

Egg composition and reproductive investment in aphidophagous ladybird beetles (Coccinellidae: Coccinellini): egg development and interspecific variation

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Abstract. Most studies of insect reproductive allocation concentrate on propagule size and number and very few consider egg composition, which is likely to be equally important. In the present study, data are provided on changes in egg lipid, glycogen, free carbohydrate and protein during embryonic development of the aphidophagous ladybird *Adalia bipunctata* (L.) and the compositions of *A. bipunctata*, *Adalia decempunctata* and *Anisosticta novemdecimpunctata* eggs are compared. In *A. bipunctata*, egg mass, lipid and glycogen decline strongly during development and egg protein declines more weakly. Free carbohydrate declines early in egg development and increases at egg hatching. Lipid is energetically the most important developmental fuel, although approximately half of the initial egg lipid remains in the neonate larva. Across the three species, energy per unit egg mass is lowest in the least specialized species, *A. bipunctata*, which also has the largest eggs, and is highest in the most specialized, *An. novemdecimpunctata*, which has the smallest eggs. Two possible explanations for the observed pattern are discussed: (i) species laying smaller eggs may incur higher developmental costs per unit mass than species laying larger eggs and (ii) more specialized species, which reproduce at lower aphid densities, may provision neonate larvae better to facilitate location and capture of aphids.

Key words. Aphid density, Coccinellidae, egg composition, embryogenesis, reproductive allocation, specialization, trade-offs.

Introduction

Insects and other arthropods are used in many studies concentrated on both inter- and intraspecific variation in the number and size of offspring, both of which are relatively easily measured (Carrière & Roff, 1995; Blanckenhorn, 2000; García-Barros, 2000; Betz & Fuhrmann, 2001; Brown, 2003; Seko & Nakasuji, 2006). In such work, it is often assumed implicitly that reproductive biomass, measured as the product of offspring size and number, is proportional to maternal energetic investment within or across species. However, offspring, and notably eggs, vary also in their composition (Diss *et al.*, 1996; Graeve & Wehrmann, 2003) and this is likely to

be important in defining maternal investment (Williams, 1994; Bernardo, 1996; Fox & Czesak, 2000). In spite of this evidence, detailed measurements of egg composition remain rare in studies of reproductive investment in insects compared with works on egg size and number. Only in recent years have physiological studies of egg composition (Needham, 1931; Babcock & Rutschky, 1961; Van Handel, 1993) included work concentrating on reproductive investment from an evolutionary or ecological viewpoint (Diss *et al.*, 1996; McIntyre & Gooding, 2000; Giron & Casas, 2003). It thus remains difficult to make clear generalizations about the ecological and evolutionary implications of egg compositional differences in insects as well as other invertebrates.

Within arthropods, it is now clear that egg composition may vary intraspecifically due to genetic, intrinsic and environmental factors (Fox & Savalli, 2000; Arcos *et al.*, 2003, 2004; Giron & Casas, 2003). Intraspecific differences in egg

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water content and composition have already been invoked to explain apparent weaknesses of genetic trade-offs based around egg size or number (Fischer *et al.*, 2006; Karl *et al.*, 2007) and the same is likely to be true for phenotypic trade-offs. However, although intraspecific compositional differences are becoming increasingly well-characterized, there remain virtually no complementary comparisons of closely-related taxa, which are also likely to display compositional variation (Graeve & Wehrmann, 2003). This is a significant oversight. Interspecific variation may not only reflect the observed effects within species, but also ecological differences between species, and, furthermore, egg nutrient content, may be important in interspecific phenotypic trade-offs. Cross-species comparisons have much to contribute in explaining how egg nutrient composition evolves and how compositional changes interact with other life-history characters.

In the present study, changes over development and interspecific variation in egg composition are considered for one tribe of ladybird beetles (Coccinellidae: Coccinellini). A considerable amount has already been published on the reproductive strategies of the Coccinellini, which comprise the majority of well-known aphidophagous coccinellids. Females lay relatively large numbers of small eggs to mitigate the risks of egg predation (Agarwala & Dixon, 1993). Egg size is considered to be constrained by the ability of small neonate larvae to capture aphid prey and, in the case of larger species, by egg to adult development time (Stewart *et al.*, 1991, but see also Sloggett, 2008a). Across species, females are assumed to allocate the same proportion of biomass to reproduction (gonads) and there is an interspecific trade-off between egg size and number across species, mediated by the number of ovarioles that females possess (Stewart *et al.*, 1991). Intraspecifically, although eggs from different females vary to some extent in size, it is egg number rather than egg size that varies in relation to available food (Dixon & Guo, 1993). More recently, Perry & Roitberg (2005) have argued that nonhatching eggs, which are ubiquitous, at least in the Coccinellini, constitute adaptive trophic eggs (i.e. a maternal means of providing additional nutrition to larvae from the same clutch, which eat them before dispersal).

