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Ecological Constraints on Female Fitness in a Phytophagous Insect

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ABSTRACT: Although understanding female reproduction is crucial for population demography, determining how and to what relative extent it is constrained by different ecological factors is complicated by difficulties in studying the links between individual behavior, life history, and fitness in nature. We present data on females in a natural population of the butterfly *Leptidea sinapis*. These data were combined with climate records and laboratory estimates of life-history parameters to predict the relative impact of different ecological constraints on female fitness in the wild. Using simulation models, we partitioned effects of male courtship, host plant availability, and temperature on female fitness. Results of these models indicate that temperature is the most constraining factor on female fitness, followed by host plant availability; the short-term negative effects of male courtship that were detected in the field study were less important in models predicting female reproductive success over the entire life span. In the simulations, females with more reproductive reserves were more limited by the ecological variables. Reproductive physiology and egg-laying behavior were therefore predicted to be co-optimized but reach different optima for females of different body sizes; this prediction is supported by the empirical data. This study thus highlights the need for studying behavioral and life-history variation in orchestration to achieve a more complete picture of both demographic and evolutionary processes in naturally variable and unpredictable environments.

Keywords: sexual conflict, life history, temperature, host preference, time limitation, parental investment.

Introduction

Knowledge of how ecological factors limit reproductive output in nature is of major importance for predicting population responses on both spatial and temporal scales. In species where parental care is restricted to the placement of eggs in suitable habitats or on suitable hosts, male fitness is often approximated as the lifetime number of matings

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acquired (e.g., Bateman 1948; Svensson et al. 2006; Delaney et al. 2007), whereas female fitness is a function of the total number of eggs laid (e.g., Wiklund et al. 2001; Roff 2002; Rosenheim et al. 2008) and the quality of the chosen egg-laying sites (e.g., Jaenike 1990; Minkenberg et al. 1992; Doak et al. 2006). In such mating systems, population growth rate is intimately related to the female reproductive strategy (Roff 2002). A more mechanistic understanding of the constraints on female reproductive output is thus crucial for predicting evolutionary responses and population persistence. However, our understanding of how these mechanisms constrain fitness in mobile organisms such as flying insects is compromised by the inherent difficulties associated with tracking the movements of individuals under field conditions for periods of sufficient duration to allow quantification of life history, reproductive behaviors, and associated reproductive output.

In insects and other ectotherms, growth rates and activity levels are typically constrained by low temperatures (Frazier et al. 2006; Angilletta 2009), and responses to such thermodynamic constraints often entail compensatory behaviors (Angilletta et al. 2003; Huey et al. 2003; Dial et al. 2008). Another obvious constraint on female reproduction is a lack of hosts or habitats that provide food and shelter for offspring (Awmack and Leather 2002). Similar to the restricting effects of low temperatures on activity and the time available for females to search for suitable egg-laying sites, other factors such as the presence of predators or courting males might reduce a female's time budget for ovipositing (Leather 1988; Gotthard et al. 2007).

Females that require more time to search for high-quality hosts than it takes for new eggs to mature are considered to be time limited, whereas females that encounter more egg-laying opportunities than they can utilize are egg limited (Rosenheim 1996; Sevenster et al. 1998; Rosenheim et al. 2008). In nature, individual females typically fall into one category or the other, because environmental

stochasticity makes a perfect match between the number of mature eggs ready for ovipositing and available egg-laying sites highly unlikely (Schaffer 1974; Ellers et al. 2000; Rosenheim 2011). In terms of increased intrinsic mortality, there are likely severe costs involved in maintaining a high rate of egg maturation (which would minimize the risk of egg limitation; Papaj 2000). Natural selection is therefore predicted to shape reproductive rates according to the relative risks of egg and time limitation (Rosenheim 1996; Sevenster et al. 1998). Furthermore, the potential fecundity of a female insect is largely determined by the amount of body reserves she can convert into eggs, which usually increases sharply with body size (Honěk 1993). Large females are therefore predicted to need more time to realize their potential fecundity, and consequently they should become time limited more often than small females (Wiklund and Karlsson 1988; Ellers and Jervis 2003; Gotthard et al. 2007). It is thus predicted that intraspecific variation in life history is likely to lead to different solutions to the trade-off between reproduction and life span (Ellers and Jervis 2003; Berger et al. 2012).

A behavioral compensatory mechanism for a time-limited female to maintain a high level of fecundity might be to include among her oviposition targets poor-quality host individuals (e.g., Minkenberg et al. 1992; Díaz-Fleischer and Aluja 2003) or low-ranked host species (e.g., Jaenike 1990; Minkenberg et al. 1992; Singer et al. 1992; Jervis et al. 2007; but see Agnew and Singer 2000; Tammaru and Javoiš 2000 for opposite results). Hence, time limitation reduces the number of host individuals a female can evaluate but does not necessarily decrease her realized fecundity if she becomes less choosy and accepts a larger proportion of encountered hosts. Conversely, egg-limited females could maximize fitness by ovipositing on only the most suitable hosts encountered. Thus, large females that carry a surplus of eggs can be predicted to be less choosy in terms of host choice, whereas small, egg-limited females should make more careful decisions about where to oviposit. In general terms, this could be seen as a behavioral compensation resulting in intraspecific body-size allometry of parental investment behaviors (Berger et al. 2012).

Phytophagous insects constitute one of the most suitable systems to study female egg-laying physiology and behavior in relation to time and egg limitation (e.g., Jones 1977, 1987; Courtney 1984; Rausher 1985; Boggs 1986; Jaenike 1990; Visser 1994; Awmack and Leather 2002; Doak et al. 2006). The activity of small flying insects is largely governed by weather conditions, and variability in these conditions inevitably infers uncertainty about future reproductive opportunities (Leather 1988; Weisser et al. 1997; Gotthard et al. 2007). When the weather allows, females typically spend time searching for suitable host plants that are unevenly distributed across a landscape. At each host plant encounter, the decision to include or exclude a certain host is likely to affect future offspring fitness, because individual host plants of the same species also vary in quality and suitability (Jones and Ives 1979; Damman and Feeney 1988; Jaenike 1990; Doak et al. 2006). Furthermore, time limitation on female fecundity can result in a sexual conflict, because any surplus male mating attempts deprive females of time and energy that could otherwise have been spent in search of host plants.

Male harassment of already-mated females has been shown to have a negative impact on female fecundity in several species, but this effect has mainly been studied in systems where males try to coerce matings (e.g., Cook et al. 1994; Watson et al. 1998; Gosden and Svensson 2007) and thereby often physically damage females (Clutton-Brock and Parker 1995). However, even females that do not risk being injured during courtship might still face costs of additional matings and courtship bouts if these behaviors deprive them of time. Most evidence of such costs comes from laboratory studies (Clutton-Brock and Langley 1996; McLain and Pratt 1999; Gibbs et al. 2005; Ojanguren and Magurran 2007) or is indirect evidence from field studies. For example, unwilling females have been shown to adopt a coy lifestyle (Wickman 1986; Wickman and Jansson 1997) or accept matings only during periods unsuitable for ovipositing (Forsberg and Wiklund 1989). Mate-searching males have also been shown to force females away from their preferred egg-laying sites (Odendaal et al. 1989; Baguette et al. 1998; McLain and Pratt 1999), while time costs involved in heterospecific courtship may have negative impacts on female fecundity, thereby increasing selection for females with color patterns that do not attract male attention (Nielsen and Watt 2000; Gosden and Svensson 2007).

