What Keeps Insects Small? Time Limitation during Oviposition Reduces the Fecundity Benefit of Female Size in a Butterfly

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ABSTRACT: Laboratory studies of insects suggest that female fecundity may increase very rapidly with adult size and that mass may often increase close to exponentially with time during larval growth. These relationships make it difficult to see how realistic levels of larval mortality can outweigh the fecundity benefit of prolonged growth. Hence, it is unclear why many insects do not become bigger. In this study, we experimentally explore the relationship between female size and fecundity in the butterfly *Pararge aegeria* and show that thermally induced time limitation during oviposition may substantially reduce the fecundity benefit of larger females. We model time-limited oviposition under natural temperature conditions and show that fecundity is also likely to increase asymptotically with female size in the field. With realistic estimates of juvenile mortality, the model predicts optimal body sizes within the observed range even when larvae grow exponentially. We conclude that one important reason for why insects with a high capacity of larval growth do not evolve toward larger sizes may be that the fecundity benefit is in fact relatively limited under natural conditions. If so, these results may help resolve some of the inconsistencies between theory and empirical patterns in explaining optimal size in insects.

Keywords: life-history theory, body size, growth trajectory, trade-off, fecundity, Lepidoptera.

Body size is one of the most studied traits in evolutionary biology and ecology, mainly because of its strong and mul-

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tiple effects on adult reproduction (Stearns 1992; Blanckenhorn 2000; Roff 2002). Life-history models typically assume that reproductive output increases indefinitely with adult body size because many laboratory studies show that a large adult size correlates with high competitive ability as well as high female fecundity. In insects, laboratory estimates indicate that there is often a positive allometric relationship between female fecundity and body size (Honek 1993; Blanckenhorn 2000; Roff 2002). Such patterns suggest that adult body size is under strong directional selection, but since size distributions of natural populations appear to be stable, it seems reasonable to expect strong counterbalancing selection against a larger size. The most widely accepted argument for why we are not surrounded by much bigger insects is that it takes time to grow large and that a long juvenile period increases the risk of mortality before reproduction (Kozlowski 1992; Stearns 1992; Blanckenhorn 2000; Roff 2002).

Many holometabolous insects have a very high capacity for growth, and larval mass often may increase close to exponentially with development time (Blau 1981; Wickman et al. 1990; Leimar 1996; Migeon et al. 1999; Margraf et al. 2003; Esperk and Tammaru 2004; Gotthard 2004). With this type of growth trajectory, a very limited increase in juvenile period or growth rate may lead to very large increases in final body size. For example, mean pupal weight of a laboratory population of the tobacco hornworm (*Manduca sexta*) increased by approximately 50% after 30 years of laboratory evolution (i.e., no direct selection on size; D'Amico et al. 2001). This was due to combined increases in larval growth rate, the critical size for metamorphosis induction, and the length of growth period in the last larval instar, showing that substantial evolutionary changes in body size by changes in the growth trajectory are possible over relatively short timescales.

With a positive size-fecundity allometry, it is difficult to see how realistic levels of larval mortality alone could balance the potential fitness benefits of a few extra days of near-exponential growth (Leimar 1996). This type of

inconsistency between theory and empirical patterns has led several researchers to conclude that it is difficult to see what keeps insects and many other animals small (Leimar 1996; Tammaru 1998; Blanckenhorn 2000; Gotthard 2004). One mechanism that has been suggested repeatedly is positive size-dependent predation during larval growth (Leimar 1996; Blanckenhorn 2000, 2005), and there is evidence that this can be important under certain conditions (Berger et al. 2006). Several researchers have also suggested that larval mortality is more likely to balance selection for increased size if the relationship between realized fecundity and female size shows a pattern of diminishing returns (Abrams et al. 1996; Leimar 1996). Empirical evidence for this type of relationship, however, is scarce.

In this study, we investigate to what degree realistic levels of time limitation during oviposition can produce size-fecundity relationships that show diminishing returns. Several models of oviposition behavior in parasitoids have suggested that allocation to fecundity should decrease with decreasing oviposition opportunities (Rosenheim 1996, 1999; Sevenster et al. 1998; Ellers et al. 2000; Rosenheim et al. 2000). In many insects, thermal conditions may put strong limits on the amount of time females can be active, and this will reduce realized oviposition rates (Courtney and Duggan 1983; Kingsolver and Watt 1983, 1984) and may also influence other aspects of oviposition behavior (Doak et al. 2006). The larger and potentially more fecund a female gets, the more likely it is that her realized fecundity will be limited by such time constraints rather than the number of eggs she has available for oviposition.

To test this idea, we explored the effect of thermally induced time limitation during oviposition on the relationship between female size and fecundity in the temperate butterfly *Pararge aegeria*. Earlier work on this species showed that larvae may grow exponentially (Nylin et al. 1989) and that there can be a positive allometric size-fecundity relationship when laboratory conditions are set to allow females to realize their full reproductive potential (Karlsson and Wickman 1990). We predicted that the relationship between female weight and fecundity should be strongly positive in thermal conditions that allow plenty of time for oviposition, whereas it should be weaker or nonexistent in more time-limited conditions. In a follow-up experiment, we established the relationship between ambient temperature and oviposition rate so that we could use temperature data from the field to estimate to what degree females are likely to be time limited in the natural situation. With these results, we constructed a model of time-limited oviposition to predict the shape of the size-fecundity relationship in the natural thermal conditions of the original field site. This allowed us to perform a life-history optimization to predict optimal sizes with and without time limitation on female oviposition.