The present study provides baseline data on changes in egg lipid, glycogen, free carbohydrate (i.e. sugars) and protein during development in *Adalia bipunctata* (L.). Egg composition in this and two other species, *Adalia decempunctata* and *Anisosticta novemdecimpunctata* is compared; these results are linked to their development and modes of life.

Materials and methods

Ladybirds and egg collection

Adalia bipunctata adults were collected in Bayreuth, Germany (49.93°N, 11.58°E); some additional adults from laboratory rearings of larvae [on the pea aphid, *Acyrtosiphon pisum* (Harris), reared on field beans, *Vicia faba* (L.)] from the wild-collected adults were also used. *Adalia bipunctata*

eggs used in the study originated from approximately 60 different females. The other species used were *Adalia decempunctata* (L.) (26 females), also collected in Bayreuth, and *Anisosticta novemdecimpunctata* (L.) (12 females) collected in Cambridge, England (52.21°N, 0.10°E). *Adalia bipunctata* is a generalist species occurring on a wide variety of herbaceous plants, shrubs and deciduous trees, whereas *A. decempunctata*, its sibling species, is more specialized, generally occurring only on shrubs and deciduous trees (Honěk, 1985; Hemptinne & Naisse, 1988; Majerus, 1994a). *Anisosticta novemdecimpunctata* is the most specialized of all, being largely restricted to *Phragmites* and *Typha* reed beds (Majerus, 1994a; Klausnitzer & Klausnitzer, 1997).

Ladybirds were sexed under CO₂, using the criteria of Randall *et al.* (1992); all ladybirds were also demonstrated to be free of *Coccipolipus* mite infection, which affects host egg viability deleteriously (Webberley *et al.*, 2004). Females of *A. bipunctata* were maintained in groups of four to five with three to four males in 140-mm diameter Petri dishes or individually in 90-mm diameter Petri dishes; females of the other species were all maintained individually. Individual females were mated every 2–3 days, which is sufficient to maintain high egg fertility (Majerus, 1994b). A sample of eggs was checked regularly to ensure that the eggs being collected exhibited high viability. In all studies, ladybirds were maintained at 20 °C under an LD 16 : 8 h photoperiod and fed daily on a diet of excess pea aphids with small pieces of apple provided additionally as a fluid source. All three species reproduce well on this aphid in the laboratory, with no obvious ill-effects (El-Hariri, 1966; Majerus & Kearns, 1989; Stewart *et al.*, 1991; Tinsley & Majerus, 2006), although this species is only a likely natural prey for *A. bipunctata*. Ladybirds were provided with fresh Petri dishes and food every day and eggs were collected from the old Petri dishes. Eggs were laid typically onto the surface of the Petri dish: for preservation, they were removed carefully as a whole using a mounted needle. Hatching larvae were also removed from egg clutches using a needle.

Changes in egg composition during A. bipunctata egg development

To examine changes in egg composition during development in *A. bipunctata*, eggs were either preserved for analysis immediately (day 1 eggs: less than 24 h old) or allowed to develop further for 24, 48 or 72 h (day 2, 3 and 4 eggs respectively) prior to being preserved. At 20 °C, eggs of *A. bipunctata* took 96–100 h to hatch. Hatching larvae were also collected and preserved before any cannibalism of nonhatching eggs occurred within egg clutches (Banks, 1956; Majerus, 1994a).

Species comparison

To compare the composition of eggs of different species, eggs less than 24 h old were also collected from *A. decempunctata* and *An. novemdecimpunctata*: these were compared with *A. bipunctata* eggs of the same age (i.e. day 1 eggs).

Weighing and preservation of eggs and quantification of compositional components

Groups of eggs were placed in a 1.5 mL Eppendorf safe-lock reaction tube with 20 mg Na₂SO₄ and 200 µL 75% MeOH in water. The number of eggs/larvae was recorded and the total egg/larval mass obtained by weighing the tube with reagents prior to and after addition of the material to the nearest 0.01 mg. Eggs were then stored frozen at -75 °C prior to analysis. Between 10.16 mg and 16.49 mg of eggs or larvae were preserved per reaction tube (i.e. 79 and 141 individuals per tube), dependent on the species and developmental stage. In a number of cases, it was necessary to add eggs to the reaction tube more than once, to obtain sufficient material for analysis. If this occurred, the reaction tube was allowed to warm to room temperature before being weighed and further eggs or larvae added, to avoid increasing the water content of the material within the tube through condensation. Numbers of individuals added and mass change of the reaction tube was recorded each time material was added; thus mass changes over *A. bipunctata* development and masses for eggs of the different species are calculated from a larger number of samples than the number of samples analysed for egg/larval composition.

