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What limits insect fecundity? Body size- and temperature-dependent egg maturation and oviposition in a butterfly

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Summary

1. Large female insects usually have high potential fecundity. Therefore selection should favour an increase in body size given that these females get opportunities to realize their potential advantage by maturing and laying more eggs. However, ectotherm physiology is strongly temperature-dependent, and activities are carried out sufficiently only within certain temperature ranges. Thus it remains unclear if the fecundity advantage of a large size is fully realized in natural environments, where thermal conditions are limiting.

2. Insect fecundity might be limited by temperature at two levels; first eggs need to mature, and then the female needs time for strategic ovipositing of the egg. Since a female cannot foresee the number of oviposition opportunities that she will encounter on a given day, the optimal rate of egg maturation will be governed by trade-offs associated with egg- and time-limited oviposition. As females of different sizes will have different amounts of body reserves, size-dependent allocation trade-offs between the mother's condition and her egg production might be expected.

3. In the temperate butterfly *Pararge aegeria*, the time and temperature dependence of oviposition and egg maturation, and the interrelatedness of these two processes were investigated in a series of laboratory experiments, allowing a decoupling of the time budgets for the respective processes.

4. The results show that realized fecundity of this species can be limited by both the temperature dependence of egg maturation and oviposition under certain thermal regimes. Furthermore, rates of oviposition and egg maturation seemed to have regulatory effects upon each other. Early reproductive output was correlated with short life span, indicating a cost of reproduction. Finally, large females matured more eggs than small females when deprived of oviposition opportunities. Thus, the optimal allocation of resources to egg production seems dependent on female size.

5. This study highlights the complexity of processes underlying rates of egg maturation and oviposition in ectotherms under natural conditions. We further discuss the importance of temperature variation for egg- vs. time-limited fecundity and the consequences for the evolution of female body size in insects.

Key-words: body size, ectotherms, egg maturation, fecundity, Lepidoptera, life-history theory, ovigenesis, oviposition

Introduction

The fitness of a female is determined by the number of viable offspring that she can produce, a factor strongly dependent upon her body size. The fecundity–body size relationship, typically quantified under laboratory conditions, is central to life-history models of age and size at maturity (see Kozlowski 1992; Stearns 1992; Roff 2002). Optimality models, however, often fail to explain why insects do not evolve to be larger (Blanckenhorn 2000). Most insects accumulate mass quickly during larval growth (Blau 1981; Wickman, Wiklund & Karlsson 1990; Leimar 1996; Migeon, Garfinkel & Edgar 1999; Margraf, Gotthard & Rahier 2003; Esperk & Tammaru 2004; Gotthard 2004). So, if females are typically able to convert all of their accumulated resources into realized fecundity, even a very small additional foraging effort would result in a substantial gain in fitness (Leimar 1996; Tammaru, Esperk & Castellanos 2002; Berger, Walters & Gotthard 2006). Thus, it

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seems unlikely that size–fecundity relationships estimated under laboratory conditions are reliable estimates of size–fitness relationships in the wild. In order to make predictions of evolutionary responses and viability assessments of natural insect populations, it is therefore crucial that we identify the factors and mechanisms that constrain female fecundity in the field.

The question of whether field fecundities are limited by female resources or by egg-laying opportunities has received considerable attention (Bouletreau 1978; Boggs 1986; Shine 1987; Leather 1988; Wiklund & Karlsson 1988; Honek 1993; Visser 1994; Blanckenhorn 2000; Papaj 2000; Jervis, Boggs & Ferns 2005; Gotthard, Berger & Walters 2007; Jervis, Ferns & Boggs 2007). In favourable environments, where thermal conditions allow activity and hosts are abundant, the amount of resources that a female can convert to eggs should directly determine her reproductive output and, hence, large females will have higher fitness than small females. However, most natural environments do not present unlimited oviposition opportunities and realized fecundity will typically be limited by the amount of time females can be active and oviposit. Under such circumstances, large females will not be able to realize their larger potential fecundity, and selection to increase body size will be weak. Moreover, as egg maturation itself is also strongly temperature-dependent, the physiological process of converting resources to eggs may also be limiting under natural conditions (Boggs 1986; Carroll & Quiring 1993; Papaj 2000; Jervis, Boggs & Ferns 2005). The relationship between these two temperature-dependent processes and their relative importance in determining realized fecundities during periods of cold and variable weather conditions is not well studied in insects. Thus, little is known about how temperature variation during periods not suitable for oviposition affect rates of egg maturation, related allocation trade-offs and, ultimately, selection of female body size.

