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Maternal age affects offspring nutrient dynamics

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ABSTRACT

The internal physiological state of a mother can have major effects on her fitness and that of her offspring. We show that maternal effects in the parasitic wasp *Eupelmus vuilleti* become apparent when old mothers provision their eggs with less protein, sugar and lipid. Feeding from a host after hatching allows the offspring of old mothers to overcome initial shortages in sugars and lipids, but adult offspring of old mothers still emerged with lower protein and glycogen quantities. Reduced egg provisioning by old mothers had adverse consequences for the nutrient composition of adult female offspring, despite larval feeding from a high-quality host. Lower resource availability in adult offspring of old mothers can affect behavioural decisions, life histories and performance. Maternal effects on egg nutrient provisioning may thus affect nutrient availability and fitness of future generations in oviparous animals.

1. Introduction

Environmental conditions experienced by a mother can have major consequences for her own fitness, but can also influence the fitness of her offspring (Marshall and Uller, 2007; Wolf and Wade, 2009). Such maternal or transgenerational effects have been studied in a wide range of animals, including water fleas (Plaistow et al., 2015), insects (Newcombe et al., 2015; Wilson and Graham, 2015), fish (Carter et al., 2015), reptiles (Nafus et al., 2015) and birds (Bouwhuis et al., 2015; Schroeder et al., 2015). Most work on maternal effects has focused on the influence of external environmental factors (Mousseau and Fox, 1998; Salinas and Munch, 2012), even though the internal maternal state constitutes a source of continual phenotypic variation (Marshall and Uller, 2007). Maternal effects are tightly linked to reproductive investment, yet resource allocation towards reproduction rarely stays constant over a mother's lifetime. Investment into reproduction is affected by trade-offs such as between offspring number and offspring size (Smith and Fretwell, 1974; Preziosi et al., 1996; Fox and Czesak, 2002; Gibbs et al., 2005), resource allocation decisions (van Noordwijk and de Jong, 1986; Gibbs et al., 2010; Niitepõld and Boggs, 2015), reduced reproductive potential later in life (Kindsvater and Otto, 2014), and diminishing availability of resources for reproduction over time (McIntyre and Gooding, 2000; Giron and Casas, 2003). Even when mothers are faced with relatively constant environmental conditions, resource allocation strategies can dramatically alter how a mother's current phenotype affects her own and her offspring's fitness.

Individuals that are larger at birth tend to have higher growth rates, larger adult body sizes and higher chances of survival, in what is known as the offspring size-performance relationship (Fox, 1993; Einum and Fleming, 1999; Fox and Czesak, 2002; Allen et al., 2008; Krist, 2011). Larger females further often have a higher lifetime reproductive success because they may produce larger offspring (Beauplet and Guinet, 2007; Steiger, 2013). It is, however, not generally true that being bigger is always better (Moran and McAlister, 2009; Blackenhorn, 2011). Other factors may alter the offspring size-performance relationship (Gaillard et al., 2000; Dibattista et al., 2007), as in common grackle nestlings (*Quiscalus quiscula*) where asynchronous hatching almost completely alleviated the benefits associated with larger offspring size (Maddox and Weatherhead, 2008). Physiological sources of variation can further directly mediate growth, survival and reproduction. Whilst offspring size is frequently used as a proxy for estimating offspring quality, underlying physiological estimates are often lacking.

Maternal resource provisioning affects both embryogenesis and nutrition of the embryo because the eggs of most oviparous animals are closed systems in terms of macronutrient content (but see Le Ralec, 1995). In insect eggs, proteins are one of the major macronutrients

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(around 40–50% of the total macronutrient composition (van Handel, 1993; Giron and Casas, 2003; Geister et al., 2008; Sloggett and Lorenz, 2008), presumably to facilitate the formation of structural parts during embryogenesis. Eggs further tend to have a high caloric value to meet the nutritional demands of the developing embryo, where long-term energy sources, such as lipids, form another large fraction of an egg's nutritional components (around 30–40% of total macronutrients), complemented by shorter-term energetic substrates such as carbohydrates (sugars and glycogen, around 10–30%). In egg rafts of the mosquito *Culex quinquefasciatus,* 90% of respiration was supported by lipid and 10% by glycogen consumption (van Handel, 1993). Lipids were also found to be the most important fuel during embryonic development of the ladybird beetle *Adalia bipunctata* and the spider crab *Hyas araneus* (Petersen and Anger, 1997), whereas proteins were used at a slower rate. Whilst these studies have considered nutrient utilization throughout the embryonic stage, it has remained unclear how nutrient dynamics are affected after hatching and during subsequent developmental stages.

The parasitic wasp *Eupelmus vuilleti* (Hymenoptera: Eupelmidae) has long served as an insect model system in nutritional ecology, with extensive studies on adult nutrient allocation strategies, behavioural decisions and life history consequences. *E. vuilleti* depends both on capital and income resources (i.e. resources obtained and stored during development and resources acquired as adults, respectively), where adult females rely on capital lipid stores for the production of eggs and on income carbohydrates to cover maintenance costs (Casas et al., 2005). Haemolymph of their host (beetle larvae) constitutes an income resource when a host larva is found, but adult females need to choose between host-feeding or egg-laying, as a host larva cannot be used for both simultaneously. Host, and thereby food availability, further affects life histories and behaviour of these wasps, as oviposition is favoured when host densities are low, while host-feeding is favoured when host densities are high (Richard and Casas, 2009). Ageing in adult females increases the time necessary to host-feed, oviposition frequency, as well as metabolic rates of foraging females (Giron et al., 2004; Casas et al., 2005, 2015). This leads to a steady reduction in nutrient reserves over time, which in turn leads older mothers to invest less in their offspring (Giron and Casas, 2003). The large knowledge-base on *E. vuilleti* nutrient dynamics in adults, behaviour and life histories make it an exceptional system for studying the consequences of reduced maternal egg provisioning on nutrient levels in the offspring.

