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Small body size in an insect shifts development, prior to adult eclosion, towards early reproduction

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Life-history theory has suggested that individual body size can strongly affect the allocation of resources to reproduction and away from other traits such as survival. In many insects, adults eclose with a proportion of their potential lifetime egg production that is already mature (the ovigeny index). We establish for the solitary parasitoid wasp *Aphaereta genevensis* that the ovigeny index decreases with adult body size, despite both initial egg load and potential lifetime fecundity increasing with body size. This outcome is predicted by adaptive models and is the first unequivocal intraspecific demonstration. Evidence suggests that a high ovigeny index carries a cost of reduced longevity in insects. Our results therefore contribute to the emerging evidence that small body size can favour a developmental shift in juveniles that favours early reproduction, but which has adverse late-life consequences. These findings are likely to have important implications for developmental biologists and population biologists.

Keywords: adaptation; ecological developmental biology; Hymenoptera; life histories; oogenesis; ovigeny index

1. INTRODUCTION

All organisms face the problem of when to commit resources to reproduction and away from other life-history processes such as growth, dispersal or maintenance (Sibley & Calow 1986; Stearns 1992). Theoretical modelling has suggested that the allocation of resources among such processes should be strongly dependent on the availability of resources (McNamara & Houston 1996). Body size is a trait that strongly covaries with the supply of energy and materials for different life-history processes (West *et al.* 1999). Body size also varies markedly intraspecifically in many species. Thus, the adjustment of life-history processes in response to an individual's body size should carry fitness benefits (e.g. Ellers & Jervis 2003; Bateson *et al.* 2004; Crespi & Denver 2005). In this paper we examine, in an insect species, the relationship between adult body size and an indication of investment in early reproduction, the ovigeny index.

In many insects, adults may eclose with a proportion of their lifetime egg complement that is already mature (Jervis & Ferns 2004). The ovigeny index measures this proportion and is the egg load at adult eclosion (initial egg load) divided by the potential lifetime egg production (Jervis *et al.* 2001). Though not a perfect measure of absolute investment in reproduction at adult eclosion (see Jervis & Ferns 2004; Jervis *et al.* 2005), the ovigeny index has a major advantage over some other potential indicators, such as the initial egg load itself, by virtue of lifetime potential fecundity being its denominator. Although the egg load at eclosion and the potential lifetime fecundity of insects typically increase intraspecifically with adult body size, the ovigeny index is a relative

measure of investment in early reproduction that controls for total lifetime egg production, and hence can trade-off with other life-history traits (Jervis & Ferns 2004). Interspecific comparative studies on three insect orders have revealed a negative association between adult lifespan and the ovigeny index, reflecting that the allocation of resources to early reproduction probably has adverse late-life consequences (Jervis *et al.* 2001, 2005; Jervis & Ferns 2004).

The commitment of resources to early reproduction therefore has fitness costs, but there are also potential benefits: females encountering high concentrations of reproductive opportunities early in life may be less egg limited. What then is the optimal developmental strategy to adopt? Dynamic programming models that address this question have been developed for parasitoids. Parasitoids are insects, mostly wasps, that develop to maturity on the still-living body of another host arthropod, eventually killing it. The models predict that the ovigeny index should be negatively correlated with body size (Ellers & Jervis 2003, 2004). Optimal initial egg loads are very similar for different sized wasps, because all should carry sufficient eggs to exploit a rich patch of hosts should they encounter one. However, large females carry more total resources than small females, and thus potential lifetime fecundity should be strongly size-dependent. Thus, the ovigeny index is predicted to be large for small females and small for large females. This prediction finds support across parasitoid Hymenoptera species (Jervis *et al.* 2003) and other orders of insects (Jervis *et al.* 2005). However, the evidence from intraspecific studies is more equivocal. Two previous studies (King & King 1994; Olson & Andow 1998) suggest some support for theory (see Ellers & Jervis 2003; Jervis & Ferns 2004). However, because these studies were designed to address other issues, they measured an equivocal proxy for potential lifetime egg production, number of offspring surviving to adult

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eclosion that assumes no developmental mortality or host limitation. In addition, the theory has not been rigorously tested statistically in these cases (see §2*e*).