Material and Methods

The speckled wood butterfly *Pararge aegeria* has a wide distribution throughout Europe, Asia, and northern Africa. The typical habitat is forests, where the butterflies are often found in clearings and along small roads and paths. Females typically mate only once soon after emergence and then spend the rest of their adult life searching for oviposition and feeding sites. Eggs are laid singly on larval host plants (various grasses), preferably in moist and shady locations (Wickman and Wiklund 1983). There are no field estimates of female lifetime expectancy in *P. aegeria*, but males in England and Sweden have a mean life span of approximately 6 days (Davies 1978; Wickman and Wiklund 1983), and females of our Swedish study population lived approximately twice as long as the males in the laboratory (Gotthard et al. 2000). Due to the forest habitat with large shady and colder areas interspaced with areas warmed by the sun, females typically encounter a highly variable temperature environment during their searches. In this study, we used butterflies from a bivoltine population in southern Sweden that has an unusually complex phenology (Nylin et al. 1989). Due to its two alternative diapausing strategies, this population of *P. aegeria* flies during three and sometimes four distinct peaks of the season and therefore experiences the whole range of temperature conditions that are present in southern Sweden between early May and September (Van Dyck and Wiklund 2002).

Size-Fecundity Experiment

Ten females were collected in September 2003 and allowed to oviposit together in a population cage. Individuals from a random subset of the resulting larvae were reared on the grass *Dactylis glomerata* in laboratory conditions that produce direct development (photoperiod 19L : 5D, temperature 17°C). We noted date of pupation and pupal weight as well as the sex of all individuals. At this point, we randomly assigned all female pupae ($N = 89$) to their respective future treatments during oviposition.

We weighed and marked all adults individually after they had released their meconium, and we kept them in a cold room (10°C) until mating. Since hatching was somewhat asynchronous, this waiting period varied between 2 and 7 days among females (4.5 ± 0.17 days [mean $\pm$ 1 SE]). Females were mated in cages (0.5 m × 0.5 m × 0.5 m) with constant access to sugar water but no host plants. The cages were inspected every 15 min to ensure that all matings were recorded (*P. aegeria* do not mate during the night). After mating, all females were transferred to the cold room, where they waited for 2 days before they were allowed to start oviposition. During the course of the ovi-

position experiment, we kept all females individually in 1,000-mL transparent plastic jars with a tuft of the host plant *D. glomerata* cultured in water through a small hole in the bottom of the jar. Throughout the experiment, all females had access to a piece of cotton soaked with sugar water (renewed every day), and they were kept in climate cabinets that were set to produce two treatments. In the nonlimited treatment (NL), the temperature was raised to 25°C for 8 h between 1000 and 1800 hours, while the rest of the time it was held at 14°C. In the limited treatment (L), we set the cycle to give six 20-min periods of 25°C between 1000 and 1800 hours every day (temperature rise began at 1000, 1100, 1300, 1400, 1600, and 1700 hours), while the rest of the time the temperature was held at 14°C. To estimate the difference in temperature and time for oviposition between treatments (time above 18°C), we logged the temperatures at 1-min intervals for 6 days (NL treatment: mean $T = 16.9$°C, oviposition time $= 8.1 \pm 0.009$ h day^{-1} [mean $\pm$ 1 SD]; L treatment: mean $T = 14.1$°C, oviposition time $= 2.2 \pm 0.02$ h day^{-1} [mean $\pm$ 1 SD]). The photoperiod in both treatments was 17L : 7D, which mimics summer conditions in southern Sweden.

We counted the eggs laid 2 days after the start of the experiment, then every third day for the first 14 days, and then twice a week until the females died. At each census time, we transferred the females to a new oviposition jar with a fresh host plant and randomized the position of oviposition jars in the climate cabinets. We calculated cumulative fecundities for each female and census point, but due to the field estimates of lifetime expectancy, we concentrated on the different estimates from the first 14 days of oviposition as well as lifetime fecundity. We analyzed differences in fecundity between treatments by ANCOVA, testing the effects of weight (covariate) and treatment (fixed factor) as well as the interaction between these factors, because this was the most direct test of our hypothesis. To account for potential effects of variation in female age at the start of the experiment, we always included this covariate in our analyses. We also examined the relationship between size and fecundity by regressions for each treatment. In no case did we find a significant allometric relationship between fecundity and size, and we therefore present results for untransformed values only.

We examined the effects of treatment, weight, and reproductive investment early in life on mortality rates by using Cox's proportional hazards regression (Cox 1972; Fox 2001). As a measure of early life reproduction, we used fecundity after 5 days of oviposition because this was the latest estimate we could obtain for all females (a few died between 5 and 8 days). All statistical analyses were performed with Stata 8.2 (Statacorp 2005).

Temperature-Threshold Experiment

For this experiment we reared the grand-offspring of 20 field-collected females on *D. glomerata* and used the same setup for mating and oviposition as in the size-fecundity experiment. We allowed 99 females to oviposit in temperature-controlled cabinets that were set to a 17L : 7D regime. We verified maturity of females beforehand by allowing individuals to freely oviposit for 8 h at 25°C on the first day, and we excluded from analysis those that did not lay any eggs during this period (nine cases). On the second day, we raised cabinet temperatures to one of eight temperatures (15°, 17°, 18°, 19°, 20°, 22°, 25°, or 30°C) for 3 h at midday; the rest of the time, temperatures were a constant 14°C. On the third day, we removed the females from their jars and counted the number of eggs laid.