To fully grasp how maternal effects determine fitness of a mother and her offspring, it is critical to consider both temporal phenotypic variation in the mother's internal physiological state and the consequences of variable egg nutrient provisioning for her offspring throughout development and up to the reproductive adult life stage. Using *E. vuilleti* wasps, this study aims to show that *1*) ageing mothers have fewer resources and reduce egg provisioning; *2*) reduced egg provisioning by a mother differentially affects nutrient dynamics in developing offspring; and *3*) reduced egg provisioning negatively affects nutrient levels in adult offspring. This work extends the existing knowledge on nutrient dynamics in *E. vuilleti* (Giron and Casas, 2003; Giron et al., 2004; Casas et al., 2005, 2015; Richard and Casas, 2009), the only insect to date for which such detailed physiological estimates are now available from freshly laid eggs to adults at death. Our work also sheds new light on the mechanistic basis of maternal effects, showing that maternal age can severely affect egg macronutrient provisioning and subsequent availability of resources for offspring in the adult life stage.

2. Materials and methods

2.1. Insects

All experiments were performed with a laboratory culture of the host-feeding, solitary, ectoparasitoid *Eupelmus vuilleti* (Hymenoptera: Eupelmidae). This parasitic wasp develops on third to fourth instar *Callosobruchus maculatus* (Coleoptera: Bruchidae) larvae that in turn feed and develop inside cowpea seeds and pods, *Vigna unguiculata* (Fabaceae). *E. vuilleti* produces anhydropic (yolk-rich) eggs, like the vast majority of oviparous animals. Only a few eggs mature before adult eclosion and the majority of eggs are produced during adult life (i.e. synovigeny). Female longevity depends on host availability and thereby feeding opportunities. Under laboratory conditions females live on average 6.6 days without hosts/food and 14.3 days with access to hosts/food (Casas et al., 2005). During their lifetime, females oviposit on average 39 times and host-feed 22 times (Casas et al., 2005), with a peak in oviposition after 3–4 days post-eclosion. *E. vuilleti* was maintained and experiments performed at a relative humidity of 65%, a photoperiod of 13:11 light:dark, and a temperature regime of 33 °C (lights on):23 °C (lights off) reflecting natural conditions.

2.2. Sample collection

A day before experiments started, *C. maculatus* host larvae of similar size (~3 mm) were extracted from seeds, placed inside perforated gelatin capsules, and refrigerated at 4 °C to slow down development (Gauthier and Monge, 1999). Gelatin capsules serve as artificial seeds that allow for the control of both host quality and number. Moreover, *E. vuilleti* females can readily form a host-feeding tube, similar to what occurs when feeding from hosts within natural seeds (Giron et al., 2002). Freshly emerged adult *E. vuilleti* females were collected, placed singly in a small Petri dish (diameter: 3 cm), and allowed access to water on cotton wool for maintenance outside of experimental hours. Females were further allowed access to males for mating during the first 24 h after emergence. For experiments each female was transferred to a Petri dish (diameter: 5 cm) twice a day from 9am to 12 pm and from 12 pm to 3 pm with access to three, randomly chosen gelatin capsules, each containing one host larva. After each interval, eggs of individual females were counted and either randomly collected for biochemical analyses (eggs 1, 2, 3 or 4 for young mothers and eggs 19, 20, 21 or 22 for old mothers) that allowed estimating nutrient quantities (protein, sugar, glycogen and lipid) or discarded (Fig. 1A). These are the first eggs laid for young mothers, and eggs laid just over the lifetime oviposition peak for old mothers. Eggs with these rank numbers were chosen based on a previous study (Giron and Casas, 2003) which revealed pronounced differences in nutrient content between eggs 1–4 and eggs 19–22. Oviposition rank is highly correlated with age of the mother in this species, due to the relatively slow egg maturation rate throughout adult life. To follow nutrient dynamics throughout development and into the adult stage, collected eggs were allowed to develop on standardized high-quality hosts for various durations after egg laying: fresh egg (3 h), developed egg (28 h), hatched larva (33 h), developed larva (8 days), and adult females (17 days)(Fig. 1A). Nutrient content of mothers was determined for females collected after laying between 1 to 4 or 19 to 22 eggs for young (n = 31) and old mothers (n = 17), respectively. All eggs used in experiments hatched successfully (similar to findings in Giron and Casas, 2003).

2.3. Nutrient composition of eggs and hatched larvae

Protein levels in eggs and hatched larvae were determined for a set of separate samples, as proteins could not be extracted from the same samples used for sugar, glycogen and lipid quantification (n = 18, 15, and 17 for fresh eggs, developed eggs and hatched larvae of young mothers; n = 15, 14 and 17 for fresh eggs, developed eggs and hatched larvae of old mothers). Protein levels in eggs and hatched larvae were determined using an adapted Bradford dye-binding micro-assay procedure described in Giron et al. (2002) using 5, randomly chosen, eggs/hatched larvae per sample (Fig. 1B). Eggs/hatched larvae were manually crushed with a pestle in a microcentrifuge tube, after which 45 µl of aqueous lysis buffer solution [100 mM KH2PO4, 1 mM dithiothreitol