In this paper, we investigate whether ovigeny index declines with body size in females of the wasp *Aphaereta genevensis* Fischer 1996 (Hymenoptera: Braconidae: Alysiinae). Our findings suggest that the adaptive variation in investment in reproduction seen across species is mirrored at the intraspecific level. This may have important implications for population biology and developmental biology.

2. MATERIAL AND METHODS

(a) *Study species*
Aphaereta genevensis is a parasitoid of *Drosophila*, known only from New York State, USA. Eggs are inserted into the host larva (i.e. it is an endoparasitoid), and the parasitoid completes development in the host pupa (i.e. it is a larval–pupal koinobiont). First instar larvae display aggressive behaviour towards conspecific larvae in the same host (Mayhew & van Alphen 1999), which results in only a single wasp normally completing development (i.e. it is a solitary species). Although one egg per host is normally the modal clutch size, adults can adaptively raise their clutch size under a variety of conditions, in common with other solitary parasitoids (see Pexton & Mayhew 2005 and references therein).

(b) *Culturing*
Details of host (*Drosophila virilis* Sturtevant) and wasp culture history are given in Mayhew & van Alphen (1999). Adult flies were kept in 30 cm plastic cages with a muslin door and laid eggs into bottles containing standard yeast-based medium. Bottles were replaced twice weekly. Wasp cultures were maintained in 6 cm diameter glass jars with foam stoppers. A 2 cm layer of agar was added to the jar and about 100 host larvae, a drop of viscous yeast and three to four mated female wasps. The jars were stored at 20 °C under constant light and at ambient humidity (range 40–70% relative humidity). Emerging wasps were aspirated into jars containing moist vermiculite and a drop of honey and stored in darkness at 4 °C.

(c) *Potential lifetime fecundity*
To estimate potential lifetime egg production, we exposed wasps to an abundance of hosts throughout their lifetimes under benign conditions of temperature and food availability, and counted the eggs laid (realized fecundity). Following Ellers & van Alphen (1997), we also added the number of developing and mature oocytes in the wasp abdomen upon death (extra potential fecundity). Newly emerged female wasps (less than 24 h old) were selected at random, and each female was placed into an individual glass tube (75 × 25 mm). Each tube also contained 1 cm depth agar, 10 *D. virilis* third instar larvae, a small amount of active yeast and a drop of honey on the plastic lid (which had air holes covered by mesh). Each wasp was moved to a new tube each day, with fresh hosts, and this was repeated until the wasp died. The wasp was then stored at −18 °C. The host larvae in the tubes were dissected under a binocular microscope and the number of eggs per host recorded (see Mayhew & van Alphen 1999 for protocols). Frozen adult wasps were later dissected to count the eggs remaining in the abdomen, and their hind tibia length was recorded to the nearest 0.01 mm. We combined the number of eggs laid per wasp over its lifetime plus the number of eggs remaining in the abdomen after death as the measure of potential lifetime fecundity. One wasp died after 18 days without having laid any eggs, and with an unusually large number of oocytes (82) remaining in its abdomen. We assumed that this individual was aberrant and excluded it from the analysis.

(d) *Initial egg load*
For initial egg load, we used previous data collected from the same culture under identical environmental conditions (Pexton & Mayhew 2002). Parasitized fly pupae were washed out of culture jars with lukewarm water and placed into clean specimen tubes. The tubes were checked at 1 h intervals during the day and at the start of each working day, ensuring that the oldest wasps were at most 12 h old. Newly emerged female wasps were killed by freezing at −18 °C. Later, their egg load and hind tibia lengths were recorded as above.