Because of the difficulties of following individual insects in the field, the few studies that do exist that examine how time limitation affects female fecundity and fitness in the wild have focused on short-term observations lasting seconds or minutes rather than hours or days (e.g., Nielsen and Watt 2000; Doak et al. 2006). This short duration makes it difficult to assess the relative costs of different ecological constraints. Moreover, any study aimed at investigating time limitation on female fitness must also take into account the possibility that females will trade off realized fecundity for host plant selectivity (Jaenike 1990; Minkenberg et al. 1992; Doak et al. 2006). In this study, we investigated different factors that affect realized fecundity and host plant selectivity in a natural population of the wood white butterfly (*Leptidea sinapis*). This slow-flying butterfly provides a rare opportunity to closely observe the life of a female insect over longer periods of time and obtain individual assessments of how behavioral and life-history variations shape female fitness in nature. We

observed female egg-laying behavior over 75 min per female. During this time, we noted female flight and nectar feeding budgets, host encounters, male courtship bouts, and successful oviposition events. Each female was captured at the end of the trial, which enabled measurements of body size and the number of eggs each female carried in her abdomen (her egg load). We were further able to assess differences in attributes of host plants that were either rejected or accepted by females. By using path analysis, we could describe the multivariate relationship between life history and behavioral and ecological variables and form a biological hypothesis of how these factors translate into female fitness. We also derived laboratory measures of the body-size scaling of potential fecundity and longevity and an estimation of the life span–reproduction trade-off in *L. sinapis* on the basis of a phenotypic correlation between early reproductive effort and longevity. Finally, we applied simulation models to the life-history and ecological data and incorporated climate data and a previously derived estimate of the temperature threshold for flight in this species (Friberg et al. 2008*b*). This allowed us to assess the respective fitness costs associated with time limitation imposed by male disturbance, host availability, and temperature constraints in nature.

Material and Methods

Study Species

Two species of *Leptidea* butterflies are found in Sweden: *Leptidea sinapis* and *Leptidea juvernica* (previously identified as *Leptidea reali* but now considered to be a third species in this cryptic complex; Dincǎ et al. 2011). In this study, we focused on the wood white butterfly *L. sinapis*, which ecloses from pupal hibernation between mid-May and mid-June in south-central Sweden (Eliasson et al. 2005) and is on the wing from mid-May to early July (Eliasson et al. 2005; Friberg et al. 2008*a*). At these latitudes, *L. sinapis* is largely univoltine and only rarely emerges into a summer generation (Friberg et al. 2008*a*). In Sweden, *L. sinapis* is a habitat generalist that thrives in both forest and meadow habitats, where it coexists with *L. juvernica*, a specialist restricted to the meadow habitat (Friberg et al. 2008*a*). Males of the two species cannot distinguish between females and will readily engage in courtship with both con- and heterospecific females, whereas females exclusively accept matings with conspecific males (Freese and Fiedler 2002; Friberg et al. 2008*c*).

The thermal threshold for flight in *L. sinapis* has been estimated to be 18°C (Friberg et al. 2008*b*). When temperatures exceed this threshold, the repertoire of mated *Leptidea* females can be divided into four behaviors (Wiklund 1977*a*, 1977*b*). They fly, and during flight they often alight on vegetation to taste the substrate by scraping the gustatory sensillae on their front feet against the plant surface. These host plant inspections can result in oviposition, but more frequently they lead to the female rejecting the plant (Friberg et al. 2008*b*). Other important aspects of a female's behavioral repertoire are nectaring and resting in the vegetation, hidden from mate-searching males. Finally, when a female is discovered by a patrolling male, it almost invariably leads to pursuit and courtship of the female. The male courtship ritual is described in detail by Wiklund (1977*a*) and Friberg (2008*c*). In short, a male who discovers a female will pursue her until she alights on the vegetation. He then lands in front of her and starts to display by oscillating his proboscis from side to side. Courtships are not physically harmful to females and end either with the female accepting the male as a mate or with the male terminating the courtship by flying away. Unsuccessful courtships often last several minutes and sometimes exceed 30 min in nature (Wiklund 1977*a*; Friberg et al. 2008*c*).

Female Time Budgets in Nature

The field study was conducted in Riala, ∼60 km north of Stockholm (59°37′N, 18°29′E). The field site is a forest landscape fragmented with meadow areas that is described in detail by Friberg et al. (2008*a*, 2008*b*). Present in the forested area and the adjacent clearing are *Vicia sepium*, *Vicia sylvatica*, and *Lathyrus vernus*, which are potential but rarely used host plants of *L. sinapis*, as well as the more commonly used host plant *Lathyrus linifolius*. The preferred host plants *Lathyrus pratensis*, *Vicia cracca*, and *Lathyrus corniculatus* grow in the meadow habitat and along the sides of the road that traverses the forest.

In order to obtain a field measure of female fecundity, individual females were followed for a standard duration of 75 min. This time interval was chosen as a compromise between a duration that is long enough to allow a quantitative assessment of individual variation in reproductive output and behavior on the one hand and the chance to observe as many females as possible on the other. Each trial started with a scanning of the area for females exhibiting egg-laying behavior. As soon as a female was discovered, the observer began to follow her. In a few cases, the trial had to be terminated before 75 min had passed so as not to lose sight of the female. The minimum duration that a female was followed was 40 min, and the mean ($\pm$SD) observation period was 73 $\pm$ 6 min.

We noted the number of eggs a female laid during the trial, and on which host plant species, and we also recorded the number and duration of male courtships from detection until male termination of courtship. The species affiliation of the courting male was often possible to deter-

mine, because *L. sinapis* males always incorporate one or a few wing strokes in courtships lasting longer than 20 s, whereas *L. juvernica* males keep their wings closed above the body throughout courtship (Friberg et al. 2008*c*). The species affiliation of males who courted females for less than 20 s without performing wing strokes was noted as "unknown." We also recorded each host plant inspection and the total time that females spent resting, nectaring, or in flight (oviposition behavior included). In the trials performed in 2007 we observed that females often inspected nonhost plants during the host plant search, and so for the 2008 trials we expanded our protocol to include nonhost plant inspections.

After a female was followed, she was caught and killed so we could perform later egg-load dissections, size measurements, and species determination via genital preparation. At dissection, both forewings and both front leg femora were cut off and digitalized, using a Canon scanner (CanoScan 9950F) at a setting of 600 dpi. Wing area and femur length were measured, using the Analyzing tool in Photoshop CS3 (Extended Version). The number of spermatophores was noted in order to confirm that all females were mated.

In our analysis of the field data, we included only those *L. sinapis* females that had been collected after the trial. This was necessary to allow species determination and post mortem measurement of size and the number of mature eggs in the abdomen (egg load). Furthermore, it was necessary that each trial be performed entirely in sunny weather so as not to confound time limitation with egg limitation, as females almost invariably alight on the vegetation during overcast conditions but can also do so when they are close to depleting their egg stores. Of the 84 females that were followed, 21 from 2007 and 52 from 2008 met the criteria for analysis. The following parameters were quantified from the field data of these 73 females: oviposition rate (eggs laid per hour), courtship rate (male courtships per hour), and courtship duration (the proportion of the total trial duration that a female was courted by a male). We also assessed female egg load (eggs remaining in the abdomen + eggs laid during the trial) and calculated female flight activity (the proportion of time spent in flight during the trial) and the number of host plant inspections each female made (inspections per hour). From the numbers of host inspections and eggs laid, we calculated female selectivity as the number of host plants a female rejected divided by the number of inspected host plants. A female selectivity of 1 means that the female rejected all potential host plants, and a value of 0 means that she oviposited on all inspected hosts.