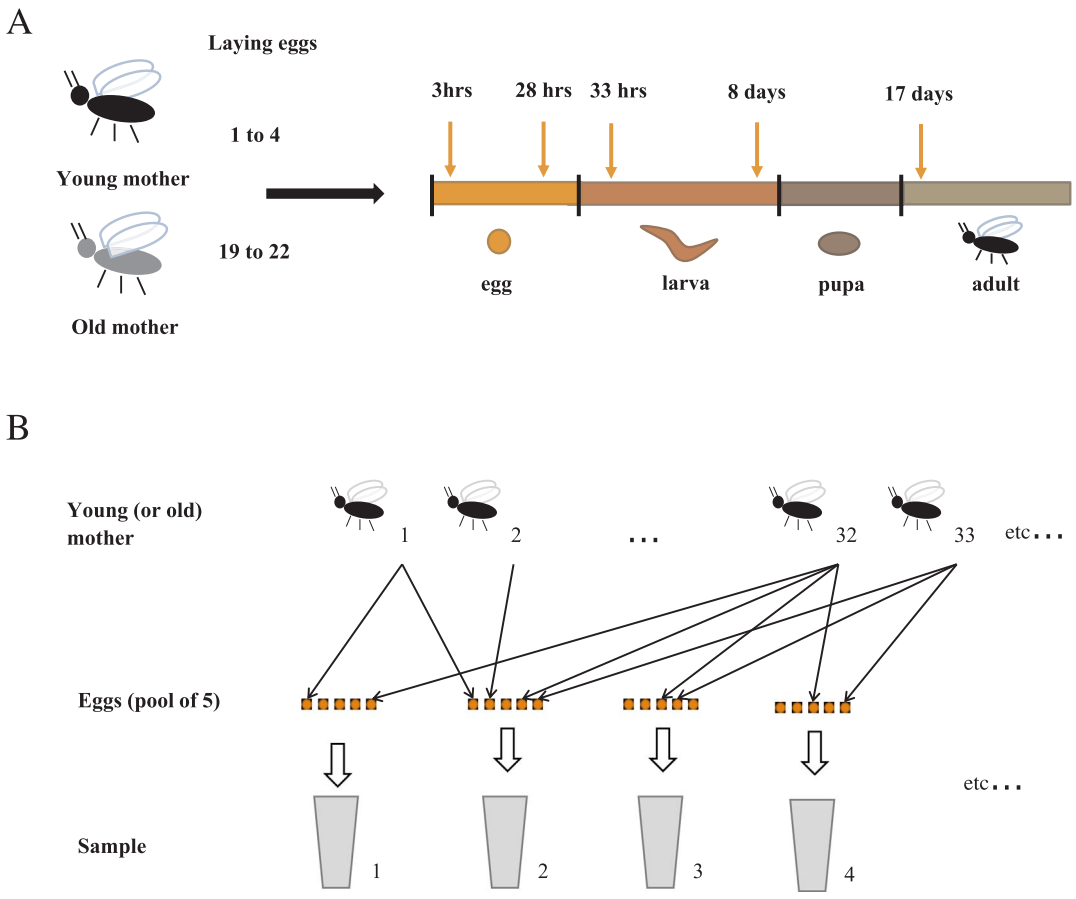

Fig. 1. Overview of sampling points, including young and old mothers, and throughout the life of their offspring (A). An example of our sampling scheme, where mothers could contribute 1, 2, 3 or 4 eggs to different pools (in this case 5 eggs per pool for protein quantification). In < 6% of cases a mother contributed the maximum number of 4 eggs to different pools (B).

(DTT) and 2 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4], 5 µl of triton X (0.01%) and 450 µl Bradford reagent (Sigma) were added. Samples were left to incubate for 5 min before absorbance was read at 595 nm using a spectrophotometer (Varian Spectrophotometer Cary 50 Scan; Agilent technologies). Bovine serum albumin was used to obtain calibration curves.

Sugar, glycogen and lipid levels in eggs/hatched larvae were determined using 10, randomly chosen, eggs or hatched larvae per sample following procedures described in Foray et al. (2012), which was adapted for nutrient quantification of smaller life stages (n = 12, 9, and 9 for fresh eggs, developed eggs and hatched larvae of young mothers, with the exception of sugars in fresh larvae for which n = 10; n = 7, 7, and 8 for fresh eggs, developed eggs and hatched larvae of old mothers). In short, 150 µl of a chloroform:methanol (1:2) solution was added to each sample, which was then vortexed, centrifuged and mechanically crushed (biovortex n°1083, Biospec Products), after which each pestle was rinsed with 300 µl chloroform:methanol solution. 60 µl of 2% sodium sulphate was added to allow glycogen to precipitate and subsequently centrifuged for 15 min (180*g* at 4 °C). To determine sugar quantities, 250 µl of supernatant was pipetted into a 1.5 mL vial and dried down until 10 µl of supernatant remained. 1 mL of freshly prepared Anthrone reagent was then added and heated for 15 min in a water bath (90 °C). Vials were cooled on ice and samples transferred to 1 mL cuvettes, after which absorbance of each sample was read at 625 nm. To determine glycogen quantities, vials were rinsed twice using 400 µl 80% methanol and vortexed, after which 1mL Anthrone reagent was added. Samples were then heated for 15 min in a water bath (90 °C) and cooled on ice, after which they were transferred to a cuvette and absorbance read at 625 nm. Glucose was used to obtain calibration curves for carbohydrate analyses. Lipid quantities were determined using 180 µl of supernatant that was completely dried down in a fresh vial. 40 µl of 98% sulfuric acid was added and vortexed vigorously. Vials were placed in a water bath (90 °C) for 2 min and cooled on ice before 960 µl Vanillin reagent was added and absorbance read at 525 nm. Triolein was used to obtain calibration curves for determining lipid quantities.