(e) *Statistical analysis*
The ovigeny index is defined as initial egg load divided by potential lifetime fecundity. Since both initial egg load and potential lifetime fecundity can only be measured destructively, we had to estimate the ovigeny index indirectly by comparing sets of different individuals. Previously, it has been suggested (see fig. 1 of Jervis & Ferns 2004) that a intraspecific decrease in ovigeny index with body size can be explained by the different slopes of the regressions of initial egg load and potential lifetime fecundity with body size detectable, for example, by a significant interaction in an analysis of covariance (ANCOVA). This is only partially true. If the ovigeny index does decrease with body size, then these two regressions will indeed have different slopes. However, even if the ovigeny index remains constant with body size (the null hypothesis), we would expect these two lines to have different slopes if either one of them is positive: if the initial egg load increases with body size, for the ovigeny index to remain constant with body size, then the potential lifetime egg production must increase with body size still more. Significantly different regression slopes are therefore possible, even expected, under the null hypothesis, and are an insufficient test of theory. Nor is it simple to predict a slope of the potential lifetime fecundity relationship under the null hypothesis because that depends on both the slope and intercept of the initial egg load relationship, neither of which are known with certainty.

We therefore took an alternative approach that addressed directly how the ovigeny index is related to body size, for which the null prediction is clear. We identified pairs of individuals from the data, one of which had its initial egg load measured and one of which had its potential lifetime fecundity measured. Pairs also had to have similar hind tibia lengths, which we ensured using an *a priori* tolerance level to limit the size differences between individuals. We selected 12 pairs of individuals at random from the data that met this tolerance level (12 being a fair sample that allows low tolerance levels), in which no datum was used more than once. For each pair, we calculated the ovigeny index and the mean hind tibia length. For the 12 pairs, we then calculated the Spearman rank correlation coefficient between ovigeny index and hind tibia length. Spearman's rank correlation was used because the ovigeny index is a proportion that breaks the assumptions of simple parametric statistics. We then repeated

the procedure 1000 times to produce the mean and boot-strapped confidence intervals of the correlation implied by the data (e.g. West *et al.* 2001). The proportion of coefficients less than or equal to 0 tests the null hypothesis that the correlation between ovigeny index and hind tibia length is not negative (the *p*-value). We repeated the analysis with different size tolerance levels to ensure that the result did not arbitrarily depend on this.

The effect of hind tibia length on longevity in the potential lifetime fecundity experiment was examined by fitting a Weibull distribution to the data in the GLIM statistical package. The shape parameter of the Weibull is greater than 1 if the risk of death increases with age, and less than 1 if the risk decreases with age. The significance of hind tibia length was estimated by the change in deviance when this variable was removed from the model using a χ^2-test (Crawley 1993). We also examined how the number of eggs laid per day varied over time using a repeated measures analysis of variance (ANOVA) in SPSS, limiting the analysis to that time frame (days 1–29) that gave sufficient error degrees of freedom for this test.

3. RESULTS

Of the 21 females examined for potential lifetime fecundity, 11 died without any eggs remaining in the abdomen. Of the remaining 10, the mean (median, s.e.) number of eggs remaining was 5.60 (4.00, 1.51). Overall, the mean (median, s.e.) number was 2.67 (0.00, 0.94). Oviposition activity was low for very young wasps, increased up to day 7, and then gradually declined again, remaining at very low values after day 25 (figure 1). A repeated measures ANOVA confirmed that there was significant heterogeneity in oviposition across days 1–29 ($F_{28,280} = 167.7$, $p < 0.0005$). The risk of death significantly increased with age (Weibull shape parameter $= 4.97$, $\chi_1^2 = 42.69$, $p < 0.001$), and declined with hind tibia length ($\chi_1^2 = 5.28$, $p < 0.025$).