It is possible that under certain thermal conditions it is the conversion rate of resources into eggs that is the main process limiting female fecundity rather than the time for general activity and oviposition, especially if there are trade-offs associated with maintaining high rates of egg maturation during periods of inactivity. If, for example, high maturation rates are associated with an overall higher metabolism, this could reduce life span (Papaj 2000; Brown *et al*. 2004; Jervis, Boggs & Ferns 2005). Females might also pay a more direct cost of reproduction because allocation of energy to eggs should leave less resources to maintain body functions, resulting in reduced female performance and survival (Papaj 2000; Carey 2001; Roff 2002; Jervis, Boggs & Ferns 2005, Jervis, Ferns & Boggs 2007). Lastly, female flight might be directly impaired by shifts of resources to the abdomen (Wickman & Karlsson 1989; Berrigan 1991; Papaj 2000). Given the potential costs of maturing and storing eggs, we may expect larger females to afford a greater surplus of eggs ready to be oviposited, due to their greater energy reserves. Thus, the decision to mature eggs during periods not suitable for oviposition might differ between large and small females.

A recent study on the satyrine butterfly, *Pararge aegeria*, showed that realized fecundity was highly temperature-dependent and that oviposition in the field was partly time-limited due to periods of low temperatures (Gotthard, Berger & Walters 2007). Theoretical results also indicated that daily variability in temperature makes it beneficial for females to have an excess of eggs ready for oviposition so that they are prepared to utilize maximally the next favourable period. In this experimental study, we explore the relationship between female body size and rates of egg maturation and oviposition under different temperature regimes in *P. aegeria*. Our main aim was to investigate to what degree thermal conditions during periods when females do not oviposit may influence realized fecundity through effects on the egg maturation process. We first estimated the temperature dependence of egg maturation and then used these estimates to predict realized fecundities of ovipositing females in experimental treatments that allowed us to separate the effects of temperature on egg maturation and oviposition. In the experimental design, we capitalized on the fact that females of this species do not oviposit in darkness. By varying the temperature during night-time (when females only mature eggs), and daytime (when females may both mature and lay eggs) we were able to decouple the time budgets for oviposition and egg maturation. This experimental set-up made it possible to disentangle the complex relationships between egg maturation and oviposition rates in a thermally variable environment. Moreover, it allowed us to assess the trade-off structure and potential size dependencies that influence optimal maturation rates in the field.

Methods

STUDY SPECIES

Pararge aegeria is a satyrine butterfly with a wide geographical distribution throughout Europe. The population used in this study was sampled from Skåne, southern Sweden. The Skåne population is bivoltine, but has a complex phenology (Nylin, Wickman & Wiklund 1989) resulting in three discrete flight periods each year in May, June and July/August (C. Wiklund, pers. comm.). Thus, this population experiences large variation in climatic conditions both within- and between-flight periods. Analysis of meteorological data suggests that all flight periods comprise many days with temperatures unsuitable for oviposition. It has been shown that under favourable laboratory conditions fecundity is strongly related to female body size in this population of *P. aegeria* (Karlsson & Wickman 1990), but recent work suggests that the qualitative nature of this relationship can vary depending upon thermal conditions (Gotthard, Berger & Walters 2007). Females usually mate only once in the field and lay their eggs singly on a variety of grass species. The individuals used in the oviposition experiment were first generation offspring from wild females from the Skåne population caught in July 2006. The females used in the egg maturation experiments were offspring of females from the same population caught in 2005. The larvae were reared in conditions inducing direct development (17 C, 19L : 5D), which produces adults in the month of July.

EGG-MATURATION EXPERIMENTS

In Experiment I, 24 females were randomly allocated at eclosion to one of three climate cabinets set to a constant temperature of 14 C,

21 C or 28 C. An additional 11 females were killed directly in order to control for eggs matured at time of eclosion. Individual females were placed in small plastic cups within cabinets programmed to give complete darkness (light stimulates oviposition), with only access to water for 2 or 4 days after which they were killed (deep frozen) and later dissected. This allowed us to investigate (i) whether, and at which rate, unmated females mature eggs in darkness without any stimuli from host plants; and (ii) if this rate declines with time as females fill up their abdomen with eggs.