2.4. Nutrient composition of developed larvae and adults

Nutrient levels in developed larvae and adults were determined following the protocol of Foray et al. (2012), which was modified for *E. vuilleti* (n = 38 and 29 for developed larvae and adult offspring of young mothers; n = 32 and 21 for developed larvae and adult offspring of old mothers, with the exception of protein for which n = 34 for developed larvae, as well as sugar and glycogen for which n = 20 for adult offspring). Protein, sugar, glycogen and lipid content were determined sequentially for the same individual. In short, 130 µl of aqueous lysis buffer solution and a stainless steel bead were added to each sample. Samples were homogenized using a bead homogenizer at 25 Hz for 1 min, and centrifuged (500*g* at 4 °C) for 5 min. Protein levels were determined using 2.5 µl (developed larvae) or 5 µl (adult) supernatant placed in a 96-wells microplate. 250 µl Bradford reagent was then added to each sample, and after 5 min absorbance was read at 595 nm. After protein quantification, 5 µl aqueous lysis buffer solution, 14 µl 20% sodium sulphate solution and 1080 µl of a chloroform:methanol solution (1:2 v/v) were added to each sample. Samples were then vortexed and centrifuged (200*g* at 4 °C) for 15 min to separate glycogen from the supernatant. To determine sugar levels, 150 µl of supernatant was transferred to a 96-well borosilicate microplate and left at room temperature until 10 µl of supernatant remained. 240 µl of Anthrone reagent was then added and absorbance read at 625 nm after samples were heated for 15 min in a water bath (90 °C) and subsequently cooled

Table 1
Results of Wilcoxon rank sum tests to compare protein, sugar, glycogen and lipid between young and old mothers (A), and linear models for all offspring life stages (B) and early (C) and late (D) life stages separated. Interaction represents the interaction between the factors life stage and mother.

Nutrient type	Protein			Sugar			Glycogen			Lipid		
Factors												
A- Mothers		*w*	*p-value*		*w*	*p-value*		*w*	*p-value*		*w*	*p-value*
Mother		373.5	**0.02**		447	**< 0.001**		514	**< 0.001**		526	**< 0.001**
B- All life stages	*df*	*F*	*p-value*	*df*	*F*	*p-value*	*df*	*F*	*p-value*	*df*	*F*	*p-value*
Life stage	4	4056.56	**< 0.001**	4	1758.51	**< 0.001**	4	467.61	**< 0.001**	4	3968.98	**< 0.001**
Mother	1	0.83	0.36	1	8.22	**0.005**	1	14.28	**< 0.001**	1	6.84	**0.01**
Interaction	4	1.99	0.10	4	3.30	**0.01**	4	1.79	0.13	4	5.34	**0.005**
C- Early life stages	*df*	*F*	*pvalue*	*df*	*F*	*pvalue*	*df*	*F*	*p-value*	*df*	*F*	*p-value*
Life stage	2	0.78	0.46	2	7.23	**0.002**	2	12.34	**< 0.001**	2	10.61	**< 0.001**
Mother	1	0.13	0.72	1	9.56	**0.003**	1	0.002	0.97	1	18.35	**< 0.001**
Interaction	2	4.61	**0.01**	2	3.68	**0.03**	2	0.29	0.75	2	4.68	**0.01**
D- Late life stages	*df*	*F*	*p-value*	*df*	*F*	*p-value*	*df*	*F*	*p-value*	*df*	*F*	*p-value*
Life stage	1	180.32	**< 0.001**	1	1.65	0.20	1	12.08	**< 0.001**	1	50.37	**< 0.001**
Mother	1	0.62	0.43	1	0.23	0.63	1	22.06	**< 0.001**	1	0.04	0.83
Interaction	1	3.30	0.07	1	1.15	0.29	1	1.74	0.19	1	0.06	0.81

Significant differences are highlighted in bold.

on ice. For estimating glycogen content, glycogen residue was rinsed twice by adding 400 µl 80% methanol, which was then vortexed and centrifuged for 5 min (200*g* at 4 °C). 1 mL of Anthrone reagent was added to each sample, after which it was vortexed and heated for 15 min in a water bath (90 °C), cooled on ice and filtered. 250 µl was transferred to a 96-well borosilicate microplate and absorbance read at 625 nm. Lipid levels were determined by placing 200 µl of supernatant in a 96-wells borosilicate microplate, which completely evaporated by placing the microplate in a water bath (50 °C). 17.5 µl of 98% sulfuric acid was then added and the microplate placed in a water bath (90 °C) for 2 min. The microplate was then cooled on ice, after which 235 µl of Vanillin reagent was added and samples shaken slowly for 15 min before absorbance was read at 525 nm.

2.5. Notes on the experimental design

The experimental design was based on that described in Giron and Casas (2003), where the oviposition rank of each egg of 70 *E. vuilleti* mothers was tracked, functioning as a measure of physiological age rather than chronological age (see Ligout et al., 2012 and Levine, 2013 for arguments on the use of physiological age). Biochemical analyses described here were similarly constrained as a pool of 5 (protein) or 10 (sugar, glycogen, lipid) eggs or hatched larvae were required to determine nutrient levels for each sample. Each mother could thus have contributed more than one egg to different samples. Pooling of eggs/hatched larvae prohibits the use of a statistical design that takes into account this type of pseudo-replication. We have, therefore, used a substantially higher number of mothers (i.e. 312). In our experimental design a mother could contribute only a single egg (or hatched larva) per pool (i.e. sample; Fig. 1B); hence there was no artificial increase in sample size (or degrees of freedom) through potential pseudo-replications. By using the mean nutrient content (in µg per individual) for each pool of eggs or hatched larvae obtained from different mothers we thus corrected for the supernumerary degrees of freedom. If a mother contributed to more than one sample, there may be a correlation between treatments (offspring of young vs old mothers at different times during development). These correlations should, however, reduce the variance between treatment modalities, which could actually mask the potential differences we aim to detect. We have included Appendix A to provide an overview of the number of times a mother contributed to different pools of each treatment.