The extraction and separation of lipid, protein, glycogen and free carbohydrate (sugars) in the samples was by a method modified after Speck & Urich (1969) and Van Handel (1965); for details, see Lorenz (2003, 2004). The colorimetric quantification of lipid, glycogen and free carbohydrate was performed as described (Lorenz, 2003) using modified sulphophosphovanillin and anthrone methods. Protein was estimated with an EL 808 Ultra Microplate Reader (Bio-Tek Instruments, Bad Friedrichshall, Germany) using the RotiQuant Universal assay (Roth, Germany) and bovine serum albumin as a standard. To monitor the efficiency of extraction and the separation into substance classes, blank samples (containing 20 mg of Na₂SO₄ and 200 µL of 75% MeOH) and internal standards (additionally containing 4 mg of cholesterol, 1 mg of bovine serum albumin, 100 µg of glycogen and 100 µg of glucose) were carried through the extraction and separation process along with the egg samples. From these blanks and internal standards, the recovery rates for the different substance classes were calculated and used to correct the values obtained for the egg samples (for details see Lorenz, 2003).

Analyses of data

Each sample (i.e. the eggs collected in a single Eppendorf) comprised the pooled eggs of a number of different female individuals. All analyses relating to the composition of eggs used these samples as replicates. Analyses of egg mass used individual subsamples of eggs as replicates (i.e. values taken every time a group of eggs was weighed; see above). Results are presented as mean ± SE. It should be noted that the standard errors given represent between-Eppendorf or between-weighing variation, due to the pooling of eggs from different females in samples.

In analyses of the individual compositional components, data for changes in egg composition over development to the larva were expressed per egg (i.e. mass of each component or total energy in each Eppendorf sample divided by the total number of eggs or larvae in that Eppendorf). This provides a picture of total compositional changes in the individual propagule. By contrast, comparisons of the egg compositional components across species were expressed as values obtained per mg fresh mass of eggs (i.e. mass of each component or total energy in each Eppendorf sample divided by the total mass of eggs or larvae in that Eppendorf). This is more informative than values per egg, which can simply reflect egg size. As all samples from both experiments were quantified in the laboratory at the same time, the same pooled group of samples from *A. bipunctata* were used as day 1 of the developmental analysis and for *A. bipunctata* in the cross species analysis: the larger sample sizes there reflect this fact.

Changes over development in egg mass, egg lipid, glycogen, free carbohydrate and protein were analysed using linear and quadratic regression. The final choice of regression (linear or quadratic) was based on the significant regression giving the highest adjusted *r*² value when linear and quadratic regressions were compared. Larvae, which will naturally be lighter due to loss of the egg chorion and vitelline membrane, were not included in regression analysis of mass changes. Regression was carried out using the median time in hours for each egg collection period (day 1, 12 h; day 2, 36 h; day 3, 60 h; day 4, 84 h) and a value of 100 h (i.e. the maximum time to egg hatch) for larvae. To examine energetic costs of development, we used energy conversion factors of 16.0 kJ g⁻¹ for protein and carbohydrate and 37.5 kJ g⁻¹ for lipid (Adrian *et al.*, 1988).

Species differences in lipid, glycogen, free carbohydrate and protein concentrations were each compared using a one-way analysis of variance (ANOVA), with Holm-Šidák post-hoc testing between species. The relationship between total energy concentration, egg size and total energetic content per egg was also analysed, based on the energy conversion factors. Energy concentration in the three species was analysed using ANOVA and post-hoc tests as previously described. However, to compare the egg masses of the three species and energetic content per egg median tests, post-hoc partitioning of samples, when required, were used due to failed normality and equality of variance tests (Siegel & Castellan, 1988).

Analyses were carried out using SIGMASTAT, version 3.10 (Systat Software, Inc., San Jose, California) and SPSS 8.0 for Windows (SPSS Inc., Chicago, Illinois), except median tests, which were carried out by hand.