In Experiment II, 40 females were mated in three small 0·2-m^3 cages with equal numbers of males and females per cage. Cages were monitored regularly and copulating pairs were isolated and allowed to separate naturally. Mated females were then placed in 0·5-L plastic cups with host plants and access to sugar water. All females experienced ideal conditions for egg laying (25 C : 14 C; 16L : 8D) for the following 3 days. Two females that did not lay any eggs during this period were later excluded from the analysis. Females may not lay eggs for two reasons: (i) they perceive the host to be of poor quality and choose to hold back eggs; and (ii) females may, on occasion, suffer from a physical deformity as a result of experimental handling that can prevent successful oviposition (D. Berger, pers. obs.). After the third day females were assigned to five different night treatments: the control and egg maturation at 10 C, 15 C, 20 C or 25 C following the same procedure as described for maturation Experiment I. The control females were killed and dissected directly in order to determine how many mature eggs females contained initially. Females in each of the temperature treatments were killed following 12 h in darkness. This method allowed us to estimate the temperature dependence of egg maturation in mated females that had already started ovipositing. The effects of temperature and experiment type (I or II) on egg maturation rates were analysed with GLM and linear regression.

OVIPOSITION EXPERIMENT

The goal of this experiment was to disentangle thermal effects on rates of egg maturation from thermal effects on rates of oviposition. In particular we aimed to quantify how the interaction between these two temperature-dependent processes influences realized fecundity in a thermally variable environment. In the experiment, the amount of time available for oviposition and egg maturation could be varied independently because oviposition in this species is restricted to daylight hours (confirmed during the egg maturation experiments and checked during this experiment). Prior to the start of the experiment female pupae were ranked by weight and split into four weight groups. Females from each group were randomly assigned to the different experimental treatments. Matings were conducted as described above. After mating, females were kept at a temperature of 10 C until the following morning, so that assignment to respective treatments could be synchronized. Oviposition was monitored in climate cabinets with a photoperiod of 16L : 8D. There were two night-time temperature treatments that were crossed with two daytime temperature treatments in a 2×2 design. In all treatments the baseline temperature of each daily cycle was set to 14 C (below the threshold for oviposition behaviour; Gotthard *et al.* 2007) before being raised to 25 C for various amounts of time during night and day. The day treatments were either 2 or 8 h of 25 C, while the night treatments were 0 or 6 h of 25 C. This design created four treatments with different amounts of time of 25 C: 2 h day + 0 h night (called 2 h in the following), 8 h day + 0 h night (8 h), 2 h day + 6 h night (2 + 6 h) and 8 h day + 6 h night (8 + 6 h). An important aspect of the experimental design is that the 8 h and the 2 + 6 h treatments have the same daily mean temperature.

It might be predicted that female fecundity will be more limited by egg maturation processes in the night treatments with constant 14 C (2 and 8 h), while female fecundity in the night treatments containing 6 h of 25 C (2 + 6 and 8 + 6 h) will be more limited by processes associated with oviposition behaviour. It might also be predicted that fecundity will be greater in the day treatments with 8 h of 25 C (8 and 8 + 6 h) where oviposition opportunities are almost unlimited, rather than in the day treatments with only 2 h of 25 C (2 and 2 + 6 h) where time for oviposition is reduced. Temperatures were logged at every 10 min for 3 days in each treatment cabinet so that these recordings could later be used together with data from egg maturation Experiments I and II to calculate the predicted number of eggs matured by females in the oviposition treatments. On the third and seventh day of the experiment, host plants were replaced and all eggs laid were removed and counted. Thereafter, egg counts and replacement of host plants were conducted on a weekly basis until the natural death of the female. Females were later dissected to calculate the number of mature eggs that remained unlaid. During dissection, we confirmed the mating status of females by checking for spermatophores; fertility was confirmed from the hatching success of eggs laid during the experiment. As a result, 2 out of 38 females were excluded from statistical analyses. All effects on fecundity were analysed with night (6 vs. 0 h, 25 C) and day (8 vs. 2 h, 25 C) treatments as categorical factors and female weight as a covariate. Results for the first week of oviposition were analysed by a repeated measures ANOVA, where the repeated measure was fecundity measured for days 1–3 and days 4–7. As a number of females died during the second week, data on fecundity for days 8–14 were not used in the repeated measures analysis. Instead we analysed this trait (fecundity during days 8–14 of females surviving to day 14) and lifetime fecundity by standard ANOVA. In all cases we started with full factorial designs before non-significant interaction effects were sequentially excluded in inverse order of their explanatory power.

