The importance of proteins and lipids as energetic and structural components of eggs and larvae has been shown in a great variety of insects and other invertebrates (Guisande & Harris 1995; Diss *et al.* 1996; Chapman 1998).

Lipids are known to be the most efficient and most commonly used energy source in insects and in other groups of animals such as birds (Van Handel 1993; Rivero & Casas 1999; Royle *et al.* 1999) and their importance in embryonic development is a well-known phenomenon (Van Handel 1993; Guisande & Harris 1995). In *E. vuilletti*, the pattern of lipid decrease with maternal age rapidly attained a stable lower value of about 0.24 μg lipids per egg, suggesting that this may be the minimum required for successful embryonic development. The quantity of lipids available to *E. vuilletti* females is limited and fixed upon emergence because this species feeds on lipid-poor host haemolymph and is incapable of lipogenesis (Giron *et al.* 2002; Giron & Casas in press). Among the nutrients, protein content (both absolute and relative) correlates best with egg size. Proteins would therefore appear to be a better predictor of offspring fitness than lipids or sugars. Yolk proteins, such as vitellin, are known to be an important source of amino acids for neonate larvae and have been found to be a good predictor

of neonate fitness in other insects (Diss *et al.* 1996; Chapman 1998).

The different nutrient classes of eggs could act on offspring fitness at different levels and points in time due to their different specific functions. Moreover, there is increasing evidence that key nutrients or trace elements may be essential for egg development and larval survival, and thus essential for offspring fitness (Jann & Ward 1999; Rivero *et al.* 2001; O'Brien *et al.* 2002). Future studies should take a multidimensional approach to egg composition (McGinley & Charnov 1988).

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