Before multivariate analysis was performed, all continuous variables were standardized to range from 0 to 1 by using the formula $(x - x_{\min})/(x_{\max} - x_{\min})$. This is rec-ommended to avoid potential bias due to disproportionate weight being given to variables with more variance (Quinn and Keough 2002). We tested the male impact on female egg-laying rate, host plant inspection rate, and selectivity in separate linear models, with the above-mentioned parameters as response variables and courtship frequency (and duration) and initial female egg load (because a female's egg-laying propensity is likely to be strongly affected by her current egg load; Papaj 2000) as continuous explanatory variables. The main effect of year was included as a categorical factor in all analyses. Nonsignificant interactions that did not markedly affect the models ($P > .1$) were removed in a stepwise fashion to attain the minimal adequate model. We analyzed relationships between the investigated variables by using linear regressions and ANOVAs. In addition, we used the structural equation modeling (path analysis) available in the sem package (Fox and Byrnes 2011) for R, version 2.13.1 (R Development Core Team 2011), to describe the ecological relationships in a multivariate context. Path analysis allows for testing of correlations that hold more than one variable as dependent, and it can be particularly helpful in identifying and distinguishing among several evolutionary hypotheses (Kingsolver and Schemske 1991). Before the analysis, we tried to specify a predictive model on the basis of our previous knowledge of butterfly ecology, physiology, and oviposition behavior. In a second step, we reduced our model by removing nonsignificant relationships between variables and then comparing maximum likelihoods of the full and reduced model to select the one that had the best statistical support.

Attributes of Accepted versus Rejected Host Plants

During trials performed between May 23 and June 3, 2008, a total of 107 *L. linifolius* host plants that were inspected by 18 females were individually marked. For each plant, we noted whether the female oviposited on the plant or whether she rejected it. We then returned to the plants twice: first after a period of between 0 and 2 weeks (on June 4) and thereafter a second time, ∼3–4 weeks after egg-laying (on June 25). On these occasions, a total of 79 plants were rediscovered. On each census occasion, we measured plant height, number of flowers and fruits, and plant condition according to a categorical five-point scale: (1) dead; (2) substantially deteriorated; (3) dark green with brown spots; (4) fresh, dark green; and (5) fresh, light green. The same observer graded the host plants on both occasions and did so without knowledge of the host plant category (female accepted or rejected). The height, condition, and number of flowers on rejected and accepted host plants were analyzed by restricted maximum likelihood (REML) in mixed models, with host plant individual

as a random effect (repeated measures), using the lme4 package (Bates et al. 2011) available for R. We used the time of inspection (early and late, after host plants had been visited by a female), host status (accepted or rejected), and their interaction as fixed factors. Height and condition were modeled assuming normally distributed data, whereas the number of flowers was modeled with a Poisson distribution. *P* values for fixed effects were calculated by comparing model likelihoods by using the profiled deviance of models, which for REML estimates in lme4 are equivalent to maximum likelihood estimates (Bates et al. 2011).

Body Size, Potential Fecundity, and Intrinsic Life Span

The size dependence of potential fecundity and intrinsic life span and the trade-off between reproduction and life span were estimated for incorporation into model simulations. We monitored egg laying throughout the adult lives of 32 females observed under optimal egg-laying conditions in the laboratory. The females used in the experiment were the offspring of nine females from the Riala population (either collected during 2007 [seven females] or taken from the laboratory stock [two females], to which new wild-caught individuals had been added every summer between 2003 and 2006). The females were reared as larvae on *L. corniculatus* in temperature and day-length conditions intended to induce direct development (25°C, 22 h day length). All females were weighed as adults immediately after the release of the meconium (the waste material generated during metamorphosis) and subsequently mated once with virgin males. The size-fecundity relationship was estimated by letting the females lay eggs in 1-L plastic jars where they had constant access to the host plant *L. pratensis*. The females were kept in a climate cabinet programmed to provide ideal conditions for oviposition (16L : 8D; 25°C for 8 h during the middle of the light period and 14°C for the rest of the time) and fed sugar solution each day throughout their life span. Each day, the females were provided access to a new host plant; eggs were counted on a daily basis until the death of the female. Each female was dissected post mortem in order to count the remaining number of mature eggs in her abdomen. These eggs were added to the number of eggs laid, in order to estimate the total potential fecundity of each female. Wing area and femur length were measured, using the protocol described above. Acquiring these additional size measures allowed us to find good scaling components for later predictions of adult mass of wild-caught females, for which adult weight at collection is an unreliable estimate of female size at metamorphosis.

We analyzed the relationship between body mass, potential fecundity, and intrinsic life span, using linear regression. We also extracted the first principal component (PC1) of wing area and femur length to obtain a good predictor of body size (weight) of wild-caught females. In addition, to obtain an estimate of the trade-off between early reproduction and life span (to be incorporated into later model simulations), we defined a new variable, early reproductive effort, calculated as the number of eggs laid per day during the first 5 days of ovipositing.

Describing trade-off structures by using phenotypic data may be hazardous if phenotypic correlations are poor estimators of the underlying genetic components (Roff 2002). This is a risk when there is relatively large variation in individual condition relative to individual differences in allocation between the two traits investigated for a trade-off (de Jong and van Noordwijk 1992). This can, for example, cause a positive phenotypic correlation between the expression of two traits, such as life span and fecundity, that rely on the same resource pool, because high-quality individuals can afford high reproductive rates and still live longer than individuals of poorer condition. To correct for this possibility, we added each female's lifetime fecundity to the model of the dependence of life span on early reproductive effort, which should control for individual differences in condition.

We also estimated age-dependent mortality rates with the software Winmodest (Pletcher 1999), by fitting the Gompertz equation to the data. This allowed for estimation of daily mortality risk $(u_x) = ae^{bx}$, where x is age, a is the baseline mortality at age 0, and b is the aging (Gompertz) parameter describing the change in mortality rate with age (Carey 2001).

Time Limitation in the Wild

To assess the relative effects of time limitation induced by male courtship, host abundance, and temperature constraints, we performed a simulation model based on the empirical data. In table 1, we summarize all model parameters calculated from the laboratory or field data and provide the major assumptions made in the simulations. The results from these simulations are not to be interpreted as precise quantifications because of the unavoidable uncertainty in the estimates of physiological/life-history (temperature threshold, relationships between body size, fecundity, and life span) and ecological (female time budgets) parameters (see "Results"). Nevertheless, this model serves to illustrate well the relative impact of time limitation on female reproductive success induced by males, hosts, and temperature.

We simulated optimal reproductive effort (*R*; calculated as the number of eggs matured per day) and resulting life span (*L*) and optimal levels of host selectivity (*s*) for females of different body sizes with corresponding potential

Table 1: Model parameters incorporated into the simulations

Parameter (variable)	Estimation	Value	Source
Life span (L)	Laboratory data	$-1.74R + .22B + 21.2$	This study
Potential fecundity (B)	Laboratory data	$4.96 \times$ body size $- 48.5$	This study
Reproductive effort (R)	Laboratory data; simulated	No. eggs per day during days 1–5	This study
Body size	Laboratory data; field data	Mean laboratory value, 27.8 mg; mean wild value, 26.1 mg	This study
Selectivity (s)	Simulated, field data	Simulated value, 0–.95; mean field value, .76	This study
Host density (H)	Field data	Mean, 13.1	This study
Host quality (q)	Field data	Mean, 3.4; accepted, 3.7; rejected, 3.2; SD, .67	This study
Offspring fitness (w)	Assumption	50% difference between accepted and rejected	Doak et al. 2006
Extrinsic mortality	Assumption	10%	Friberg et al. 2008a
Temperature threshold	Lab data	18°C	Friberg et al. 2008b
Operating temperatures	Climate data	Mean, 17.3; SD, 5.9	SMHI, temperature loggers

Note: SMHI, Swedish Meteorological and Hydrological Institute.

fecundity (B). The relationships between female body size and potential fecundity (fig. 3a), potential fecundity and life span (fig. 3b), and reproductive effort and life span (fig. 3c) were all derived from our laboratory estimates. Female life span and early reproductive effort traded off according to a linear relationship:

$$L = k_1 R + k_2 B + m, \quad (1)$$

where k_1, k_2, and m are the constants estimated from the laboratory data (see "Results"). In the simulations, females were allowed to exhibit any combination of selectivity and reproductive rate. The optimal strategy for each female size class was found by maximizing lifetime reproductive success (LRS), defined as

$$\text{LRS} = Fw, \quad (2)$$

where F is lifetime female fecundity and w is larval offspring survival. Lifetime fecundity is a function of female reproductive effort (R) and the daily number of suitable hosts (H) a female encounters, taken over her life span (L):

$$F = \sum_{i=1}^{L} f(R, H) = \begin{cases} R, & R < H \\ H, & R \geq H \end{cases}. \quad (3)$$

If males, number of suitable hosts, and temperature do not limit egg-laying opportunities, a female will lay her daily egg load (R). Otherwise, she will oviposit as many eggs as allowed by the number of host plants encountered that are of high enough quality. In our model, we made the assumption that females can retain eggs not laid on one day and then lay them on the following days. While this assumption is not likely to hold indefinitely, it is in

line with observations of females being able to store eggs over multiple days and lay them at later occasions in the laboratory (M. Olofsson, M. Friberg, and D. Berger, personal observation).