PREDICTED FECUNDITY

Predicted fecundities for each of the four oviposition treatments were calculated using linear regressions of egg maturation rate against temperature fitted to the data from egg maturation Experiments I and II, in combination with temperature records taken within cabinets during the oviposition experiment. To predict the number of eggs matured during the 3 days of mating prior to the start of the oviposition experiment, the data from egg maturation Experiment I was used (unmated females), whereas the predicted number of matured eggs during oviposition was estimated based on data from egg maturation Experiment II (mated females allowed to oviposit prior to maturation period). All statistical analyses were carried out in R (R Development Core Team 2005).

Results

EGG MATURATION

The 11 virgin females killed directly after eclosion in Experiment I contained no eggs, while virgin females allowed to mature eggs in the dark showed a strong temperature-dependent rate of egg maturation ($R^2 = 0{\cdot}88$, $t_{1,21} = 12{\cdot}49$, $P < 0{\cdot}001$) (Fig. 1). The maturation rate of females left for 2 days was not significantly greater than for those left for 4 days

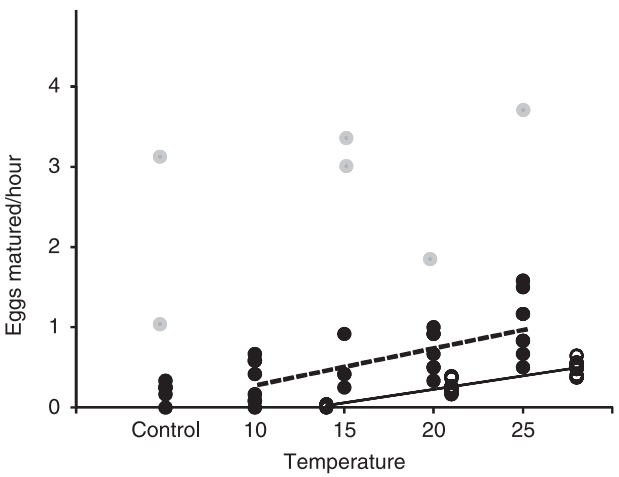

Fig. 1. Hourly egg maturation rates in Experiment I (white circles) and Experiment II (black circles). Grey circles represent the six outliers in Experiment II that were excluded when predicting fecundities of ovipositing females. The control females were used to estimate the number of eggs contained by females at the start of the maturation period in Experiment II.

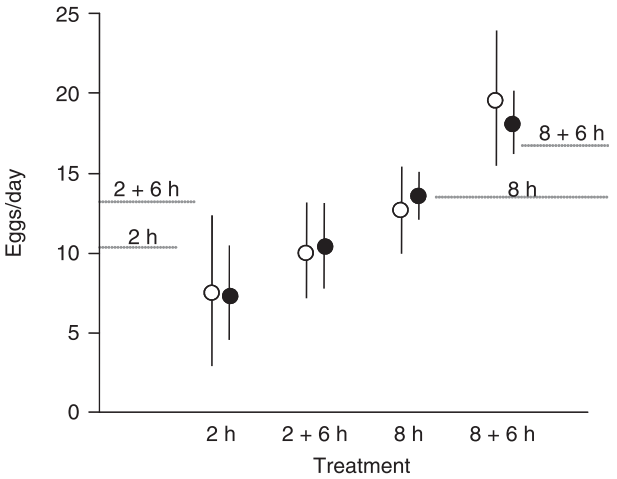

Fig. 2. Rates of egg laying per day (means ± 2 SE) in the four oviposition treatments during the first week of egg laying. White circles denote rates calculated for days 1–3 and black circles days 4–7. Grey hatched lines represent predicted fecundities in the corresponding treatments estimated from temperature–egg maturation relationships calculated from maturation Experiments I and II.