Both potential lifetime fecundity and initial egg load were positively related to hind tibia length (table 1; figure 2). The slope of the regression of potential lifetime fecundity against hind tibia length was significantly steeper than that of initial egg load (table 1; figure 2). The regression equations are: potential lifetime fecundity $= -218.79 + 579.00 \times$ hind tibia length (mm) ($r^2 = 0.632$) and initial egg load $= 22.30 + 76.25 \times$ hind tibia length (mm) ($r^2 = 0.256$). The relationship between ovigeny index and hind tibia length is significantly negative with a size tolerance level (see §2e) of both 0.02 mm (mean $r_s = -0.544$, 95 percentile -0.301, $p < 0.001$) and 0.04 mm (mean $r_s = -0.541$, 95 percentile -0.224, $p = 0.002$; figure 3).

The individual at $x = 0.59$ mm, $y = 10$ eggs (figure 2) has a standardized residual from the potential lifetime fecundity regression of -2.54. To assess the influence of this individual, we repeated the ANCOVA and bootstrap analysis without it. The interaction between treatment and eggs (figure 2) was still significant ($F_{1,47} = 22.12$, $p < 0.001$, regression potential lifetime fecundity $= -187.78 + 540.23 \times$ hind tibia length (mm), $r^2 = 0.698$). The bootstrap analyses were still significant at a tolerance of 0.02 mm (mean $r_s = -0.520$, 95 percentile -0.311, $p < 0.001$) and 0.04 mm (mean $r_s = -0.504$, 95 percentile -0.189, $p = 0.009$).
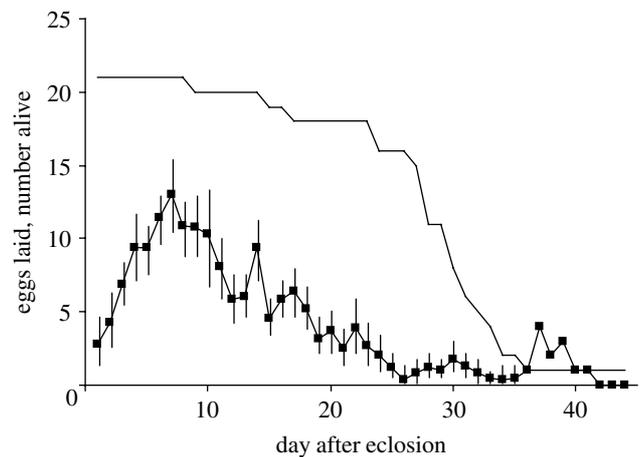
Figure 1. The mean ($\pm$s.e.) number of eggs laid per day during life in *A. genevensis* (filled squares), in individuals supplied with 10 fresh hosts per day. Means are calculated only from individuals still alive (sample size given by the solid line, representing the survival curve).

Table 1. ANCOVA of number of eggs against treatment (initial egg load or potential lifetime egg production measured), with hind tibia length as the covariate. (Values computed by subtraction of a term from the full model.)

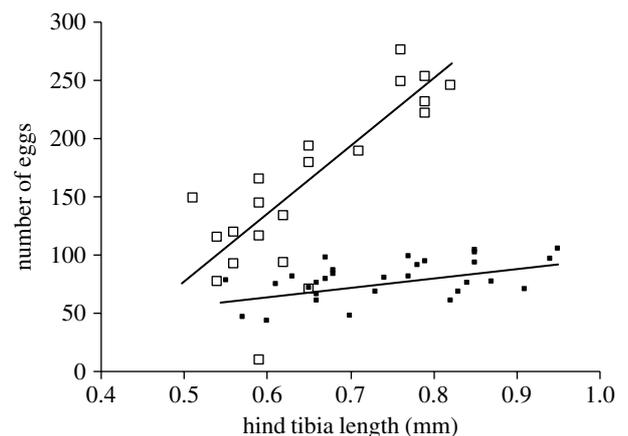| source	SS	d.f.	MS	F	p
treatment	15 120	1	15 120	12.39	0.001
residual	58 538	48	1 220		
treatment × tibia length interaction	31 488	1	31 488	20.17	<0.001
residual	74 907	48	1 561		
model total	144 291	3	144 291	156.16	<0.001
total error	43 419	47	924		
total	187 710	50			

Figure 2. Egg load at eclosion (filled squares) and potential lifetime egg production (open squares) against hind tibia length in *Aphaereta genevensis*. Regression lines are shown.