We found a clear difference in condition between plants accepted or rejected by *Leptidea* females (see "Results"), but we do not know to what extent this variation translates into larval offspring fitness (w). We therefore made an assumption on the basis of the results of a previous study (Doak et al. 2006) that showed that larval fitness (in terms of survival to the last instar) was 50% higher on hosts accepted by females of another pierid butterfly species, *Pieris virginiensis*. Translating this to our data on the conditions of rejected and accepted hosts, larval survival increases by 90% for every standard deviation of improvement in host quality. The proportion of hosts that are accepted is equal to the area under a standardized cumulative density distribution with a total area of 1, in a range defined by host qualities equal to and greater than the critical value of host quality (q_{crit}) that is directly related to female selectivity (s):

$$w = f(s), \quad (4)$$

$$s = 1 - \int_{\infty}^{q_{crit}} f(q) \, dq. \quad (5)$$

Thus, having high selectivity increases the survival of each individual offspring (w in eq. [4]) but at the same time reduces the number of hosts available for ovipositing (H in eq. [3]) to $H(1 - s)$, resulting in a trade-off between host quality and number.

We have no precise estimate of extrinsic mortality factors for these butterflies. Without extrinsic mortality, natural selection will always favor a low reproductive rate because long life buffers against environmental stochasticity in reproductive opportunities and a missed opportunity can always be compensated for later in life (Ellers et al. 2000; Berger et al. 2012). However, for butterflies (and insects in general), extrinsic mortality can be quite high, as implied by the relatively short intrinsic reproductive life span of these organisms (Carey 2001). A minimum estimate of average life span for *Leptidea* females at the field site during a period with weather conditions favorable for activity was estimated as 6 days (Friberg et al. 2008*a*), and in our laboratory experiments, when we applied similar conditions for female egg laying but excluded extrinsic mortality factors, the average life span was ~20 days (see "Results"). By making the simplifying assumption that mortality risk does not change with female age and by noting that average life span (L) is equal to the inverse of daily mortality risk, we can approximate the level of daily extrinsic mortality as $1 - (1 - 1/L_{wild})/(1 - 1/L_{lab})$ (Carey 2001). Considering that the butterflies in the study by Friberg et al. (2008*a*) had spent, on average, 5.5 days in the laboratory before being released in the field, the daily extrinsic mortality value for that study was $1 - (1 - 1/6)/[1 - 1/(20 - 5.5)] = 0.105$. This approximation seems reasonable, and so for our analysis we assumed a daily extrinsic mortality risk of 10%. We later checked model sensitivity to this assumption by increasing or decreasing mortality (5%–15%). Although variation in extrinsic mortality will affect optimal rates of reproduction (Schaffer 1974), the qualitative conclusions concerning the relative effects of time limitation from hosts, males, and temperature on females of different body sizes remained. Similarly, because we have a rather modest quantity of laboratory data, to be able to estimate age-dependent mortality rates (see "Results") we also evaluated whether results were robust against deviations from our assumption of a constant mortality rate throughout a female's life. Specifically, we simulated a scenario where mortality increases with age (Gompertz parameter $b = 0.2$).

Finally, we simulated hourly temperatures experienced by each female butterfly by using a climate data set of sun hours and temperatures from 1996 to 2008 (excluding 2003, because of missing data). The climate data were generated at a nearby weather station (in Svanberga, ~25 km north of the field site) and were provided by the Swedish Meteorological and Hydrological Institute. By calibrating this data set with temperature measurements from four different temperature loggers located at the field site in Riala in 2007 and 2008, we could estimate the operating temperatures of females exhibiting oviposition behavior. We assumed that sunshine increases ambient temperature by 4.25°C for a butterfly in flight (see Gotthard et al. 2007 and references therein for further justification). The threshold for flight activity in *L. sinapis* is ~18°C (Friberg et al. 2008*b*). Thus, if the hourly temperature was less than 18°C, a simulated female could not lay any eggs, and if the temperature was 18°C or higher, she laid eggs according to equation (3).

We could approximate the relative constraints imposed by temperature, host plant abundance, and male courtship by relaxing these constraints one at a time in our model while keeping all other variables equal. For temperature, we accomplished this by allowing females to be active irrespective of the ambient temperature; for host plants, we let females encounter 10 times more hosts per hour than estimated in the field; and for male courtship, we gave females 15% longer time budgets for flight during sufficiently warm periods (15% was the average proportion of the female time budget for flight that was occupied by male courtship). We simulated 100,000 females in each of the four scenarios. The reduction in relative fitness for each factor was calculated as $1 - Fw/Fw^*$, where Fw is average lifetime reproductive success in the natural scenario incorporating time limitation from all three factors and Fw^* is the average lifetime reproductive success in a scenario where constraints from a specific factor had been removed. The realized fecundity for females in each scenario was calculated as the average number of eggs laid by each simulated size class divided by the potential fecundity of that size class.

Results

Female Time Budgets in Nature

Independent censuses of *Leptidea* butterflies showed that males were more commonly observed than females throughout the season and were observed both earlier and later in the day during trials (results not shown). Thus, females are not likely to experience any periods completely absent of the risk of encountering a male. Only two of the 238 male courtships resulted in mating, illustrating the strong asymmetry in mating propensity between the sexes. In 146 (61%) of the 238 courtships, the female was courted by a conspecific *Leptidea sinapis* male, and in 26 (11%) of the courtships, a *Leptidea juvernica* male courted the female. In the remaining 66 courtship bouts (28%), the male terminated courtship after less than 20 s, thereby precluding determination of his species affiliation. Uncertainty in estimating the exact fraction of heterospecific courtships is, however, unlikely to have affected the results, since males of both species court con- and heterospecific females as readily and for as long a duration (Friberg et al. 2008*c*). During the trials, females spent an average

($\pm$ SD) of 34 $\pm$ 13 min per hour in flight (57%), 8 $\pm$ 7 min drinking nectar (13%), and 11 $\pm$ 11 min resting (18%), whereas the average time spent in courtship per hour was 6 $\pm$ 7 min (10% of the total time and 15% of the time available for flight) spread over 2.4 $\pm$ 2.0 courting males. The average female laid 3.4 $\pm$ 2.4 eggs and visited 13.1 $\pm$ 7.9 host plants per hour. The overall egg-laying rate was similar between years, but females encountered a significantly higher number of host plants in 2007 (16.5 $\pm$ 10.3 plants per hour) than in 2008 (11.75 $\pm$ 6.25 plants per hour; table 2). A majority of eggs were laid on *Lathyrus linifolius* (165) and *Lathyrus pratensis* (110), whereas *Vicia cracca* (16), *Lathyrus corniculatus* (9), and *Vicia sylvatica* (1) were used less often. Females displayed the highest average acceptance rate when visiting *L. pratensis* (0.36 $\pm$ 0.34 eggs per landing), followed by *L. corniculatus* (0.32 $\pm$ 0.38), *L. linifolius* (0.28 $\pm$ 0.21), *V. cracca* (0.28 $\pm$ 0.38), and *V. sylvatica* (0.03 $\pm$ 0.018). No eggs were laid on *Vicia sepium* or *Lathyrus vernus*, although these plants were sometimes inspected by females and have been determined to be suitable hosts for *L. sinapis* larval development (Friberg and Wiklund 2009). Overall, females laid eggs on 26% $\pm$ 14% of the inspected host plant individuals. On average, females landed and inspected 23 $\pm$ 18 nonhosts per hour, which included several grass species and the herbs *Melampyrum sylvaticum*, *Geranium sylvaticum*, and *Galium* spp. The most commonly used nectar plants were *L. linifolius*, *G. sylvaticum*, *Viola riviniana*, and *V. sepium*: these four species were the targets of 78% of 726 observed nectaring bouts.