($t_{2,21} = -0{\cdot}49$, $P > 0{\cdot}6$). In Experiment II, six outliers were identified (see Fig. 1) using Grubbs' test (available in the 'Outliers package' for R). These individuals contained far more eggs than observed on average and most likely these eggs had matured prior to the onset of the 12 h-maturation period. Two of these individuals belonged to the control, two to the 15 C, one to the 20 C and one to the 25 C treatments. To deal with these outliers, we first performed a robust ANOVA (available in the 'Robustbase package' for R) that identifies and corrects for variation caused by extreme outliers in the data. Egg maturation was significantly affected by temperature ($t_{1,31} = 4{\cdot}40$, $P < 0{\cdot}001$) (Fig. 1). We then used the guidance of the Grubbs' test and excluded the six outliers before estimating the linear relationship between temperature and maturation that was later used for calculating the predicted number of eggs matured by females in the oviposition experiment. By subtracting the mean number of eggs found in dissected females belonging to the control from the number of eggs found in females belonging to the remaining temperature treatments in Experiment II, we calculated hourly maturation rates comparable to those of Experiment I. The rate of egg maturation was found to be higher in mated (Experiment II) vs. virgin females (Experiment I), but there was no significant difference in the slope of the temperature–maturation relationship (experiment: $F_{1,47} = 29{\cdot}7$, $P < 0{\cdot}001$; temperature: $F_{1,47} = 54{\cdot}6$, $P < 0{\cdot}001$; experiment × temperature interaction: $F_{1,46} = 1{\cdot}31$, $P = 0{\cdot}26$) (Fig. 1).

PREDICTED FECUNDITIES

The number of eggs matured per hour at temperature x during mating is given by the relationship: $0{\cdot}0336x - 0{\cdot}444$ ($R^2 = 0{\cdot}88$) (white circles, full line in Fig. 1). The predicted average of matured eggs during the 3-day mating period prior to the maturation experiment was calculated to 20·1 eggs. Thus, each individual was assumed to start with a batch of 20·1 eggs ready for oviposition. The predicted daily fecundity during the first week of oviposition is given by the relationship: $0{\cdot}046x - 0{\cdot}376$ ($R^2 = 0{\cdot}46$) (black circles, dashed line in Fig. 1). Based on these relationships, the predicted fecundities in the different oviposition treatments were calculated: 2 h = 10·6 eggs, 2 + 6 h = 13·4 eggs, 8 h = 13·8 eggs and 8 + 6 h = 16·6 eggs (Fig. 2).

OVIPOSITION

There were strong effects of both day (2 and 2 + 6 vs. 8 and 8 + 6 h) and night (2 and 8 vs. 2 + 6 and 8 + 6 h) treatment on fecundity during the first week of oviposition, with females experiencing longer periods of 25 C laying more eggs (night: $t_{31} = -3{\cdot}25$, $P = 0{\cdot}0028$; day: $t_{31} = -6{\cdot}75$, $P < 0{\cdot}001$; weight: $t_{31} = 1{\cdot}12$, $P = 0{\cdot}27$; oviposition census time: $t_{34} = -0{\cdot}11$, $P = 0{\cdot}91$, repeated measures ANOVA) (Fig. 2). However, realized fecundity was not strictly dependent on the relationship between temperature and egg maturation. Female fecundity after the first week was higher in the 8 h treatment compared to the 2 + 6 h treatment despite equivalent mean daily temperatures ($t_{19} = -2{\cdot}90$, $P = 0{\cdot}009$), indicating that females in the 2 + 6 h treatment were oviposition-limited rather than egg-limited (Fig. 2). The number of eggs laid by surviving females between days 8 and 14 differed between day treatments ($F_{1,20}$, $P = 0{\cdot}015$), while there was no discernible effect of night treatment ($F_{20} = 0{\cdot}21$, $P = 0{\cdot}65$). Lifetime fecundity was not significantly affected by treatment or female weight although there was a strong trend towards higher lifetime fecundity in the day treatments with 8 h of 25 C (night: $F_{1,32} = 0{\cdot}06$, $P = 0{\cdot}81$; day: $F_{1,32} = 3{\cdot}94$, $P = 0{\cdot}056$; weight: $F_{1,32} = 0{\cdot}30$, $P = 0{\cdot}59$, 2 h = 113 ± 35 eggs, 2 + 6 h = 112 ± 30 eggs, 8 h = 133 ± 21 eggs and 8 + 6 h = 151 ± 32 eggs). The lack of treatment effects were likely due to the fact