Due to the small size and vulnerability of the smaller life stages, mass could not be determined for individuals used for biochemical analyses, but mass data were collected for a set of separate individuals (Appendix B). The only difference in mass between offspring of young and old mothers was found for fresh eggs, but these differences in mass were not due to a difference in size, as egg volume was similar for fresh eggs laid by young and old mothers. We, therefore, did not correct for weight and used absolute nutrient quantities, in line with all previous work done on *E. vuilleti* (Giron and Casas, 2003; Casas et al., 2005, 2015; Giron et al., 2004, 2002

2.6. Statistics

Wilcoxon rank sum tests were performed to test for differences in nutrient levels (protein, sugar, glycogen, and lipid) between young and old adult mothers. To test for differences in nutrient levels in offspring, linear models (LMs) were used with life stage (egg to adult), mother (young and old) and their interaction as factors. Due to major size differences between early and late life stages, a highly significant effect of life stage was found for all nutrients. To take size differences into account, LMs were further performed on two separate datasets, one only including the smaller, early developmental stages (eggs, hatched larvae) and another only including the later developmental stage (developed larvae) and emerged adults. Data that did not meet the assumptions of residual normality and homoscedasticity were log transformed. Reported P values were those of the saturated models or the simplified models when factors and/or interactions were non-significant. All analyses were performed using R Project 3.1.0 (R Core Team, 2014).

3. Results

3.1. Nutrient levels in mothers

The quantity of all nutrient types was significantly lower in old compared to young mothers (Table 1; Fig. 2). Over time, old mothers used 17% of their original protein stores, 36% sugars, 70% glycogen and 77% lipids compared to quantities in young mothers. Protein and lipid were the predominant nutrient types, together constituting 87% and 90% of total nutrients in young and old mothers, respectively.

3.2. Nutrient dynamics in offspring

Protein – Old mothers provisioned their eggs with less protein, and protein dynamics were differentially affected in eggs and hatched larvae of young versus old mothers (Table 1; Fig. 3). Protein dynamics in offspring of young and old mothers was similar during later developmental stages (developed larvae and emerged adults)(Table 1; Fig. 4), but adult female offspring of young mothers emerged with

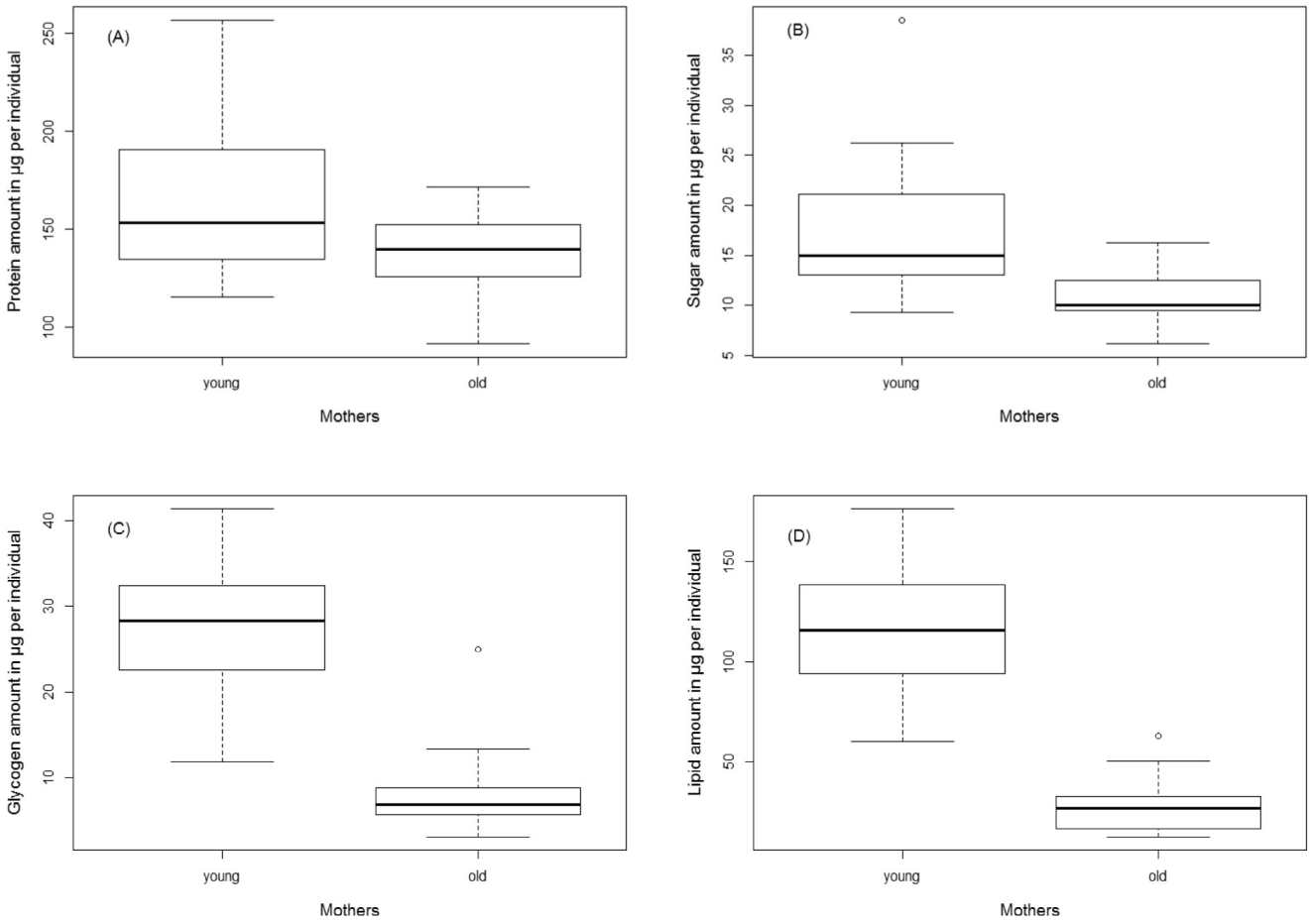

Fig. 2. Boxplots of nutrient levels in young and old mothers of the hymenopteran parasitoid *Eupelmus vuilleti*: proteins (A), sugars (B), glycogen (C) and lipids (D) in µg per individual.

higher protein levels compared to offspring of old mothers.

Sugar – Lifetime sugar dynamics were affected by age of the mother (Table 1). Old mothers allocated fewer sugars towards their eggs, levels that remained stable during early developmental stages (Fig. 3). Feeding from the host throughout development subsequently led to similar sugar levels and dynamics in developed larvae and emerged adult females (Fig. 4).