Each female had at least one mature egg in her abdomen at the termination of the trial, and female abdomens contained 12.7 $\pm$ 5.5 mature eggs at the beginning of the trial. The average initial egg load did not vary between years ($F_{1,68} = 0.0104$, $P = .92$). There was a weak decline in egg load as the season progressed ($F_{1,68} = 5.64$, $P = .020$), perhaps due to the older age of females that were followed later in the season. As expected, large females had larger egg loads ($F_{1,68} = 31.9$, $P < .001$) and egg loads decreased

over the day ($F_{1,68} = 37.6$, $P < .001$). There was also a significant albeit weak interaction between time of day and female size, because larger females started the day with more mature eggs but all females finished the day with similarly low numbers of eggs remaining in their abdomens ($F_{1,68} = 4.15$, $P = .045$; total model: $R^2 = 0.46$, $F_{5,68} = 16.6$, $P < .001$). Females with large egg loads encountered more host plants and had higher egg-laying rates and lower host selectivity than females with smaller egg loads. When we statistically controlled for female egg load, selectivity was higher earlier in the day than later in the day, when less time remained for oviposition activity, suggesting that female host evaluation is highly state dependent (table 2; fig. 2).

Females courted by a higher number of males visited fewer potential hosts than females that were allowed to search for host plants while encountering less male disturbance (table 2; fig. 1*a*). Female egg-laying rate was negatively correlated with male courtship rate (table 2; fig. 1*b*) but male courtship rate was not correlated with selectivity (table 2). When analyzing the egg-laying rate and host plant encounter rate models and taking into account the time spent in courtship instead of using the courtship rate, the impact of males on the egg-laying rate was not significant ($F_{1,67} = 0.50$, $P = .48$), whereas the time spent in courtship had a negative impact on host plant encounter rate ($F_{1,67} = 6.50$, $P = .013$). It does, however, seem likely that the relationship between female host encounter rate and male courtship is dichotomous, because of the common dependence on female flight: females with a higher propensity to fly are likely to visit more hosts and be detected by more males. We therefore performed additional analyses by calculating a new standardized variable for the proportion of the female time budget used for flight (subtracting the time used for nectaring and resting) that is occupied by courting males. The relationship between this new variable and female host encounter rate was stronger, as expected ($R^2 = 0.26$, $F_{1,71} = 24.6$, $P < .001$). In addition, the relationship between the new variable and the

Table 2: Results from the models testing the impact of year, egg load (EL), time of day (T), courtship rate, and the interaction between EL and T on egg-laying rate, host plant encounter rate, and female host plant selectivity

	Oviposition rate				Host plant encounter rate				Selectivity			
	SS	df	*F*	*P*	SS	df	*F*	*P*	SS	df	*F*	*P*
Year	.02	1	.79	.38	.21	1	6.18	**.015**	.07	1	2.46	.12
EL	1.82	1	63.8	**<.001**	.61	1	17.7	**<.001**	.88	1	29.0	**<.001**
Courtship rate	.12	1	4.26	**.04**	.28	1	7.97	**.006**	.04	1	1.25	.27
T	.04	1	1.44	.23	.015	1	.42	.52	.32	1	10.5	**.002**
T $\times$ EL	.29	1	20.3	**.002**	.21	1	5.99	**.017**	.11	1	3.67	.06
Residuals	1.94	67			2.32	67			1.97	66		

Note: df, degrees of freedom; SS, sum of squares. Statistically significant effects are indicated in bold.
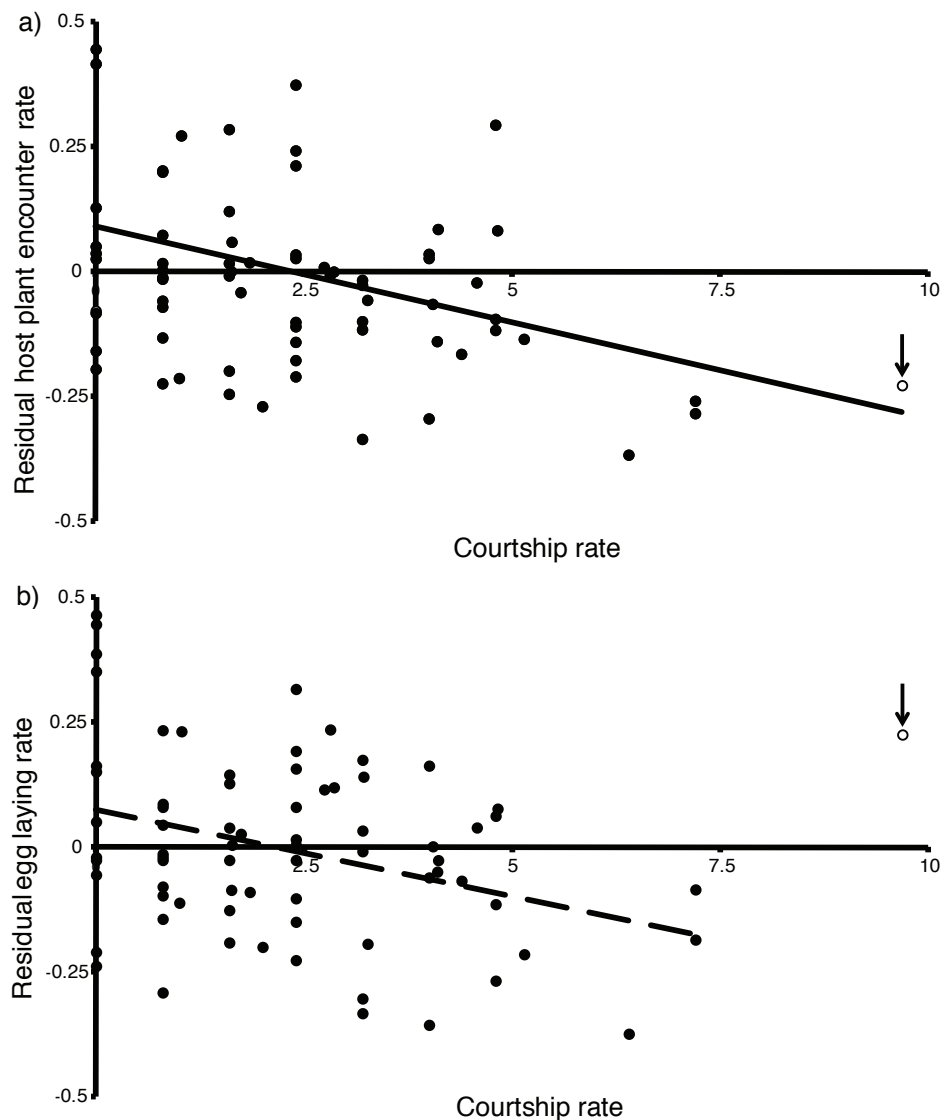
Figure 1: Relationships between *a*, host plant encounter rate and male courtship rate, and *b*, egg-laying rate and male courtship rate. The egg-laying rate was measured with the residual values from the significant relationships between the egg-laying rate and the initial egg load, and the host plant encounter rate was measured with the residual values from the significant relationships between the host plant encounter rate and the initial egg load. The solid line in *a* is based on the entire sample, whereas the dashed line in *b* describes the regression slope when the impact of the extreme outlier female (open circle, indicated by arrow) has been removed.

number of eggs laid per hour was now significant ($R^2 = 0.12$, $F_{1,71} = 9.74$, $P = .003$); however, there was still no effect on selectivity ($R^2 = 0.01$, $F_{1,70} = 0.68$, $P = .41$).