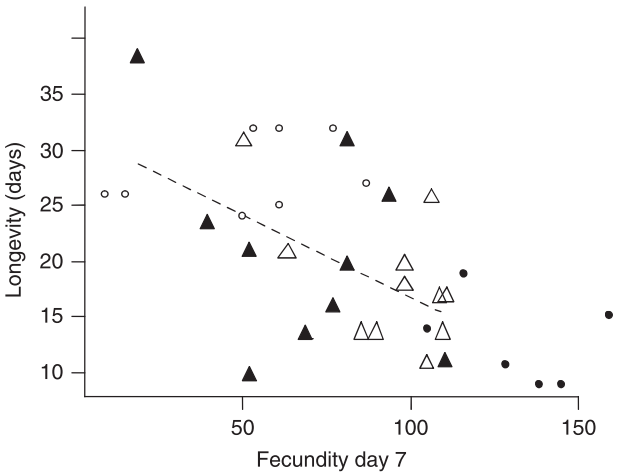

Fig. 3. Allocation trade-off between reproduction and life span estimated by the relationship between fecundity at day 7 and longevity. The regression line is fitted only to the data from the 2 + 6 and 8 h treatment sharing the same temperature average. Open circles = 2 h; filled triangles = 2 + 6 h; open triangles = 8 h; filled circles = 8 + 6 h.

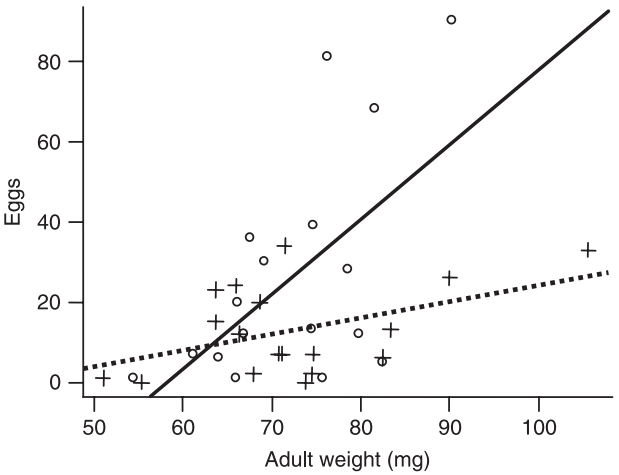

Fig. 4. Number of dissected eggs as a function of female body size in the oviposition-limited 2 and 2 + 6 h treatments (circles, unbroken line) and the less limited 8 and 8 + 6 h treatments (crosses, dotted line).

that females in the colder treatments lived longer (night: $F_{1,32} = 6{\cdot}08$, $P = 0{\cdot}019$; day: $F_{1,32} = 18{\cdot}2$, $P < 0{\cdot}001$; weight: $F_{1,32} = 0{\cdot}05$, $P = 0{\cdot}83$, 2 h = 28·0 ± 2·4 days, 2 + 6 h = 20·9 ± 5·8 days, 8 h = 18·5 ± 3·5 days and 8 + 6 h = 12·1 ± 3·2 days) and therefore could produce eggs over a longer period, in part compensating for the reduction in rates of egg maturation and oviposition. Since females in the 8 h treatment had laid more eggs over the first week than females in the 2 + 6 h treatment, independently of the temperature sum, we tested if there was also a difference in longevity between these two treatments (in order to detect possible costs associated with the allocation of resources to eggs). No such difference was found ($F_{1,17} = 0{\cdot}31$, $P = 0{\cdot}58$), however among individuals within these two treatments there was a significant negative relationship between fecundity at day 7 and life span (treatment: $t_{2,18} = -0{\cdot}47$, $P = 0{\cdot}65$; fecundity day 7: $t_{1,19} = 6{\cdot}31$, $P = 0{\cdot}026$) (Fig. 3). When testing across all temperature treatments, this effect was found to be highly significant (treatment: $t_{1,36} = 2{\cdot}56$, $P = 0{\cdot}12$; fecundity day 7: $t_{1,36} = 20{\cdot}5$, $P < 0{\cdot}001$) (Fig. 3), but as temperature probably has direct effects on both life span and fecundity it is questionable whether the effect of fecundity is correctly estimated by this analysis. The number of unlaid eggs dissected from dead females was higher among individuals from the day treatments with only 2 h of 25 C ($F_{1,27} = 7{\cdot}39$, $P = 0{\cdot}011$); heavier females also contained more eggs overall ($F_{1,27} = 11{\cdot}99$, $P = 0{\cdot}002$), but this effect was particularly pronounced in the 2 and 2 + 6 h treatments (day × female weight interaction: $F_{1,27} = 8{\cdot}42$, $P = 0{\cdot}007$) (Fig. 4). Night treatment had no effect on the remaining number of eggs found at dissection ($F_{1,27} = 2{\cdot}21$, $P = 0{\cdot}15$).