4. DISCUSSION

We have demonstrated for the first time using a rigorous methodology that the ovigeny index decreases with body size within an insect species. Interspecific variation in the ovigeny index spans the entire spectrum from 0 to 1 in insects (i.e. no eggs mature on eclosion through to all eggs mature). Documented intraspecific variation is much
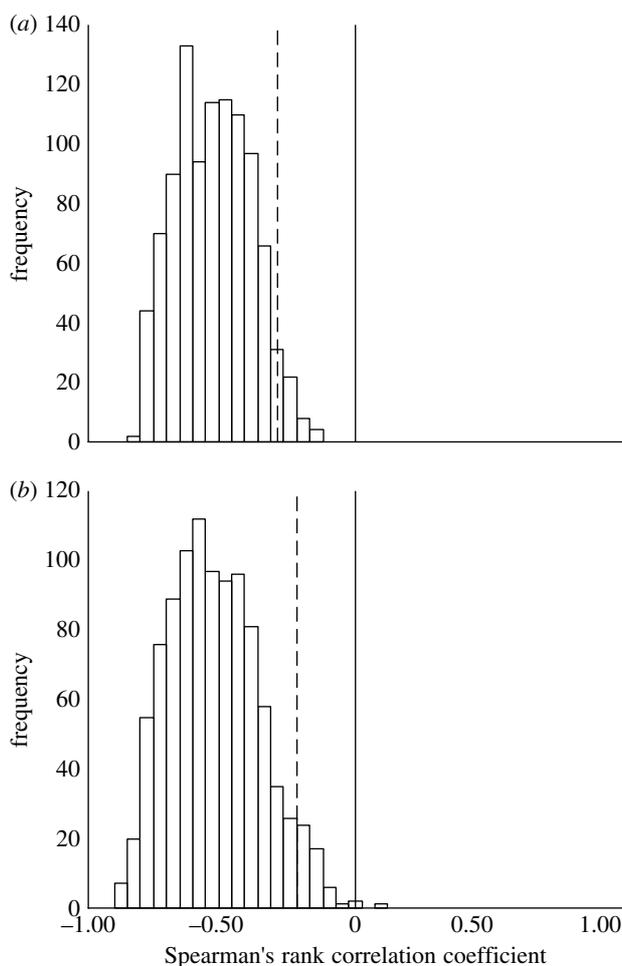
Figure 3. The Spearman rank correlation coefficient of ovigeny index against hind tibia length. Values are calculated by 1000 bootstrap samples from the data in figure 2, using an arbitrary size tolerance level from which to choose 12 pairs of individuals of similar body size. The distribution of coefficients is shown for tolerance levels of (a) 0.02 mm (the smallest possible tolerance that will give 12 pairs) and (b) 0.04 mm. The solid vertical line indicates the null expectation of zero correlation and the dashed vertical line the 95 percentile of the bootstrap replicates.

more limited, with the greatest range across 67 species of Hymenoptera and 57 species or higher taxa of Lepidoptera examined being 0.16 (Jervis et al. 2001, 2005). Using the individual regressions drawn in figure 2, and avoiding extrapolation from either, a female of hind tibia length 0.55 mm is predicted to have an ovigeny index of 0.65, while a female of 0.82 mm hind tibia length is predicted to have an ovigeny index of 0.33. This gives an estimated range of 0.32, the highest known range to date among these orders.