Results

Changes in egg composition during A. bipunctata egg development

Data on compositional and mass changes through *A. bipunctata* egg development to the first instar larva are shown in Figure 1. Individual egg mass declined during

development (days 1–4: $F_{1,28} = 24.3$, $P < 0.001$). Both of the main energy storage compounds, lipid and glycogen, declined also throughout development to the larval stage (day 1 larva, lipid: $F_{1,33} = 85.8$, $P < 0.001$; glycogen: $F_{1,33} = 192.6$, $P < 0.001$). The decline in glycogen was particularly marked: only 7% of day 1 egg glycogen occurred in the first-instar larva. By contrast, lipid in the larva was 48% of that in a day 1 egg. The regression curve for free carbohydrate throughout egg development was weakly U-shaped (day 1 larva: $F_{2,32} = 3.35$, $P < 0.05$), with an apparent decline in free carbohydrate in the young egg and an increase in the hatching larva. There was also a weak decline in protein during development (day 1 larva regression: $F_{1,33} = 4.49$, $P < 0.05$).

Based on the differences between day 1 eggs and neonate larvae, during development an egg used $6.74 \pm 0.19 \mu\text{g}$ ($= 252.8 \pm 7.2 \text{ mJ}$) of lipid, $2.12 \pm 0.03 \mu\text{g}$ ($= 33.9 \pm 0.5 \text{ mJ}$) of glycogen and $1.38 \pm 0.21 \mu\text{g}$ ($= 22.1 \pm 3.4 \text{ mJ}$) of protein. Thus, although glycogen reserves were depleted almost entirely, lipid was energetically the most important fuel (81% of energy used).

Species comparison

Compositional data for the three species analysed are shown in Figure 2. Egg lipid concentration differed between the three species with lipid concentration lowest in *A. bipunctata*, intermediate in *A. decempunctata* and highest in *An. novemdecimpunctata* (one-way ANOVA: $F_{2,33} = 44.7$, $P < 0.001$; Fig. 2A). Egg glycogen concentration did not differ between the two *Adalia* species but was also higher in *An. novemdecimpunctata* (one-way ANOVA: $F_{2,33} = 10.7$, $P < 0.001$; Fig. 2B). For free carbohydrate, the difference between *A. decempunctata* and *An. novemdecimpunctata* was significant, but *A. bipunctata*, which was intermediate, did not differ significantly from either (one-way ANOVA: $F_{2,33} = 5.3$, $P < 0.02$; Fig. 2C). Egg protein concentration was significantly lower in the generalist *A. bipunctata* than in either of the other species, which exhibited very similar values (one-way ANOVA: $F_{2,33} = 8.0$, $P < 0.001$; Fig. 2D).

The relationship between energy concentration (i.e. per unit mass), egg mass and energy per egg for the three species is shown in Figure 3. When all contents were considered together, total energy concentration significantly different for all three species, being lowest in *A. bipunctata* and highest in *An. novemdecimpunctata* with *A. decempunctata* intermediate between the two (one-way ANOVA: $F_{2,33} = 30.9$, $P < 0.001$; Fig. 3A). The eggs of all three species differed significantly in mass from each other (median test, due to failed normality and equality of variances, $\chi^2 = 38.7$, 2 d.f., $P < 0.001$; Fig. 3B). The largest eggs were those of *A. bipunctata*, the largest species; those of *A. decempunctata* were intermediate and those of the smallest species, *An. novemdecimpunctata*, were smallest. However, the estimated energetic content of individual eggs did not differ significantly between species at the conventional 5% level, though was close to significance (median test, due to failed equality of variances, $\chi^2 = 5.4$, 2 d.f., $0.10 > P > 0.05$; Fig. 3C).

Discussion

After oviposition in *A. bipunctata*, there is strong and consistent decline in both lipid and glycogen, although energetically lipid is by far the most important embryonic fuel, as is the case in other insects (Allais *et al.*, 1964; Van Handel, 1993). The main function of glycogen is probably to provide sugars for cuticle synthesis (Zaluska, 1959) and virtually no glycogen remains in the neonate larva. However, a lipid energy resource of approximately half that recorded in day 1 eggs remains available to the larva after hatching. After dispersing from their egg clutches, the tiny neonate larvae of aphidophagous ladybirds face considerable difficulty in locating and catching their first aphid prey (Banks, 1954, 1957; Dixon, 1959) and the lipid remaining in the larva probably facilitates survival after dispersal, but prior to catching their first aphid. Free carbohydrate appears to decline initially in egg development and remains constant until the egg hatch when it increases again. Its mobilization from energy storage compounds reflects immediate short term needs, which appear to be relatively constant for much of development. The mobilization of free carbohydrate in the larva is indicative of the high imminent costs of foraging. Protein, which may have an energy-storage role as well as a structural one, declines weakly over development, and is the least important energy source: that which is lost may represent protein loss during conversion from yolk protein (vitellin) to larval protein (but see also Van Handel, 1993). Some of the protein remaining in the larva may serve an energy-storage function similar to that of lipid, rather than a structural one: length of survival in gypsy moth (*Lymantria dispar*) caterpillars is positively correlated with the levels of vitellin and glycine rich egg yolk proteins (Diss *et al.*, 1996), indicating that protein can form a significant energy source for hatching insect larvae.