Discussion

Typical variation in experienced oviposition opportunities is likely to be too high and unpredictable to allow a perfect corresponding match of matured eggs (Papaj 2000). Thus, in order never to be egg-limited, females must always carry a surplus of eggs ready for oviposition. Allocation theory predicts that investment in eggs is traded off against the maintenance of the mother's condition (Papaj 2000; Roff 2002; Jervis, Boggs & Ferns 2005), and the relationship between early reproductive output and life span found in the oviposition experiment supports this prediction for *P. aegeria* (Fig. 3). Similar results have been found in other butterflies (Jervis, Boggs & Ferns 2005) and other insects (Carey 2001). Thus, assuming a general trade-off between survival and reproduction, one important question that still remains largely unanswered is to what extent additional temperatures outside the time window for flight activity are utilized for egg maturation and how this might affect female fecundity and correlated life-history traits?

Our results show that warm night-time temperatures increased early reproductive output by effects on egg maturation (2 vs. 2 + 6 h and 8 vs. 8 + 6 h), whereas a shortening of the time for oviposition during day time decreased reproductive output (2 vs. 8 h and 2 + 6 vs. 8 + 6 h) (Fig. 2). Thus, fecundity showed signs of both oviposition- and maturation-limitation, depending on the experimental conditions. We argue that these results, although not representing true field conditions, give important information on the physiological and behavioural mechanisms that determine realized fecundity in the wild. Although females contained no eggs at eclosion, they started to mature eggs prior to mating (maturation Experiment I, Fig. 1). Egg maturation rates were higher if females where allowed to mate and oviposit (maturation Experiment II, Fig. 1) and were always strongly dependent on temperature (maturation Experiments I and II, Fig. 1). Although realized fecundity was lower in the oviposition-limited 2 + 6 h treatment than in the less limited 8 h treatment, it is unclear whether there was a difference in maturation rates. Rather, females in the oviposition limited treatments simply might not have been able to lay all their matured eggs. The fact that oviposition limited females had a large number of mature eggs at death indicates that these females also matured eggs at high rates. In addition, there were no effects of night-time

temperatures on the number of eggs dissected from females post-mortem. Nonetheless, additional warm night temperatures had strong effects on realized oviposition rates (2 vs. 2 + 6 h and 8 vs. 8 + 6 h). It seems that the machinery driving egg maturation has a working rate strongly dependent on temperature, which under natural conditions also should be a good predictor of oviposition opportunities as oviposition behaviour by itself is temperature-dependent. If so, selection on further fine tuning of maturation rates might be rather weak. One could also speculate that in this species, which oviposits on a variety of grasses and relatively seldom would find itself limited by access to host plants, natural selection would benefit a relatively low temperature threshold for egg maturation and high rates of egg maturation during times not suitable for oviposition. Our calculations from the egg maturation experiments show that the threshold lies approximately between 8·2 C (for mated females assuming a linear relationship with temperature) and 14 C (for virgin females), and the observation that females in the temperature-limited 2 h treatment had matured an excess of eggs not laid at death support this hypothesis. In other species with a narrower range and poorer abundance of host plants, a low temperature threshold for egg maturation should be less beneficial because the risk of egg limitation is less likely while at the same time a low threshold could be associated with costs. Besides allocation trade-offs and costs of high metabolism, such as ageing, that might be associated with high maturation rates (Brown *et al*. 2004), a lowering of the threshold for egg maturation could itself be subjected to counteracting selection. It seems unlikely that different aspects of a temperature reaction norm are free to evolve completely independent of each other (Angilletta *et al*. 2003). Accordingly, selection for a lower thermal threshold may lead to reduced performance at warmer temperatures.