To better understand how ecological time constraints affect females in nature, we provide complementary multivariate statistical descriptions of the relationships by using path analysis. The estimated relationships are qualitatively the same as those suggested by the analyses above; their standardized partial correlation coefficients (*r*) along

with significance levels are depicted in figure 2. Apart from the paths incorporated in the final model presented in figure 2, we also included relationships between the deviation (in minutes) from 1:00 p.m. (when the sun reaches its zenith at the study site) and male courtship and between the number of hosts encountered and female selectivity. In addition to the indirect effects of male courtship on host plant encounter rate, egg-laying rate, and selectivity through the reduction in flight, we also inspected whether there were any additional direct effects on these three var-
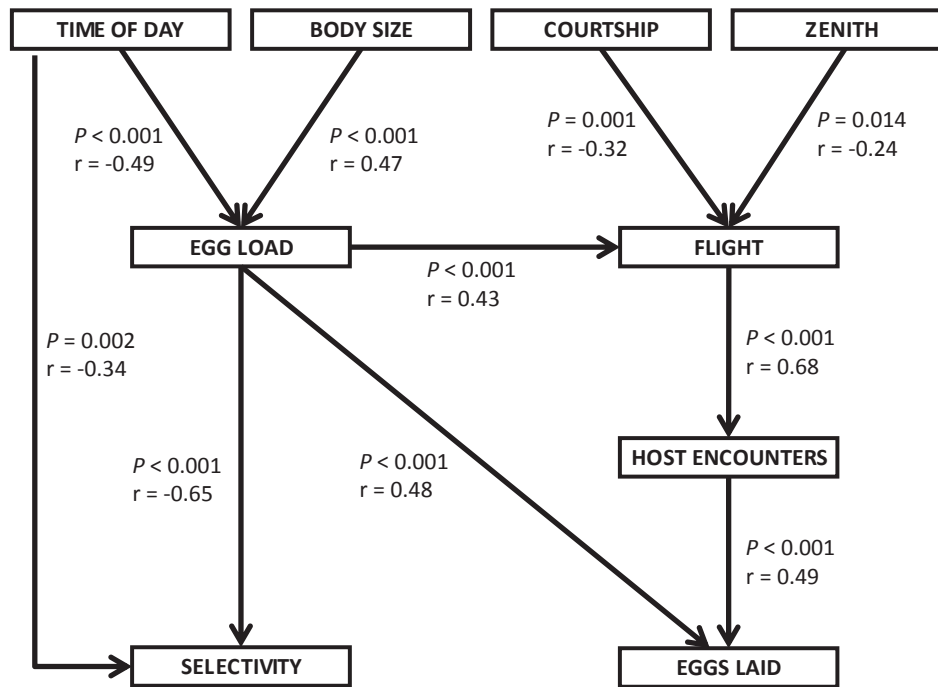
Figure 2: Path analysis of a statistical and a biological hypothesis of how behavior and life history, together with ecological constraints in terms of male disturbance and host plant availability, affect female reproductive success. Arrows indicate causality. Standardized partial correlation coefficients (r; with associated P values) give the strength and sign of each relationship while controlling for impacts from other factors.

iables from male courtship. Incorporating the five additional correlations did not improve model fit (comparison for significant improved fit compared with original reduced model: $\chi^2 = 6.28$, degrees of freedom [df] = 5, $P = .28$; all five individual path correlations: $P > .05$). The simpler model presented in figure 2 also received better support, as indicated by lower Akaike and Bayesian information criteria, and therefore we excluded the additional paths. Our final model had a χ^2 value of 116.7 with 26 df, compared with the model incorporating the five additional paths, which had a χ^2 value of 110.4 with 21 df, and the null model assuming no covariance between any of the studies variables, with a χ^2 value of 332.9 and 36 df. Our final model does not describe the data perfectly, as indicated by a significant difference between our specified model and the observed data (likelihood ratio test, $P < .001$) and relatively poor measures of model fit: the standardized root-mean-square residual (SRMR) was equal to 0.11, whereas an SRMR of less than 0.10 is recommended. Similarly, the goodness-of-fit (GFI) value was 0.80, whereas the recommendation is for a GFI value greater than 0.90 (for a more detailed description of these and similar measures of fit in structural equation models,

see Hooper et al. 2008). However, because the data were collected in nature, thereby making it impossible to control for all possible influential exogenous variables, a poor fit when compared with that of a fully saturated model is not surprising. Furthermore, because we have a modest quantity of data, GFI measures are unreliable (Hooper et al. 2008). Thus, any quantitative differences in the estimated partial correlations of our model should be interpreted with caution. Nevertheless, additional inspections of multiple regression coefficients and variance inflation factors indicate that collinearity between the investigated variables was not a problem in the analysis.

*Attributes of Accepted versus Rejected Host Plants*

Virtually no eggs were laid on flowering host plants, and females only rarely inspected host plants that had flowers. The numbers of flowers or fruits on individual inspected host plants were higher at the second inspection but did not differ between plants that were accepted or rejected by females (time: $\chi^2 = 18.8$, $P < .001$; status (accepted or rejected): $\chi^2 = 0.09$, $P = .76$; time × status: $\chi^2 = 0.06$, $P = .80$, $n = 78$). Plant height did not differ significantly

between accepted and rejected plants or between inspection times (time: $\chi^2 = 1.58$, $P = .21$; status: $\chi^2 = 1.31$, $P = .25$; time × status: $\chi^2 = 0.0004$, $P = .98$, $n = 78$). However, for both inspections, plants that were accepted as hosts had, on average, higher condition values (were less deteriorated) than plants that were rejected by females (time: $\chi^2 = 21.2$, $P < .001$; status: $\chi^2 = 8.48$, $P = .0036$; time × status: $\chi^2 = 0.054$, $P = .82$, $n = 78$).

Body Size, Potential Fecundity, and Intrinsic Life Span

The laboratory-reared females produced 98 ± 31 eggs (eggs laid [90.8 ± 32.7] plus mature eggs dissected from abdomens post mortem [6.9 ± 6.8]) during their life spans, which lasted 20.5 ± 7.75 days. Potential fecundity was significantly positively correlated with adult mass ($R^2 = 0.39$, $t = 4.36$, $n = 32$, $P < .001$; fig. 3a). To estimate the trade-off between early reproductive effort and survival, we analyzed the relationship between life span and the three explanatory variables early reproductive effort, adult mass, and potential fecundity. Potential fecundity was included to control for variation in female condition, because a possible trade-off between reproduction and life span can be masked by high-quality females with higher fecundities and longer lives and that lay more eggs early in life. The total model was very successful in explaining variation in female life span ($R^2 = 0.69$, $F_{3,29} = 24.0$, $P < .001$). Because of the strong correlation between female mass and potential fecundity (above), female mass was found to have a weak (but nonsignificant) negative relationship with longevity ($t = -1.99$, $b = -0.46$, $P = .056$) and potential fecundity was strongly positively correlated with longevity ($t = 7.45$, $b = 0.22$, $P < .001$; fig. 3b). Interestingly, when controlling for potential fecundity and body mass, the early reproductive effort of a female was strongly negatively correlated with longevity ($t = -5.54$, $b = -1.76$, $P < .001$; fig. 3c). In short, females that live fast die young. Early reproductive effort showed only weak correlations with the other two explanatory variables (variance inflation factor, 1.22), indicating that potential collinearity was not a problem in the model. We also performed all analyses using a data set in which the largest and most fecund female (see fig. 3) had been removed, to check the robustness of our results. In this case, all regression slopes remained unchanged and significant P values retained significance in the alternative analyses.