We fitted a Boltzmann sigmoidal function (eq. [1]) to all available data on oviposition rates using the nonlinear least squares function in R (R Development Core Team 2005):

$$y = \frac{A + (B - A)}{1 + \exp\left[(C - T)/D\right]}, \tag{1}$$

where T is temperature (°C), A is the bottom and B the top of the asymptotic function, C is equal to $T = 0.5(B - A)$, and D is the slope. To further analyze the relationship between oviposition rate and temperature at and above 19°C, we used linear regression.

Results

Size-Fecundity Experiment

There was one extremely small female (<40 mg), and to make sure that this outlier did not have a disproportionate influence on significance tests, we repeated all analyses without this data point. Unless it is specified, this exclusion did not affect any conclusions, and all reported statistics are calculated on the total data.

There were significant positive effects of female weight on cumulative fecundity already at the first two censuses (ANCOVA after 2 and 5 days of oviposition, $P < .01$ in both cases) but no significant effects of treatment ($P > .4$ in both cases) or the interaction ($P > .15$ in both cases). There was also a positive effect of female age at the start of oviposition at these two early censuses ($P = .04$ and .07, respectively), but at subsequent census points, female age at the start had no significant influence on fecundity ($.15 < P < .56$). The cumulative fecundity at day 8 was significantly affected by the interaction between female weight and treatment, and this was also true for all subsequent census points (days 11, 14, 17, and 20, but only results from days 8 and 14 are shown; ANCOVA at day 8: weight
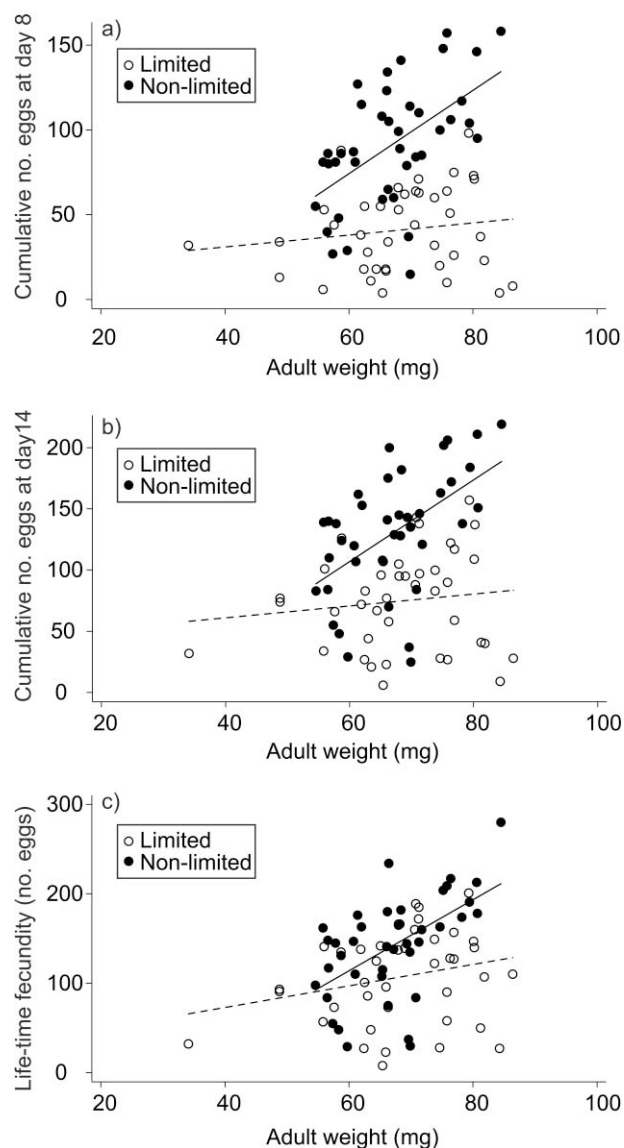
Figure 1: Results of the oviposition experiment: filled symbols show the nonlimited treatment and open symbols show the limited treatment. Solid lines show linear regressions for the nonlimited treatment (all slopes significantly different from 0), whereas dotted lines show relationships for the limited treatment (none of the slopes differed significantly from 0). *a*, Relationship between fecundity and female weight after 8 days of oviposition. *b*, Relationship between fecundity and female weight after 14 days of oviposition. *c*, Relationship between lifetime fecundity and female weight.

$F = 15.8$, df $= 1, 76$, $P = .0002$; treatment $F = 3.5$, df $= 1, 76$, $P = .065$; weight × treatment $F = 8.9$, df $= 1, 76$, $P = .0039$; ANCOVA at day 14: weight $F = 13.2$, df $= 1, 76$, $P = .0005$; treatment $F = 3.6$, df $= 1, 76$, $P = .063$; weight × treatment $F = 7.4$, df $= 1, 76$,

$P = .0082$). The significant interactions were in all cases due to a strong and significant effect of weight on fecundity in the nonlimited treatment (fig. 1*a*: linear regression coefficients [1 SE]: fecundity [day 8] $= -73$ [42] $+ 2.5$ [0.62] × weight, $r_{adj}^2 = 0.27$, $F = 15.6$, df $= 1, 38$, $P = .0003$; fig. 1*b*: fecundity [day 14] $= -93$ [59] $+ 3.3$ [0.87] × weight, $r_{adj}^2 = 0.26$, $F = 14.6$, df $= 1, 38$, $P = .0005$) and a nonsignificant effect of weight in the limited treatment (day 8, $F = 0.36$, df $= 1, 38$, $P = .36$; day 14, $F = 0.64$, df $= 1, 38$, $P = .43$). This pattern was also found already after 2 and 5 days of oviposition, although the differences in relationship among treatments did not attain statistical significance. Oviposition rate declined with female age, and the vast majority of eggs were laid during the first 20 days of oviposition in both treatments (eggs laid before day 20: nonlimited $= 97.8\%$, limited $= 86.9\%$).