It should be noted that in the population of *P. aegeria* investigated here, temperatures very rarely get as high as 25 C outside the periods when oviposition is possible (e.g. during rainy weather or at night). The experimental treatments were chosen to maximize our chances to decouple egg maturation and oviposition independent of temperature regime. Still, in the field females spend long stretches of time at temperatures that are above the temperature threshold for egg maturation but below the threshold for oviposition (estimated to be *c.* 18 C; Gotthard, Berger & Walters 2007). Thus, given our results, temperature variation below the threshold for oviposition will be of great importance in determining realized fecundities and fitness-linked oviposition decisions, such as host plant selectivity. In southern Sweden, the first cohort of *P. aegeria* starts flying in late April/early May when mean temperature and especially night-time temperatures are low, generally corresponding to the 2 h treatment or even colder, whereas the subsequent cohorts flying in June and July experience considerably warmer conditions corresponding to the 8 h or, more rarely, the 8 + 6 h treatment (Gotthard, Berger & Walters 2007). This indicates that realized oviposition rates in May will often be limited by temperature-dependent egg maturation and oviposition. In the warm month of July, however, thermal conditions that allow females to mature eggs are not in short supply. Instead it seems that body resources and host plant availability in combination with the more restricted weather conditions for female flight will ultimately limit realized fecundity (Gotthard, Berger & Walters 2007). The different adult cohorts of *P. aegeria* are the result of individuals following different developmental pathways, and due to plasticity in growth and development there are substantial morphological differences among cohorts (Van Dyck & Wiklund 2002). It is therefore tempting to speculate that adults of the different cohorts may also develop adaptive differences in thermal reaction norms that would maximize realized fecundity in the expected thermal conditions of each respective cohort. If so, a clear prediction is that selection should favour a lowering of the temperature threshold for both activity and egg maturation in the May generation of butterflies, as this would increase realized fecundity substantially (Kingsolver, Izem & Ragland 2004; Gotthard, Berger & Walters 2007).

If females suffer viability costs by allocating resources to egg production, heavier females might afford to mature a larger surplus of eggs ready to be oviposited at any time because they have more body resources to invest. Therefore, it seems likely that the strategy to mature eggs at certain rates during periods of inactivity should depend on the size of the female (Thorne *et al*. 2006). This prediction is supported by the dissection data showing that large females in the oviposition-limited day treatments (2 and 2 + 6 h) had matured more eggs than smaller females at death. There are several ways in which the observed pattern can come about. Large females may be able to store more mature eggs because of their greater abdomen volume. If so, large females may mature eggs at the same rate as small females but continue for a longer period of time. However, as large females carry more resources it is possible that they mature eggs at faster rates (see Carroll & Quiring 1993), or even mature eggs at lower temperatures (e.g. night temperatures). These alternative hypotheses are by no means exclusive and it seems likely that the maturation rate and egg storage capacity are interdependent and that both are important in creating size-dependent differences in egg loads. Large females that carry more eggs are nevertheless dependent on ample oviposition opportunities in order to capitalize on their larger egg loads and, therefore, a large egg load does not necessarily translate into a high fecundity. However, in environments where conditions for oviposition in general are limited but vary stochastically in pulses, the build up of a bigger egg load will make large females better prepared for periods offering generous egg laying opportunities, while small females at these times will find themselves egg-limited (Ellers & Jervis 2003; Ellers, Sevenster & Driessen 2000; Gotthard, Berger & Walters 2007). In conclusion, warm temperatures outside the time window for flight activity might benefit large females with more resources to invest in eggs, and, likewise, cold temperatures might put greater limits to the fecundity of large individuals, if they need longer periods of sufficient temperatures to convert all their allocated resources to eggs. Thus, temperature variation experienced outside periods for oviposition are likely important in determining size–fecundity relationships and selection on body size in ectotherms.

Paradoxically, due to the problem of estimating the temperature dependence of processes under fluctuating conditions, many laboratory studies estimating size–fecundity relationships practise the use of constant and therefore unrealistic temperature regimes. Our hope is that this predicament will receive more attention in investigations of the complex relationship between temperature variation, fecundity and related size-dependent allocation trade-offs in ectotherms.

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