Glycogen – Despite similar provisioning and dynamics of glycogen levels in eggs and throughout early development, age of the mother affected lifetime glycogen levels differently (Table 1; Figs. 3 and 4), where developed larvae and adult female offspring of young mothers contained more glycogen.

Lipid – Lifetime lipid dynamics were differentially affected in offspring of young and old mothers (Table 1). Young mothers invested more lipids in their eggs and offspring continued to use lipid resources throughout development, unlike the offspring of old mothers where lipid levels remained stable (Table 1; Fig. 3). For offspring of young and old mothers, a similar amount of lipids was carried over by feeding from the host and adult female offspring emerged with the same quantity of lipids (Fig. 4).

4. Discussion

Maternal effects have received much attention over the years (Mousseau and Fox, 1998; Salinas and Munch, 2012), but only few studies have considered the consequences of temporal variation in a mother's internal state (Marshall and Uller, 2007; Plaistow et al., 2007). Using physiological measurements of four macronutrients we studied how temporal variation in nutrient availability of a mother affected nutrient dynamics in her offspring from egg to adult. Our results confirm that internal maternal resources diminish steeply with age and that old mothers reduce egg provisioning (Giron and Casas, 2003). We further show that age of the mother affects nutrient dynamics of her developing offspring, and that offspring of old mothers contain less resources at the reproductive adult life stage. As mothers get older, fewer resources are available for allocation into reproduction, and as a consequence old mothers invest substantially less protein, sugar and lipid into their eggs. By the time eggs have hatched, nutrient quantities reached similar levels between offspring of young and old mothers; hence the offspring of young mothers were able to utilize more nutrients at a faster rate during embryogenesis. As *E. vuilleti* is a parasitic wasp, it feeds on a host larva from hatching until pupation. The offspring of old mothers may, therefore, be able to overcome initial nutritional disadvantages by feeding from the host. This was not the case for glycogen where fully developed larvae of old mothers contained 30% less glycogen than similarly aged larvae of young mothers. Moreover, both protein and glycogen stores were 20% lower in adult female offspring at emergence, revealing that negative effects during early life extend up into the reproductive life stage. Nutrient dynamics throughout development is thus affected differently depending on age of the mother, ultimately leading the offspring of old mothers to have fewer resources available for investment into their own offspring.

Nutrients are critical for maintaining vital cell functions, particularly in the egg and developing embryo where high nutritional demands can be expected. A study by Pettersen et al. (2015) revealed that larger offspring use more energy compared to smaller offspring in two bryozoan species. Similarly, young *E. vuilleti* mothers allocate more resources into their eggs and their offspring utilize more resources during embryonic development compared to offspring of old mothers (Fig. 3). At hatching, however, offspring of young and old mothers