The analysis of age-dependent mortality showed that mortality rate increased with female age (Gompertz parameter $b = 0.12$ [95% CI, 0.09–0.17]). However, in further analyses using the eha package (Broström 2012) for R, in which model fits were compared by using different error distributions and incorporating the covariates life-
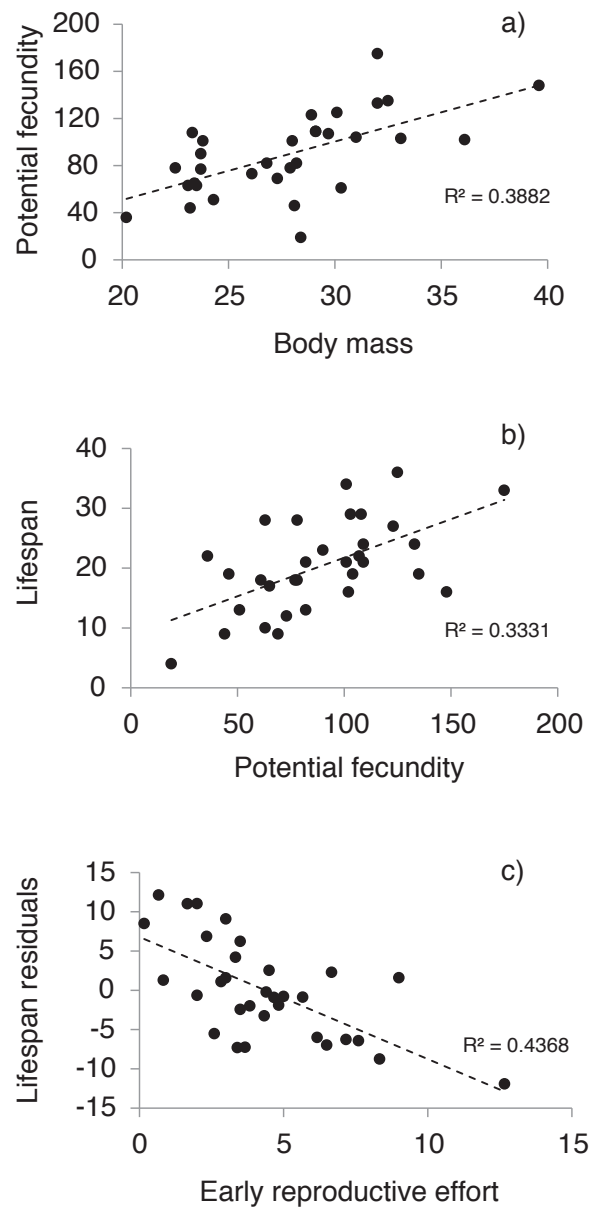
Figure 3: Laboratory data indicating the relationships between *a*, female body mass and potential fecundity; *b*, potential fecundity and life span; and *c*, the residuals of the regression of potential fecundity on life span and early reproductive effort.

time fecundity and reproductive effort (not possible in Winmodest), it became apparent that the statistical power to accurately estimate age-dependent mortality was low, although all analyses suggested increases in mortality with age (results not shown; see "Time Limitation in the Wild" for further handling of age-dependent mortality).

All three size measurements (femur length, wing area,
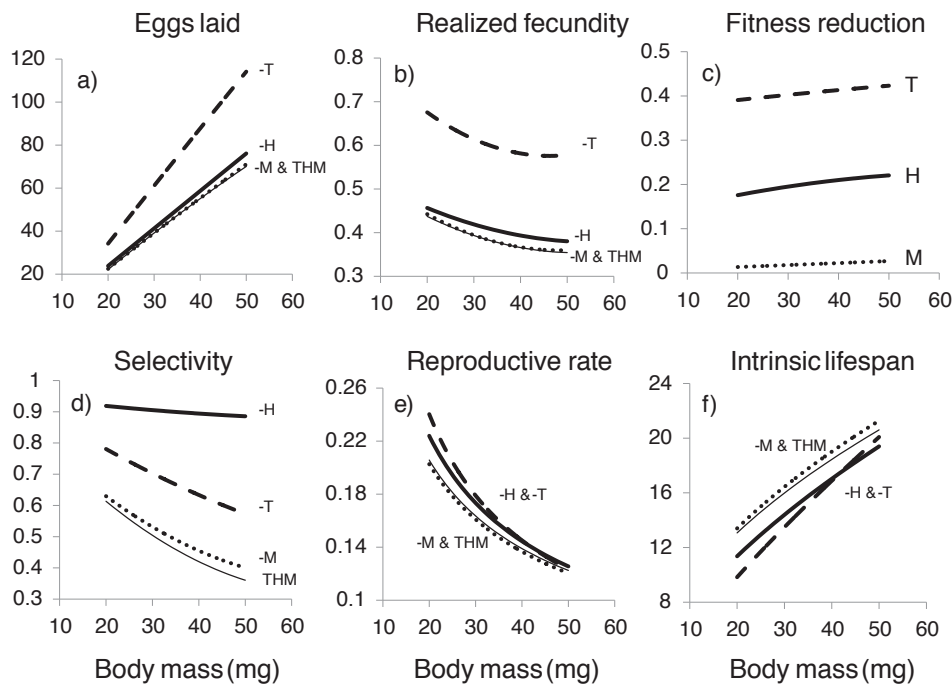
Figure 4: Predictions from model simulations of four scenarios: temperature-, host-, and male courtship–induced time limitation (the natural situation; THM); without thermal constraints ($-$T); without host limitation ($-$H); and without males ($-$M). *a*, Eggs laid; *b*, realized fecundity (eggs laid divided by potential fecundity); *c*, relative fitness reduction ($1 - Fw/Fw^*$); *d*, host selectivity (rejected hosts divided by encountered hosts); *e*, reproductive rate (reproductive effort divided by lifetime potential fecundity); and *f*, life span measured in days. See text for further details.

and their PC1) were positively correlated with adult body mass, but their PC1 was the best predictor (PC1: $R^2 = 0.52$; wing area: $R^2 = 0.46$; femur length: $R^2 = 0.45$) and was therefore used as a measure to translate the body mass of laboratory females to the size of wild-caught individuals for which only wing and femora measurements were available.

Time Limitation in the Wild

The results presented below were obtained by assuming constant mortality rates throughout a female's life, because there was large uncertainty in the estimation of the age-dependent mortality rate (see above). Therefore, we tested the sensitivity of the results given the assumption of constant mortality by simulating a scenario where the aging parameter *b* was set to 0.2 (greater than the upper 95% confidence limit of 0.17 estimated by the Winmodest analysis). However, the results of the simulations remained qualitatively the same (results not shown).

The most striking feature of these results is the devastating effect of cold temperature on female fitness. When

we relieved this constraint, female fecundity increased by 60% on average, and fitness increased by ∼40%. Unlimited access to hosts increased female fecundity by ∼6% and fitness by ∼20%, while male courtship had no effect on female fecundity and reduced fitness by an average of only 2% (fig. 4*a*–4*c*).