Total lifetime (LT) fecundity was also significantly affected by the interaction between female body weight and treatment (ANCOVA: weight $F = 17.3$, df $= 1, 76$, $P = .0001$; treatment $F = 3.1$, df $= 1, 76$, $P = .081$; weight × treatment $F = 5.0$, df $= 1, 76$, $P = .028$). Again, this difference was due to a significant and positive relationship between oviposition and body weight in the nonlimited treatment (fig. 1*c*; linear regression coefficients [1 SE], LT fecundity $= -125$ [66] $+ 4.0$ [0.98] × AW, $r_{adj}^2 = 0.29$, $F = 16.6$, df $= 1, 38$, $P = .0002$) and a nonsignificant effect of female size on oviposition in the limited treatment ($F = 2.5$, df $= 1, 38$, $P = .12$).

Mortality rate was higher in the nonlimited treatment (Cox proportional hazards regression; treatment: hazard ratio ± 1 SE $= 0.41 \pm 0.13$, $z = -2.9$, $P = .004$, average life span ± 1 SE in the nonlimited treatment $= 18.9 \pm 1.1$ days, $N = 40$; limited treatment $= 26.8 \pm 1.2$ days, $N = 40$). There was a negative relationship between adult size and mortality rate (adult weight: hazard ratio ± 1 SE $= 0.97 \pm 0.01$, $z = -2.1$, $P = .025$) but no significant effect of oviposition rate the first 5 days of life ($P = .39$). The total model was highly significant (likelihood ratio $\chi^2 = 22.7$, df $= 3$, $P < .0001$). However, excluding the smallest outlying female from this analysis had a substantial influence on the probability of the effect of adult weight (without outlier, $P_{weight} = .093$). Considering this and the lack of relationship between reproduction in early life and mortality, we conclude that the difference in life span between treatments was mainly a direct effect of different thermal environments. The longer life span of females in the limited treatment explains why there was a relatively small difference in total number of eggs laid between treatments despite the relatively large difference in cumulative fecundity earlier in life (fig. 1).

Temperature-Threshold Experiment

The rate of oviposition showed a threshold response to temperature (fig. 2). Only a single egg was laid in each treatment when daytime temperatures were raised to 15° or 17°C. The estimated temperature at which mean oviposition rates reach half their maximum is 19.14°C, though oviposition rates measured at 19°C and above showed no significant relationship to temperature ($F = 1.085$, $df = 1, 53$, $P = 0.302$, $r^2_{adj} = 0.02$).

Modeling Procedures

To ascertain what effects field temperatures may have on the fecundity-size relationship, we built a life-history model based upon our results. First, we assume that potential lifetime fecundity E_{total} increases as a linear function of adult weight (w_a) and can be calculated as potential daily oviposition rate e_i summed up to the maximum life span l of an individual, assuming a linearly decreasing survival function:

$$E_{total} = m + kw_a = \sum_{i=l}^{l} e_i\left(1 - \frac{i}{l}\right). \tag{2}$$

Second, we assume that potential daily oviposition rate e_i decreases as a linear function of age (fig. 3):

$$e_i = a - bi, \tag{3}$$

where a is the intercept, b is the slope coefficient, and i is mature adult age (days). Finally, based on the results of our experiments, we assume that an increase in potential
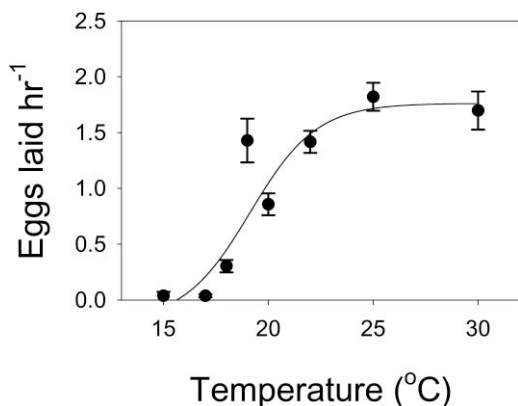

Figure 2: Oviposition rate as a function of temperature. Symbols and bars represent mean ± 1 SE. Fitted curve is a Boltzmann sigmoidal function (parameter ± 1 SE: top = 1.762 ± 0.2917; bottom = −0.1923 ± 0.7381; V_{50} = 19.14 ± 1.565; slope = 1.579 ± 1.446).
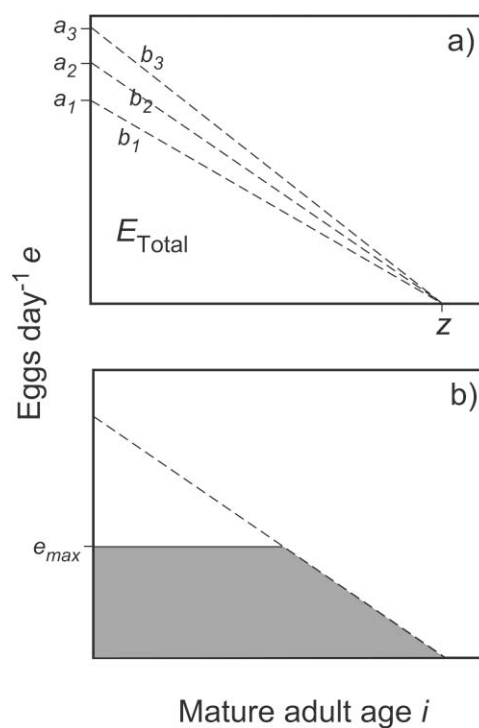