A tacit assumption of many studies concentrated on propagule size or number is that biomass is an accurate reflection of energetic content. At some level, this is clearly true: a chicken's egg contains more energy than a *Drosophila* egg. However, there is no good evidence for isometry between egg size and composition within species. In insects, Giron & Casas (2003) show that older females of the parasitoid *Eupelmus vuilleleti* lay eggs that are not only smaller, but differ in their chemical composition from younger females' eggs. Similarly, Fischer *et al.* (2006) and Karl *et al.* (2007) report differences in water content and composition in size-selected eggs in the butterfly *Bicyclus anynana*. The data in the present study indicate that isometry between egg size and composition also does not occur interspecifically: energy concentration increases across species with decreasing egg size making the energy content of all species' eggs similar in spite of significant differences in egg size. This is particularly noteworthy for the very closely related *A. bipunctata* and *A. decempunctata*, which are sibling species (Iablokoff-Khnzorian, 1982) that can be hybridized in the laboratory (Ireland *et al.*, 1986). Overall, the eggs of aphidophagous ladybirds develop rapidly (Dixon, 2000) and species with smaller eggs appear to hatch faster than those with larger

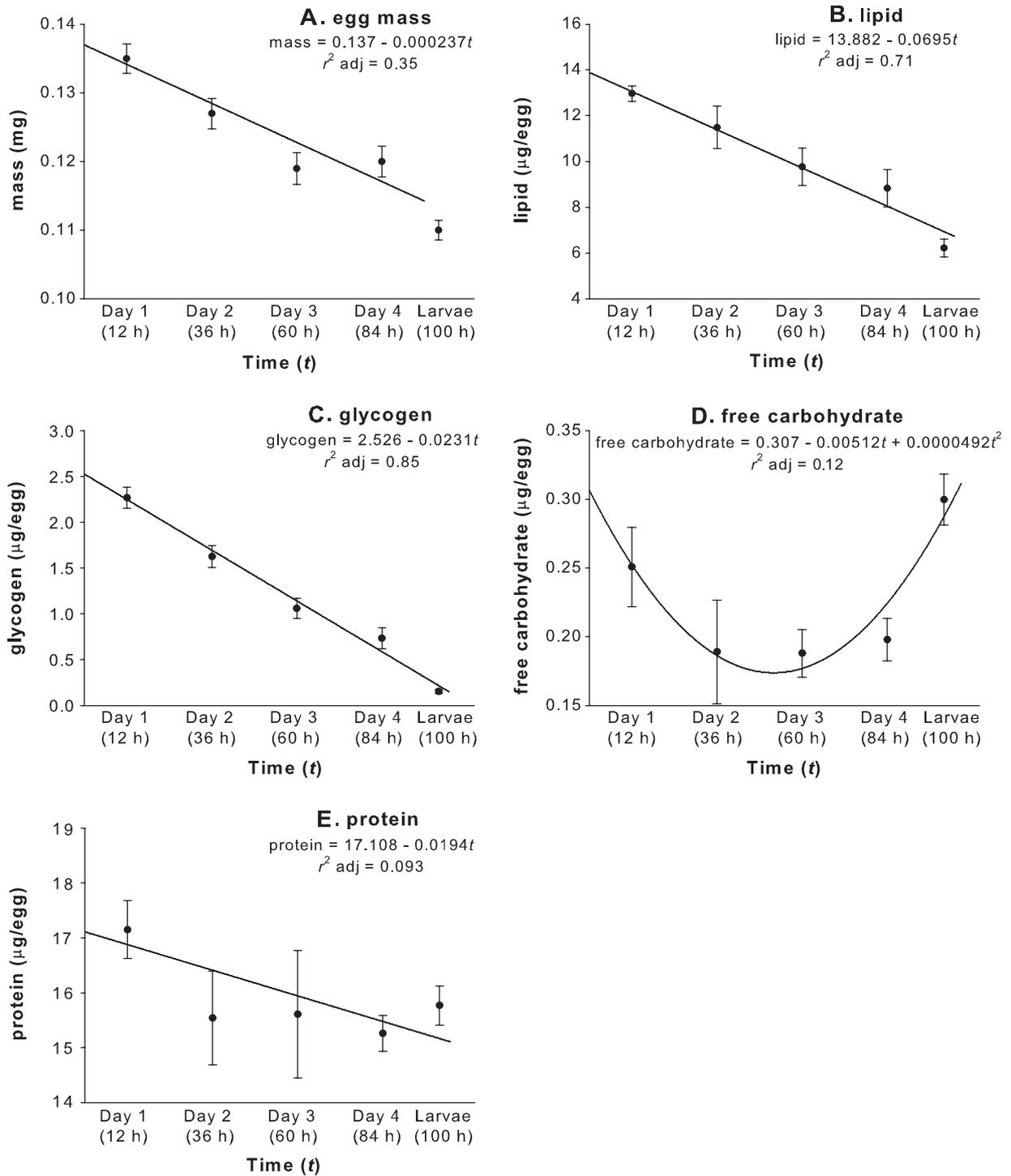


Fig. 1. Changes in *Adalia bipunctata* mass and composition (per egg) during development of the egg and in the neonate larva. Linear or quadratic regressions are shown, based on the significant regression giving the highest adjusted r^2 value. For all compositional data, day 1, $n = 15$, for all other samples, $n = 5$. For egg mass data, day 1, $n = 24$; days 2 and 4, $n = 7$; day 3, $n = 6$; larvae, $n = 13$. (A) Egg mass: regression shown does not include the larval data (see text). (B) Lipid. (C) Glycogen. (D) Free carbohydrate. (E) Protein.