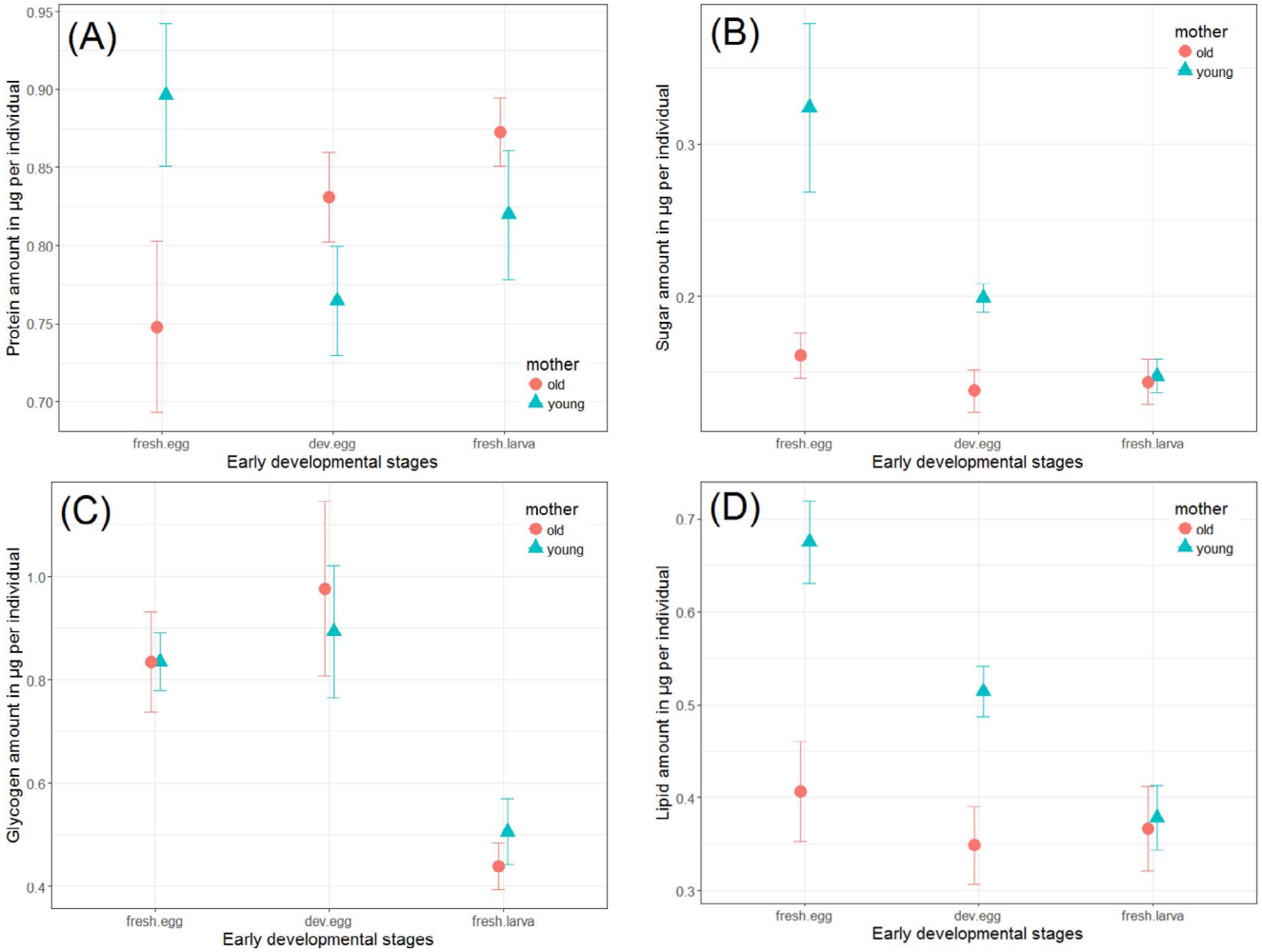

Fig. 3. Nutrient dynamics of the offspring of young and old *Eupelmus vuilleti* mothers during early development. Mean (± 1SE) level of proteins (A), sugars (B), glycogen (C) and lipids (D) in µg per individual.

show similar nutrient levels despite steep initial differences in quantity and rate of utilization. Embryos of old mothers thus seem better able to conserve nutrients during embryonic development. An explanation may be found in respiration rates. Generally, respiration rates in eggs increase as embryonic development progresses, with a steep rise in respiration after the halfway point (Woods et al., 2005; Woods, 2010). Moran and Allen (2007) showed that artificial reduction of sea urchin eggs led to proportional differences in metabolic rate, i.e. eggs that were half or a quarter the size of control eggs had metabolic rates that were approximately 50 and 75% lower. *E. vuilleti* embryos of old mothers that contain less resources may conserve nutrients in a similar fashion by lowering metabolic rate during embryonic development, an interesting topic for future studies.

Embryonic stages have been of prime interest to those studying maternal effects, but remarkably little is known about nutrient dynamics during later developmental stages. *E. vuilleti* larvae carry over a large amount of protein from their host with about 70% of all nutrients constituting protein just prior to pupation. This result is somewhat unexpected, as the majority of parasitoids are unable to synthesize lipids *de novo* (Giron and Casas, 2003; Visser and Ellers, 2008; Visser et al., 2010, 2017); hence lipids were predicted to be the predominant nutrient acquired from the host. Insect storage proteins are, however, critical for successful metamorphosis in holometabolous insects (Haunderland, 1996). During development and prior to pupation these proteins are accumulated in dense protein granules within the fat body, which can be used as amino acid resources during the pupal stage to complete development into adulthood (Haunderland, 1996). Notwithstanding the importance of capital lipid reserves, our results suggest that the carry-over of large amounts of protein may indeed be critical for metamorphosis and adult reproduction. Another interesting finding is that maternal age negatively affected glycogen levels in developed larvae, but that glycogen is provisioned equally in eggs of young and old mothers. Hosts available to mothers are of equal quality; hence offspring of both mothers should readily take over similar glycogen quantities from their host, which is completely consumed. This suggests that, prior to pupation, larvae of old mothers have used glycogen resources at a higher rate throughout development. In butterfly larvae, increased glycogen consumption during development was attributed to an increase in chitin formation, which is required for the formation of the insect's cuticle (Siegert, 1987; Muthukrishnan et al., 2012). Reduced provisioning of other nutrient types may force developing larvae of old mothers to tap into their glycogen reserves to preserve protein and lipid, while sustaining cuticle formation. The strength of resource allocation trade-offs may thus increase for offspring with lower resources available early in life.

Ageing can dramatically alter a mother's phenotype, which in turn can have major consequences for the phenotype and performance of her offspring (Hercus and Hoffmann, 2000). In *E. vuilleti* ageing reduces egg provisioning by the mother, a result similar to that found in house flies (McIntyre and Gooding, 2000). In moths, however, egg size decreased with age of the mother, but protein and lipid levels remained stable (Poykko and Manttari, 2012). Increasing maternal age can further negatively affect offspring performance. For example, parental ageing negatively affected offspring performance of *Daphnia* clones, where