Figure 3: Graphical representation of the oviposition model showing how potential fecundity is influenced by female weight and time limitation during oviposition to produce realized fecundities. In each case, the dashed line represents potential daily fecundity due to egg limitation that decreases with female age until it is 0 at age z. The area under each line equals potential lifetime fecundity E_{Total}. a illustrates how an increase in E_{Total} for three different adult sizes, corresponding to three different sets of the coefficients a and b in equation (3), affects potential daily fecundity. In b, the solid line represents realized daily fecundity as a consequence of time constraints (e_{max}); the shaded area represents realized lifetime fecundity of an individual surviving to its maximum life span l.

lifetime fecundity due to an increase in adult size is realized through an increase in oviposition rate per day and not through an increase in the length of the period of oviposition (fig. 3). For all females, $e_z = 0$, where z is a constant number of days since start of oviposition (estimated in the size-fecundity experiment to be on average 33 days). Given this third assumption, we can determine the coefficients of age-dependent oviposition rate for a given adult size (eq. [3]).

Next, we introduce time constraints on daily fecundity in a simulation model where realized daily fecundities are determined by both the capacity to lay eggs (eqq. [2], [3]) and the time available to lay eggs (fig. 3*b*). To calculate actual time constraints on potential daily fecundities under field conditions, we combined the temperature-threshold function of oviposition rate (fig. 2; for values >0) with variation from an hourly time series of ambient temper-

ature for the summer of 2005 and mean monthly temperatures for the period 1961–1990, available from the nearest meteorological station (table 1; the Swedish Meteorological and Hydrological Institute). To account for this insect's potential use of radiant heat to thermoregulate, we assume that females can increase their body temperature during periods of sunshine (data on hours of sun from the Swedish Meteorological and Hydrological Institute) and added this to ambient temperature. Studies of the thermal biology of *Pararge aegeria* (Van Dyck and Matthysen 1998; Berwaerts et al. 2001; Kemp and Wiklund 2004) and other satyrid butterflies (Heinrich 1986) indicate that individuals may increase their body temperature 8.5°C above ambient while basking but lose up to two-thirds of this temperature excess as soon as they initiate flight. We therefore assume that females on average have a body temperature excess of half the maximum (4.25°C) during periods of sunshine but also calculate optimal sizes for the two most extreme points of thermoregulation (0°C or 8.5°C rise in internal temperature). We simulated 100,000 females for each 1-mg weight interval and constructed size-fecundity functions for different thermal conditions. Note that at this point we did not assume any external sources of mortality (e.g., predation) other than an intrinsic survival function governed by maximum lifespan *l*.

The results of the size-fecundity experiment strongly suggest that thermal conditions influence the mean life span of females, whereas there was weak support for a positive size-survival relationship. The temperature effect is in line with other studies of *P. aegeria* (Karlsson and Wiklund 2005), and to at least crudely include this effect in our model, we estimated mean female life expectancy (*l*/2) for the months May to July as a function of mean monthly temperature and used these estimates in the model (appendix). In addition, we investigated how the potential positive relationship between size and life expectancy would influence predicted optimal size (appendix).

To assess what effects time constraints on potential fecundity have on predictions of optimal adult size, we calculated total fitness for females as

$$F = 0.5M(1 - \mu_1)^d (1 - \mu_2)^p \sum_{i=l}^{l} e_i \left(1 - \frac{i}{l}\right)(1 - \mu_3)^i, \quad (5)$$

where *F* is fitness; 0.5 represents genetic contribution of a female; *M* is a constant egg survivorship (proportion); μ_1, μ_2, and μ_3 are juvenile, pupal, and adult mortality rates (proportion day^{-1}), respectively; *d* is development time from hatching to pupal stage (days); *p* is duration of pupal stage (days); and *e* is potential daily fecundity (eggs day^{-1}) as a function of mature adult age *i* since first day of ovipositioning up to maximum expected life expectancy *l*. To calculate development time *d* for different adult sizes, we modeled the relationship between larval mass and time as an exponential growth trajectory (see appendix for details and parameter values).

Modeling Results

The model shows that thermal time constraints on potential daily fecundity can significantly reduce lifetime fecundity, particularly so for larger adult sizes, causing the fecundity-size relationship to approach an asymptote (fig. 4*a*, 4*b*). When optimal adult size is modeled assuming time constraints on potential daily fecundity, predictions fall within the upper range of observed body size, and optimal adult size is relatively stable over a range of natural thermal conditions and assumptions of thermoregulatory capacity (fig. 4*c*, 4*d*; table 2). The effect of a positive size-survival relationship of the magnitude present in our data had a limited effect on predicted optimal sizes (the maximum increase in optimal size, predicted for the month of July, was just 5 mg). In contrast, without time limitation on oviposition, fitness is expected to increase indefinitely with female size (fig. 4).

To verify that the simplified assumptions of the model were reasonable, we tested how well the model could recreate lifetime fecundities in the laboratory experiment. In the nonlimited treatment, the empirical estimates of lifetime fecundity was between 95 and 215 eggs for female sizes ranging from 55 to 85 mg, whereas the model predicts fecundity to range from 99 to 195 eggs, assuming no effect of adult size on longevity, or 120–242 eggs, assuming a positive effect of adult size on longevity. In the limited treatment, there was no significant effect of female size on total fecundity, which on average was 110 eggs (1 SE = ±8; fig. 1*c*). The predicted shallow slope of fecundity against adult size is consistent with this observation, and predicted fecundities are between 68 and 74 eggs, or between 79 and 89 eggs for the range of adult sizes above,

Table 1: Mean daily temperatures at the weather station closest to the field site for the period 1961–1990

	May	June	July	August	September
Mean daily temperature (°C)	11.2	15.2	16.8	16.7	13.5

depending on whether the effects of adult size on longevity are assumed. These predictions suggest that the role of adult size on life span may be important under poor thermal conditions. In both treatments, model predictions are within the observed ranges, suggesting that the simplifying assumptions of the model are reasonable.