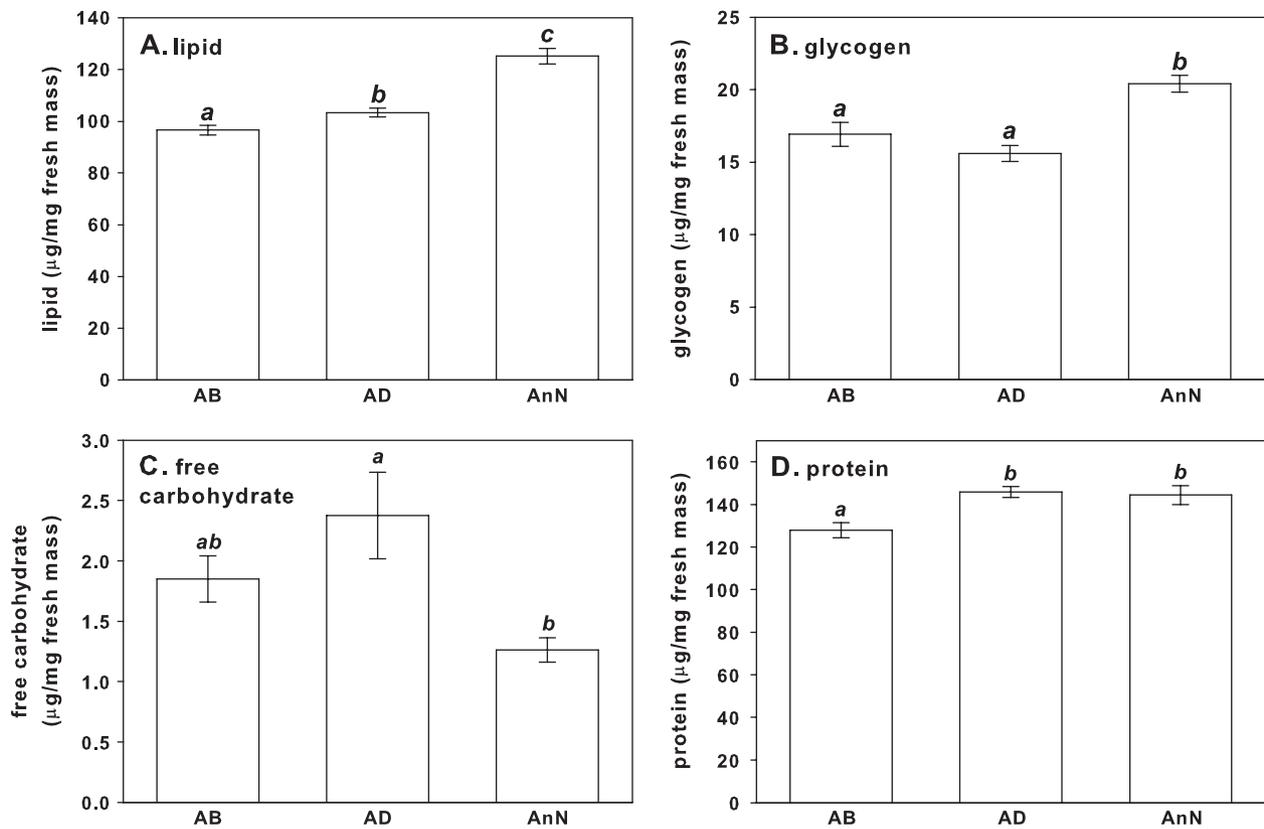


Fig. 2. Composition (per unit mass) of < 24-h-old eggs of *Adalia bipunctata* (AB, $n = 15$ samples), *Adalia decempunctata* (AD, $n = 10$) and *Anisosticta novemdecimpunctata* (AnN, $n = 11$). For each graph, bars with different letters above them differ significantly in Holm–Šidák post-hoc testing after one-way analysis of variance. (A) Lipid. (B) Glycogen. (C) Free carbohydrate. (D) Protein.

eggs (J. J. Sloggett, personal observation). If this is the case, it is unlikely that the higher concentration of nutrients in smaller eggs results from fixed costs of respiration associated with longer development times.

However, smaller eggs might require more energy per unit mass for some other aspect of development. As an example, cuticle is energetically costly: due to the higher surface to volume ratio of small larvae, proportionately more cuticle is required by them; thus potentially the energetic costs of producing cuticle are higher per unit mass for small larvae. Such a relationship between egg size and developmental energetic costs is consistent with Fischer *et al.* (2006) and Karl *et al.* (2007) who report increased water per unit mass and marginally, although not significantly, lower energetic investment per unit mass in *B. anynana* lines selected for larger egg size. Similarly, Giron & Casas (2003) suggest that a minimum amount of lipid was required for egg development in *E. vuilletti*, leading to enhanced lipid allocation per unit mass to the smallest eggs. Further work is required, however, to fully elucidate developmental energetic allocation in eggs of different sizes and to clarify the energetic relationship between egg size and development across species.

Differences in egg composition per unit mass are also likely to reflect differences in energetic allocation to larvae

after hatching. This in turn is likely to reflect differences in larval ecology. Within comparable habitats, more specialized ladybirds persist and reproduce at lower aphid densities than generalists (Sloggett & Majerus, 2000; Sloggett, 2008b). This clearly implies an overall association between increasing specialization and decreasing minimum aphid density required to sustain a high reproductive rate. Of the three ladybirds compared in the present study, the more specialized *A. decempunctata* is known to exploit lower aphid densities than its sibling species *A. bipunctata* (Honěk, 1985), and it is highly likely that the stenotopic *An. novemdecimpunctata* exploits even lower aphid densities. In general, ladybird larvae possess very limited sensory capabilities for finding aphid prey, which are only detected on contact, and searching for prey is nondirectional (Banks, 1954, 1957); additionally, first-instar larvae are extremely ineffective aphid predators due to their small size (Dixon, 1959). For specialists, the problem of locating and ultimately capturing an aphid after dispersal from the egg clutch is likely to be intensified by the lower aphid densities that specialist larvae experience. The encounter rate between predator and prey will be lower for these species than for generalists, making the average time between hatching and first eating an aphid longer. For this reason, it is expected that females of more specialized