The ovigeny index is negatively associated with adult longevity across species in the Hymenoptera, Trichoptera and Lepidoptera (Jervis et al. 2001, 2005). In *Asobara tabida*, a close relative of *A. genevensis*, the ovigeny index and lifespan are negatively correlated (reported in Jervis et al. 2001; Jervis & Ferns 2004—based on data in Ellers & van Alphen 1997). Furthermore, the congeneric *Aphaereta pallipes* has higher initial egg loads than *A. genevensis* of the same body size, but lower fat reserves and reduced longevity in the absence of adult food (Pexton & Mayhew 2002). In our data, large females have lower ovigeny indices, but greater longevity than small females.

This therefore suggests that increases in the initial egg load generally come at a cost of reduced survival, and hence late reproduction.

Our data imply variation in ovarian development during the pre-adult life-history stages. Adaptive changes in development, which improve fitness in early life despite adverse late-life consequences, are also known in a wide range of organisms from annual plants (Kudoh et al. 2002) to vertebrates (Crespi & Denver 2005) possibly including humans (Bateson et al. 2004). The latter case is often described as the 'foetal origins' hypothesis. Underlying our data is a, so-far undescribed, developmental mechanism that must act prior to adult eclosion. It is possible that understanding the mechanisms behind our data will provide useful perspectives on cases that have a more direct impact on human livelihoods. Our data are also likely to have significance for population biologists. It is becoming more widely appreciated among vertebrate ecologists that conditions experienced during development can have important long-term impacts on population growth (e.g. Lindström 1999). The processes in this paper concern important population growth parameters such as longevity, fecundity and the timing of reproduction. One recent study has shown seasonal fluctuations in the body size of a closely related parasitoid species, *A. tabida* (Ellers et al. 2001), which implies parallel fluctuations in the vital rates of these populations. Thus, we propose that an awareness of the likely developmental consequences of body size variation may also improve our understanding of insect population dynamics.

While adaptive changes in egg maturation during adult life are well known in insects (see Papaj 2000), our data refer explicitly to processes that occur prior to adulthood. Some observations suggest that similar patterns in egg maturation may occur after adult eclosion. In the damselfly *Coenagrion puella*, for example, clutch size is negatively correlated with body size, but lifetime reproductive success is positively correlated with body size because large adults survive to lay more clutches (Banks & Thompson 1987). All Odonata are completely synovigenic (ovigeny index of zero), as far as is known (Corbet 1999), and female *C. puella* normally lay all of their mature eggs in a single clutch, so clutch sizes probably indicate different rates of egg maturation during the adult stage. It would be interesting to extend the theoretical models to examine how body size affects optimal egg maturation during adult life in obligatory synovigenic insects.

The pattern of oviposition during life, with a lag both before and after peak oviposition activity, is interesting. The lag after the peak is predicted by dynamic programming models that assume pro-ovigeny (Iwasa et al. 1984). As egg load depletes, females become more selective to avoid egg limitation. Such a trend has been reported in numerous other species (Godfray 1994; Papaj 2000). It is interesting to note that oviposition activity declines to very low levels by about day 25, which is also the time when the risk of mortality increases substantially (figure 1). This is circumstantial evidence that oviposition and survival both depend on a common resource, and that oviposition activity is modulated in response to the levels of that resource. The lag before the peak (see also Traynor & Mayhew 2005) has been less widely reported or investigated in species that have an ovigeny index greater than 0 (see Jervis et al. 2001). It maybe due to physiological or

other constraints surrounding egg maturation, or learning with respect to host finding, handling or density.

There are several opportunities for further work. The adaptive nature of association between body size and ovigeny index is presently inferred only through its consistency with adaptive models. Direct tests would require measurements of the fitness consequences of different phenotypes in different environments. The evidence for an intraspecific trade-off between the ovigeny index and longevity is only inferential (the former is negatively correlated with body size while the latter is positively related to body size). Selection experiments may provide a way forward in this respect. The developmental mechanisms underlying our results and their consequences for population dynamics hold the potential for interesting new discoveries.

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