To investigate the effect of variation among days per se, we also performed an analysis where we included variation around the mean conditions of each laboratory treatment (variation added was equivalent to the field situation). This exercise suggested that fecundity increases more slowly with size and reaches the asymptote at a larger size when there is variation among days (fig. 4*b*). Hence, when the daily temperature regime varies among days, optimal adult sizes are predicted to be larger than when there is no variation among days (fig. 4*d*).

Discussion

When thermal conditions allowed a large amount of time for activity, there was a strong positive relationship be-

Table 2: Predicted optimal sizes for three typical months during the flight period, including the full range of potential thermoregulatory efficiency of *Pararge aegeria*

	Optimal female size (mg)		
Thermoregulation	May	June	July
None (ambient temp.)	75 (.20)	93 (.73)	99 (.98)
Mean (+4.25°C)	78 (.37)	99 (1.02)	106 (1.23)
Maximum (+8.5°C)	94 (.88)	113 (1.36)	120 (1.68)

Note: Predicted fitness at the respective optimal size is given in parentheses.

tween body size and fecundity, whereas there was no significant fecundity benefit of large size in thermal conditions that restricted oviposition opportunities (fig. 1). Although oviposition rates in general were lower in the limited treatment, the reduction in oviposition was clearly most prominent in the largest females (fig. 1). Hence, with this level of time limitation, the higher potential fecundity of large females will be of minor value. It is possible that differences in time for feeding and egg maturation rate
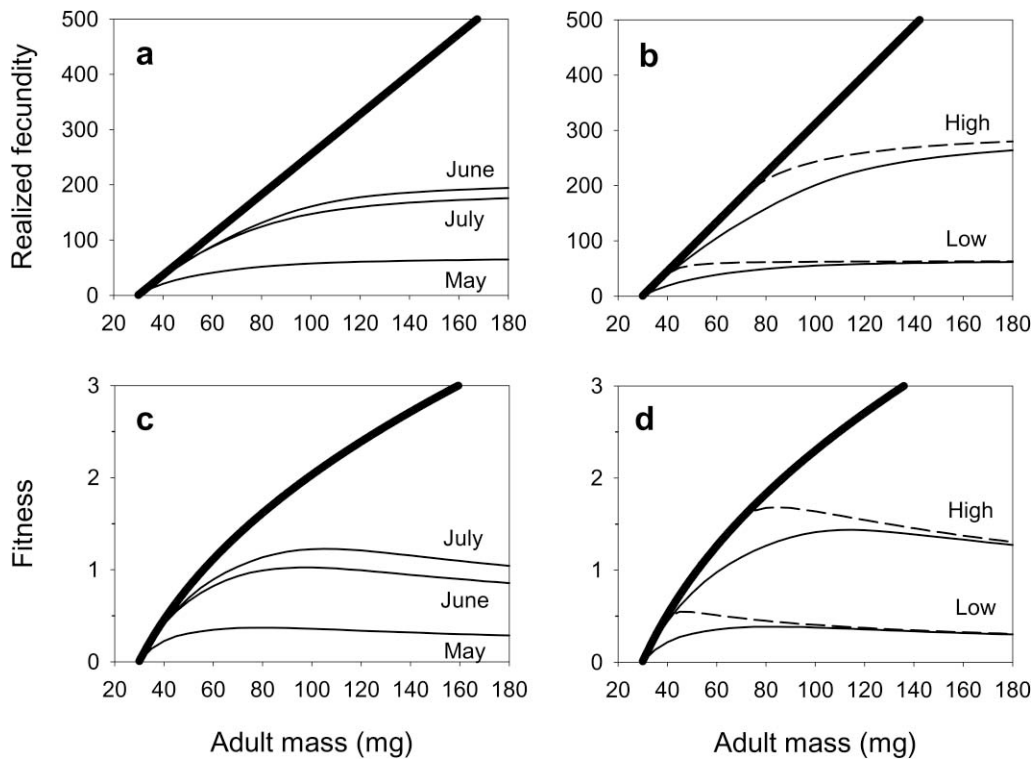

Figure 4: Predicted size-fecundity (*a*, *b*) and size-fitness (*c*, *d*) relationships for *Pararge aegeria*. In each case, bold lines indicate predictions of lifetime fecundity assuming no time limitation during oviposition, whereas thin lines indicate predictions assuming time constraints for various thermal conditions. *a* and *c* show mean effects of natural variability in temperature and sunshine hours between days for the months May to July; *b* and *d* show mean effects of constant (*dashed lines*) versus natural variability (*solid lines*) in mean daily temperatures that are representative of the limited and nonlimited treatments. For the field estimates, female body temperature was 4.25°C above ambient during all periods of sunshine. See appendix for list of model parameter values.

may also have influenced the results. For example, large females may be capable of sustaining higher rates of egg maturation compared with smaller females, but the thermal conditions in the limited treatment did not allow them to fully realize their high maturation rates (Carroll and Quiring 1993). The direct measurement of the thermal threshold for oviposition suggested that females needed an ambient temperature of approximately 18°C to be able to oviposit (fig. 2). In combination, these results show that thermal conditions are likely to create time limitation that may have strong effects on the fecundity benefit of a large female size.