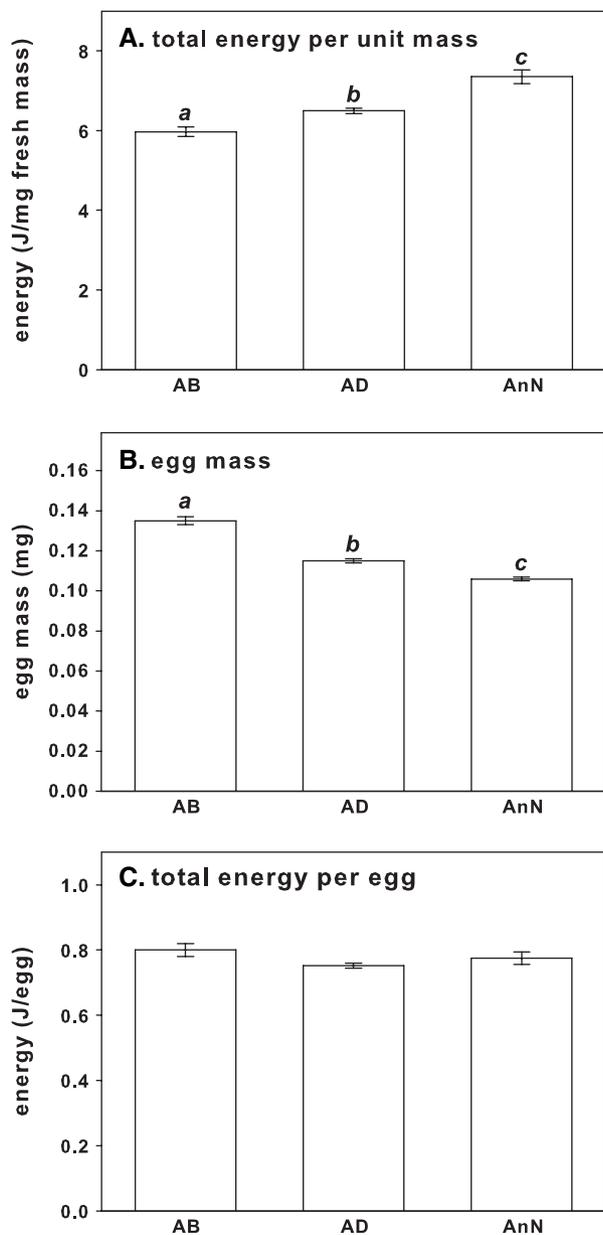


Fig. 3. The relationship between (A) estimated concentration of energy, (B) egg mass and (C) the energy content per egg for the three ladybird species analysed. AB, *Adalia bipunctata*; AD, *Adalia decempunctata*; AnN, *Anisosticta novemdecimpunctata*. For (A) and (C), sample sizes are the same as in Figure 2; for (B), $n_{AB} = 1924$; $n_{AD} = 15$; $n_{AnN} = 22$. Within graphs, bars with different letters above them differ significantly in Holm-Šidák post-hoc testing after one-way analysis of variance (A) or in post-hoc partitioning after a median test (B).

species will provision larvae better to decrease mortality from starvation, which is apparently intense, even in young larvae of generalists (Kirby & Ehler, 1977; Obrycki *et al.*, 1997). The higher energy concentrations observed with increasing specialization are consistent with this hypothesis.

Extra lipid could compensate for longer foraging times for aphids that neonate larvae of specialists experience. The same could also be true for both glycogen and, notably, protein (see above).

For the three species investigated in the present study, egg size decreases as specialization increases. Thus, on the basis of the data obtained, it is difficult to distinguish whether egg provisioning is related to high costs associated with development of small eggs or because more specialized ladybird species provision their larvae better. Both factors are likely to contribute to overall egg composition, especially as *An. novemdecimpunctata* eggs appear to contain marginally more overall energetic content per egg (albeit nonsignificantly) than the larger *A. decempunctata* eggs. In ladybirds, there is a relationship between adult (and possibly egg) size and dietary specialization, with extreme specialists tending to be smaller or larger than generalists, which are medium sized (Sloggett, 2008a). The occurrence of both large and small specialists makes this group of considerable value in teasing apart the effects of pre- and post-hatching energetic costs on interspecific variation in egg composition.

A limitation of the present study is that it is not possible to examine intraspecific differences between the eggs of individual females, nor between eggs in a clutch. It is probable that some variation exists between the eggs of individual females, as is the case for egg size (Dixon & Guo, 1993). Intraclutch variation seems less likely, although it cannot be ruled out. Yasuda & Dixon (2002) show that male and female eggs of *A. bipunctata* do not differ in size and their arguments may also be applied to sex-related differences in egg composition. Perry & Roitberg (2005) suggest that the non-hatching eggs of ladybird clutches, which are consumed by larvae from eggs that do hatch, are adaptive trophic eggs that potentially might differ compositionally from viable eggs. If different ladybird species possessed different proportions of such trophic eggs, this might lead to the measured interspecific differences in egg composition. However, overall, the evidence for adaptive trophic eggs in ladybirds remains very limited and alternative perspectives abound (Lusis, 1947; Blackman, 1967; Hurst, 1993; Majerus, 1994a, b; Mishra & Omkar, 2004; Webberley *et al.*, 2004; Roy *et al.*, 2007). It is, however, worth noting that whether nutrients are heterogeneously or homogeneously distributed with egg clutches, net parental investment, and arguably its ecological rationale, remain the same.

The present study of ladybird egg composition provides an insight into the potential developmental, ecological and evolutionary implications of this factor for studies on reproductive allocation in insects and other arthropods. The limited work in this area largely reflects the difficulty of such analyses in comparison to studies on fecundity and clutch or propagule size. Nonetheless, relatively simple techniques are now available to simply and comprehensively analyse the composition of insect eggs (Van Handel, 1993; Giron *et al.*, 2002; Lorenz, 2003); these now render such studies easier, so that compositional parameters may be better incorporated into ecological and evolutionary models of reproduction.

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