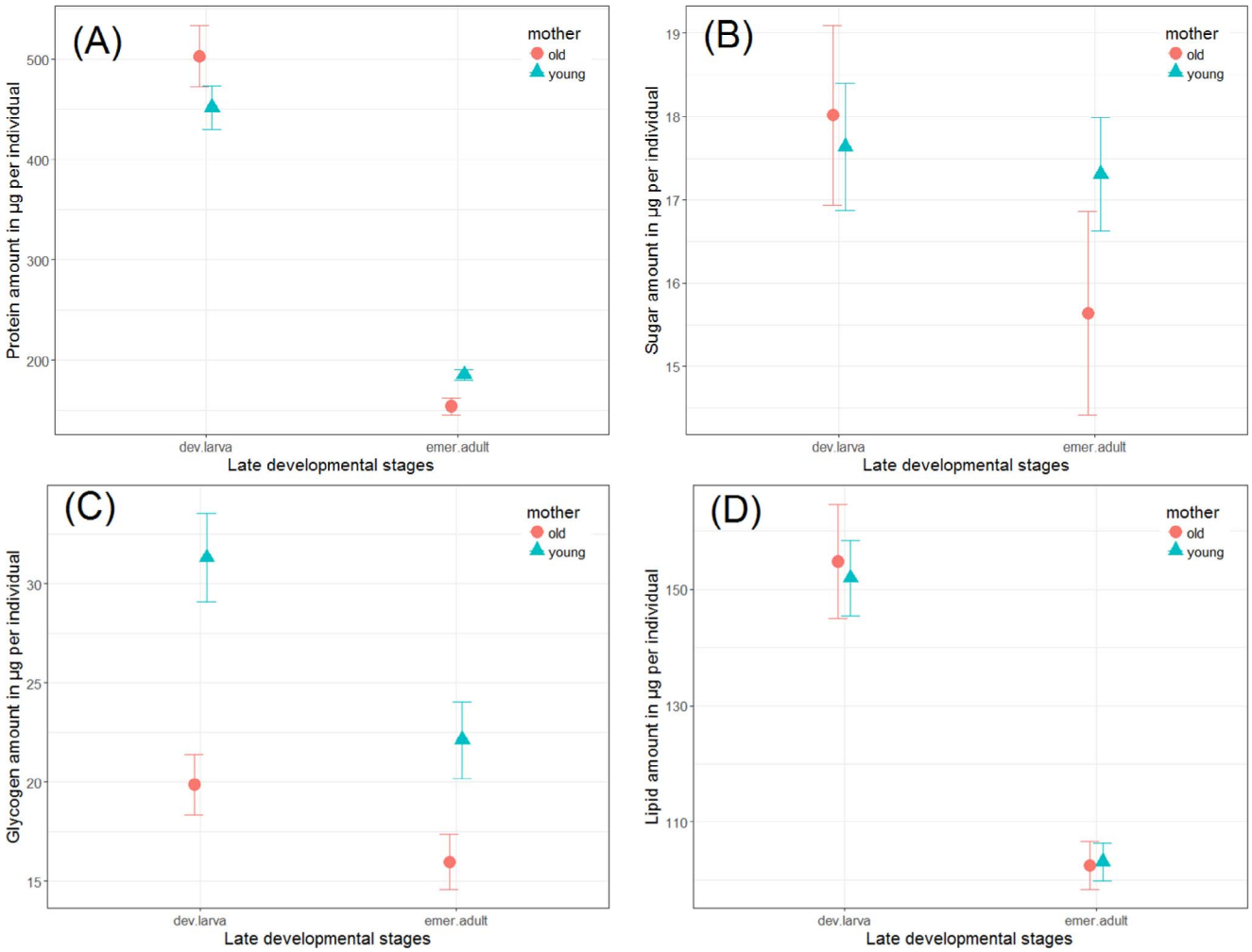

Fig. 4. Nutrient dynamics of the offspring of young and old *Eupelmus vuilleti* mothers during late development and early adult life. Mean (± 1SE) level of proteins (A), sugars (B), glycogen (C) and lipids (D) in µg per individual.

offspring of older individuals increased early-life reproduction shortening their lifespan (Plaistow et al., 2015), and late-life parental reproduction in wild birds negatively affected offspring fitness (Schroeder et al., 2015). In aphids and other hemimetabolic insects, egg size generally increases with age (Dixon et al., 1993), but in the oleander aphid *Aphis nerii* offspring of older mothers developed faster, matured at a smaller size and lived shorter (Zehnder et al., 2007). In insects, a general trend exists where offspring longevity decreases as age of the mother increases, i.e. the Lansing Effect (Lansing, 1947; Fox et al., 2003). In *E. vuilleti*, offspring of old mothers suffer from reduced egg provisioning, because adult female offspring have fewer resources available already at the onset of adult life. Maternal age may thus affect reproductive success of offspring, for instance because less glycogen is available for flight enabling dispersal (Amat et al., 2012) or because females will have less energy available to fight off potential competitors (Mohamad et al., 2015). As daughters will in turn provision their offspring with less resources, the negative consequences of reduced provisioning can extend into future generations. Maternal effects on egg nutrient provisioning may thus underlie variation in offspring performance in many other oviparous animals.

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Appendix A

Number of times a mother contributed eggs to different pools for each treatment and nutrient type.

	Young mothers				Old mothers			
	1×	2×	3×	4×	1×	2×	3×	4×
Protein Fresh egg	14	10	12	5	17	16	6	2

Developed egg	26	12	7	1	12	11	8	3
Hatched larva	8	12	11	5	6	11	15	3
Developed larva	10	11	2	0	12	5	4	0
Emerged adult	12	7	1	0	5	5	2	0
Sugar								
Fresh egg	43	10	8	3	21	12	7	1
Developed egg	31	18	5	2	8	17	8	1
Hatched larva	30	16	4	4	12	7	10	6
Developed larva	10	11	2	0	11	6	3	0
Emerged adult	12	7	1	0	5	4	1	1
Glycogen								
Fresh egg	49	14	10	3	21	12	7	1
Developed egg	31	18	5	2	8	17	8	1
Hatched larva	30	16	4	4	12	7	10	6
Developed larva	10	11	2	0	11	6	3	0
Emerged adult	12	7	1	0	5	4	1	1
Lipid								
Fresh egg	50	14	10	3	21	12	7	1
Developed egg	31	18	5	2	8	17	8	1
Hatched larva	30	16	4	4	12	7	10	6
Developed larva	10	11	2	0	11	6	3	0
Emerged adult	12	7	1	0	5	5	2	0

Appendix B

Mass data for mothers (A) and mass (and volume) data for offspring (B) for each treatment.

A- Mothers

Mother	Stage	Age	Average mass (mg)	1 s.e.	Average volume (mm3)	1 s.e.	Sample size
Young	Mother	Adult	1.8383	0.0981	–	–	10
Old	Mother	Adult	1.8098	0.0876	–	–	12
			B- Offspring				
Young	Fresh	Egg	0.0111	0.0002	0.0114	0.0002	30
Old	Fresh	Egg	0.0099	0.0002	0.0111	0.0002	20
Young	Developed	Egg	0.0085	0.0002	0.0085	0.0003	30
Old	Developed	Egg	0.0085	0.0002	0.0077	0.0002	20
Young	Hatched	Larva	0.0067	0.0005	–	–	14
Old	Hatched	Larva	0.0081	0.0013	–	–	6
Young	Developed	Larva	3.7118	0.3706	–	–	10
Old	Developed	Larva	3.9275	0.4584	–	–	10
Young	Female	Adult	2.2209	0.0527	–	–	12
Old	Female	Adult	2.1762	0.1072	–	–	10

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