Our modeling of the field situation confirms that natural levels of time limitation is likely to produce size-fecundity relationships that show a diminishing returns pattern (fig. 4). The size-fecundity function will most likely vary over the season and typically reach an asymptote at lower fecundities in May conditions compared with the situation in June and July. However, with some level of time limitation, the model always predicts a pattern of diminishing returns, and this result is relatively insensitive to exact model assumptions. With this type of size-fecundity function and realistic mortality estimates, the life-history model predicts that a considerable increase in female size would typically lead to reduced fitness even when larval growth is exponential (fig. 4). In contrast, without time limitation on oviposition, fitness is expected to increase indefinitely with female weight. The predicted optimal sizes are within the upper range of observed sizes, even for a wide range of thermoregulatory capacities (table 2). At the highest level of thermoregulation (+8.5°C), females can essentially oviposit whenever there is sun, and the amount of sunshine becomes the limiting factor. The additional assumption of a positive size-survival relationship causes a relatively limited increase in optimal size. The reason for this is that both the decline in oviposition rate with age and the risk of predation reduce the benefit of oviposition late in life.

Interestingly, the results of the model suggest that variation in thermal conditions in itself may select for a larger female size (fig. 4d). This is because the potential costs and benefits associated with variable time constraints are asymmetric. Consider a typical day, where only 3 h are available for oviposition; both small and large individuals may be time constrained and will lay an equal number of eggs. Hence, we expect no relationship between fecundity and size. Now consider the scenario where, again, on average only 3 h are available for oviposition but where time available for oviposition between days may range from 0 to 8 h. On many days, both small and large individuals will still be time constrained and will lay an equivalent number of eggs, but on days where there is ample time to oviposit, larger individuals will lay more eggs than smaller individuals. Over their lifetimes, the larger individuals become more productive than small individuals, and hence, there is a positive relationship between fecundity and size. This general result is in agreement with models on parasitoids, suggesting that increased variation in oviposition opportunities may select for a higher potential fecundity (Ellers et al. 2000).

Our results suggest that the evolution toward a larger body size is constrained by the available opportunities for oviposition, which, in this case, is ultimately limited by the thermal threshold for oviposition. As a lowering of the threshold is predicted to increase realized fecundity, particularly for the months of May and June, we would expect that the threshold is itself subject to directional selection (Kingsolver et al. 2004; Walters and Hassal 2006). It is therefore important to consider what factors are likely to influence the evolution of thermal thresholds. It is clear that thermal thresholds for activity may vary among and within insect species but also that the evolutionary trajectories of these thresholds are influenced by trade-offs (Gilchrist 1995; Angilletta et al. 2003). One important class of such trade-offs arises because thermal thresholds are likely to be correlated with other aspects of the thermal reaction norm, such as optimal or maximal temperatures for adult behavior (Ratte 1985; Gilchrist 1995; Angilletta et al. 2003; Kingsolver et al. 2004). For example, the nocturnally active spruce bud moth *Zeiraphera canadensis* readily oviposits at an ambient temperature of 10°C, but at a point between 20° and 25°C, an increase in ambient temperature starts to have negative effects on oviposition parameters (oviposition rate, egg viability; Carroll and Quiring 1993). In comparison, *Pararge aegeria*, with a threshold for oviposition of approximately 18°C (fig. 2), shows reduced oviposition performance at an ambient temperature of 30°C (Karlsson and Wiklund 2005). Although it is not possible to generalize from this particular observation alone, it still indicates that different aspects of the thermal reaction norm are unlikely to evolve completely independently. Indeed, it seems likely that the thermoregulatory behaviors displayed by many insects (e.g., basking, shivering) are adaptations for escaping these types of thermal constraints (Gilchrist 1995). These adaptations permit a high degree of fine tuning of body temperatures (heating and cooling) and allow behavioral flexibility in a thermally variable environment. However, at this point we can conclude only that our results draw attention to a need to better understand the evolution of thermal thresholds for oviposition and activity in insects.

Empirical support for the notion that thermal conditions may limit oviposition and that this may influence the evolution of potential fecundity is given by work on *Colias* butterflies from different altitudes (Springer and Boggs 1986). A high-altitude population experienced ther-

mal conditions that restricted the amount of time females could oviposit, and females of this population emerged with fewer oocytes, yielding lower potential fecundity compared with a low-altitude population. Although female size did not differ between the populations, the results suggested that a temperature-dependent reduction of oviposition opportunities might select for a lower potential fecundity in insects. Moreover, in a comparative study, Wiklund et al. (1987) found that satyrid and pierid butterfly species adapted to sun-exposed environments are more fecund and lay smaller eggs for their body size compared with satyrids from low-temperature environments. The authors suggest that fecundity of the more cold-adapted species is constrained by their thermal environment and that selection for increased fecundity through reduced egg size is relatively weak; instead, egg size scales allometrically with female size. In contrast, species from sun-exposed habitats are expected to experience strong selection to minimize egg size in order to increase total fecundity, and there is no covariation between female size and egg size in these species (Wiklund et al. 1987).

Field studies of parasitoid wasps have found fitness–body size relationships that show (1) diminishing returns patterns (Kazmer and Luck 1995; West et al. 1996), (2) that fitness increases more slowly with size in the field than in the laboratory (Visser 1994), and (3) that the relationship between size and fecundity may vary over the season (Ellers et al. 2001). In lepidopterans, field estimates of the weight-fecundity relationship have shown strong positive linear relationships (Tammaru et al. 1996, 2002). However, these lepidopteran species have very pronounced capital breeding strategies. For example, females in one of the investigated genera are wingless and deposit all their eggs on their pupal cocoon (Tammaru et al. 2002). In general, it may be expected that the link between potential and realized fecundity is particularly strong in capital breeders, but along the continuum from capital to income breeding, this link is likely to become weaker as an increasing number of adult activities (e.g., feeding, predator avoidance) obscure the correlation (Leather 1988; Boggs and Ross 1993; Tammaru and Haukioja 1996; Boggs and Freeman 2005).

In our study, we manipulated oviposition opportunities by the thermal environment only, but the suggested mechanism holds great generality. It is clear that ambient temperature is not the only factor that may limit oviposition opportunities, and the same reasoning can be applied to other ecological factors, such as predator presence, host availability (Sevenster et al. 1998; Ellers et al. 2000; Rosenheim et al. 2000), and male harassment (Gibbs et al. 2005). In the experiments reported here, there was always a host available as soon as temperatures allowed oviposition, and this is obviously an unrealistic scenario. Therefore, it is possible that real oviposition rates in the field are lower than the estimates provided here and that this could further diminish the fecundity benefit of an increased female size.

In a review of the evolution body size, Blanckenhorn (2000) recognized the central question in the field is what keeps organisms small and concluded that there is a substantial lack of empirical evidence that a large adult size is costly. This question is particularly pertinent for insects that have a very high growth potential during the larval stage. The approach taken here is not to search for a cost but to scrutinize the presumed benefit of a larger size in insects under more natural conditions. We argue that time limitation during oviposition is likely to influence the relationship between female size and realized fecundity so that the fecundity benefit of an ever-increasing female size will be substantially lower than what is usually expected. Moreover, with this type of size-fecundity relationship, the conventional costs of a longer development (Kozlowski 1992; Stearns 1992; Blanckenhorn 2000; Roff 2002) and high larval growth rates (Arendt 1997; Gotthard 2001) are typically strong enough to stabilize selection for a larger insect size even when larval growth is exponential. If so, these results may help resolving some of the inconsistencies between theory and empirical patterns and may be one important explanation for why many butterflies and other insects do not evolve to larger sizes.

Acknowledgments

APPENDIX

Table A1: Details and summary of model parameters

	Description	Values used in model	Observed values	Details	Source
M	Egg survivorship (proportion)	.90	.90	*Anthocharis cardamines*	Courtney and Duggan 1983
μ_1	Juvenile mortality rate (proportion day^{-1})	.10	.12, .14, .11	*Pararge aegeria*, *Pieris brassicae*, *Papilio polyxenus*	Kristensen 1994; Feeny et al. 1985; B. Karlsson, unpublished data
μ_2	Pupal mortality rate (proportion day^{-1})	.03	.02, .10	*Papilio machaon*, *P. polyxenus*	Wiklund 1975; Feeny et al. 1985
μ_3	Adult mortality rate (proportion day^{-1})	.07*	.15, .10, .12–.21	Male *P. aegeria*; females of *P. aegeria*, assuming they live up to twice as long as males in the field; male *A. cardamines* between years	Wickman and Wiklund 1983; Courtney and Duggan 1983; Gotthard et al. 2000
d	Larval development time (days)	Variable	31–35	Modeled by growth function: $w_a = (1-\beta)w_h\exp^{(gd)}$	Nylin et al. 1993
p	Pupal development time (days)	15	15–16	Means for different photoperiods (18–22 h; 17°C)	Nylin et al. 1993
e_i	Potential daily fecundity (eggs day^{-1})	Variable	0–28	Parameter freely varied in the model	
l	Maximum adult life expectancy, estimated as 2 × mean adult life span (days)	Nonlimited = 38, limited = 54	37.8, 53.6	Nonlimited (16.9°C) and limited (14.1°C) treatments	See "Results"
		Months May–July calculated as a function of mean temperature T	53.6, 37.8, 31.4, 22.6, 19.2, 11.4, 2.6	For mean temperatures: 14.1, 16.9, 20.0, 25.0, 30.0, 35.0, and 40.0°C: $l = -.0062T^3 + .5464T^2 - 16.892T + 200.26$	See "Results"; Karlsson and Wiklund 2005
		Adjusted for effects of adult size ($b = .21$)	$b_{\text{Nonlimited}} = .22$, $b_{\text{Limited}} = .20$	If $l = a + bw_a$, then $l_{wa} = (l - 67.5b) + bw_a + l$, where, $67.5 = $ mean w_a	See "Results"
z	Mean adult age where $e_i = 0$ (days)	33	33	Calculated by linear regression of oviposition rate on age	Data on all individuals in the size-fecundity experiment
β	Mass lost during pupal stage (proportion)	.57	.57	When reared under "standard conditions" (17°C, 20 h)	Nylin et al. 1993
w_h	Hatching mass (mg)	.4	.4	Little phenotypic variability	K. Gotthard, unpublished data
w_a	Adult mass (mg)	Variable	34–87	Modeled by growth function: $w_a = (1-\beta)w_h\exp^{(gd)}$	Figure 1
g	Growth rate (mg mg^{-1} day^{-1})	.22	.19–.22	Means for different photoperiods (18–22 h, 17°C): $\log(g) = [\log(w_a) - \log(w_h)]\ d^{-1}$	Nylin et al. 1993

Note: Estimates of mortality rates are taken from field and field cage experiments for *Pararge aegeria* and other species of temperate butterfly. Values for life-history traits are taken from laboratory experiments on the directly developing generation of a south Swedish population of *P. aegeria*. The asterisk denotes that the parameter value represents the predation rate required for overall adult mortality rate to equate to 0.1, given the assumption of an intrinsic adult life span for June.

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