As predicted, relieving time constraints always had the strongest effects on large females with high potential fecundity, which require more time to realize their full reproductive potentials (fig. 4*a*, 4*c*). Indeed, time constraints on female fecundity that are induced by cold temperature have previously been hypothesized to enforce stabilizing selection on female body size (Gotthard et al. 2007; Berger et al. 2008). A size dependence of time limitation also results in large females being less choosy about which host plants to lay eggs on, while small females are predicted to exhibit higher levels of host selectivity (fig. 4*d*). This effect was also seen in our empirical data set, which indicated that small egg loads are associated with females being more selective (table 2; fig. 2)

Host limitation versus temperature constraints had very different effects on female strategies to maximize repro-

ductive success. The strong autocorrelation between hourly temperatures experienced throughout a female's life (autocorrelation = 0.95, calculated from the climate data set) means that females are often completely restricted on cold days, whereas on warm days they always encounter plenty of hosts. Therefore, increasing the number of hosts or relieving females of males does not result in any large increase in female fecundity: on good days, all females laid close to their entire egg complement. This illustrates the importance of considering the temporal scale on which time limitation is measured and assessed. Thus, even though we observe clear effects of male courtship on female time budgets during observations lasting just over an hour, this constraint simulated over an entire day was not severe enough to result in any large reduction in realized female fecundity: if a female is heavily courted by males in the beginning of the day, she is likely to still have plenty of egg-laying opportunities as the day progresses. In the simulations, providing 15% more time available for flight allowed females to see 15% more host plants and become slightly more selective in their choice of host plants; however, this increase was only marginal (fig. 4d), and so the effect of males on female fitness is therefore predicted to be small (fig. 4c). Female fitness was more strongly affected by increased host abundance (fig. 4c), as females could increase their selectivity and oviposit on hosts of only the absolute highest quality (fig. 4d).

Extrinsic mortality can cause the death of a female before she has visited enough hosts to deplete her egg stores or, alternatively, before she has had time to convert all of her reproductive resources into eggs in a situation where hosts are abundant (Rosenheim 1996; Sevenster et al. 1998; Papaj 2000). In accordance, our simulations predict that small females should therefore invest a higher proportion of total reproductive reserves into early reproduction and thus display higher reproductive rates (reproductive effort divided by potential fecundity) in response to a higher risk of becoming egg limited (fig. 4e). This result is also in line with previous theoretical evaluations of the relationship between intraspecific variation in reproductive effort and body size in insects (Ellers and Jervis 2003; Berger et al. 2012). Consequently, small females are also predicted to have shorter life spans (fig. 4f) as a result of the reproduction–life span trade-off (eq. [1]; fig. 3). Removing the time limitation on female oviposition from low temperature or low host abundance therefore selects for an increased reproductive rate (fig. 4e) and, consequently, a shorter intrinsic life span (fig. 4f). However, when compared with the change in behavioral regulation in terms of host selectivity that results from removing the time limitation (fig. 4d), these changes in life span and reproductive rate are small.

Discussion

The field data generated in this study provide an illustrative and rare example of how life history and behavior may interact to maximize the reproductive success of female insects in nature. Large butterflies carried more eggs and had a much higher reproductive output both in the laboratory and in nature. The strongest predictor of egg-laying behavior was the number of mature eggs a female was carrying in her abdomen (the egg load): females with large egg loads were more likely to spend time in flight searching for hosts and laying eggs on the hosts they evaluated. Female host selectivity also decreased throughout the day, further indicating that females indeed make strategic oviposition decisions in response to the risk of egg or time limitation.

We were able to observe female behavior only on sunny days, since females are inactive and virtually impossible to detect in cold and cloudy weather conditions. However, by incorporating the detailed information on female behavior that was collected during benign weather conditions and previous data on the temperature threshold of female activity (Friberg et al. 2008b), we could estimate the impact of time limitation on different ecological factors and then partition these effects on female fitness. This analysis showed that, as is commonly argued for other ectotherms (e.g., Brown et al. 2004; Frazier et al. 2006; Angilletta 2009), temperature seems to be the ecological factor that most constrains reproduction. In female *Leptidea*, it therefore seems that fitness would be dramatically increased by a lowering of the thermal threshold for flight activity. Parallel arguments could be made for other thermal thresholds that are likely to evolve in orchestration with the threshold for activity, such as that for egg maturation (Berger et al. 2008). However, it is also predicted that a lowering of the thermal threshold would result in a correlated response in the upper threshold for thermal tolerance (Huey and Kingsolver 1989; Angilletta 2009). This could result in decreased egg-laying performance at warm temperatures (Karlsson and Wiklund 2005; Gotthard et al. 2007) or harmful effects on other life stages where hot temperatures are experienced, given a correlated thermal physiology across the life cycle (Bowler and Terblanche 2008; Berger et al. 2011).

The large impact of temperature on fitness might have been slightly overestimated in our simulations, because we assumed that mortality rates were the same on both cold and warm days. It is unclear how activity patterns affect mortality, as it can be argued that low temperature slows down the physiological machinery and reduces mortality associated with activity but at the same time decreases feeding opportunities and makes it difficult to escape attacks from predators. To explore the impact of the as-

sumption of constant mortality risk, we performed additional simulations where the extrinsic mortality risk was set to 0 on cold days, when females were completely inactive. In this scenario, the average reduction in fitness was 30%, compared with 40% in the original scenario, and thus still markedly higher than the 20% reduction in fitness that is associated with host plant availability or the 2% reduction that is inflicted by males.

In our model, we did not allow females to exhibit plasticity in egg maturation rates. However, unless future conditions for egg laying are highly predictable and evolutionary costs and physiological limits on plasticity in egg maturation rates are low, pronounced phenotypic plasticity is not predicted to be a favored strategy and female egg maturation rates are unlikely to be flexible enough to keep at a negligible level the mismatch between daily egg-laying opportunities and daily egg load (Papaj 2000; Rosenheim et al. 2000; Berger et al. 2008). Interestingly, stochastic variation in female oviposition opportunities owing to temperature far exceeds that of variation owing to daily host availability or male disturbance. This is because temperature conditions can vary on a relatively large scale with extended periods of either good or bad weather. However, because of the trade-off between reproduction and life span, increasing reproductive rates to accommodate the ample egg laying opportunities available on good days necessarily comes at the cost of not surviving longer periods of bad weather. Females are therefore expected to exhibit reproductive rates that more closely correspond to average host encounter rates, rather than higher reproductive rates that correspond to the situation on good days. As such, females typically either encounter no hosts on days with bad weather and become completely host limited or encounter plenty of hosts on days with good weather and become egg limited. Therefore, the increased host plant availability that females experienced on warm days in the simulations results in only a very small increase in realized fecundity. As a result of this mechanism, relieving females of courting males had a negligible effect on female fitness in our simulations over entire female life spans, even though negative effects of potential sexual conflict were detected over the 75-min observation interval in the field study.

In addition to the importance of considering the interactive effects of different ecological factors that can inflict time limitation on female reproduction, this study also highlights how different ecological constraints on female egg laying can select for compensatory responses in behavior. Our simulations showed that females that are relieved from time limitation, especially in form of host limitation, can increase fitness by increasing host selectivity. Host limitation accounts for only a 6% reduction in realized fecundity but a 20% reduction in fitness because

of its effects on female selectivity (fig. 4). This illustrates the general difficulty of assessing female fitness when measuring only realized fecundity. For example, the fecundity of one outlier in the field study, female xM1, was particularly unaffected by the male courtship rate (fig. 1), as she was courted by the most males but still managed to maintain a very high egg-laying rate. At the same time, this female showed the lowest selectivity of all of the females we observed, ovipositing on 75% of the host plants she encountered.

A few other studies have shown the importance of female selectivity by assessing larval survivorship on plants that were accepted or rejected for egg laying. Offspring fitness on accepted hosts tended to be higher either (1) because of a reduced risk of predation compared with that of host plants in nonpreferred microhabitats (Wiklund and Friberg 2008) or (2) because they have a higher nutritional value than rejected plants (Damman and Feeny 1988; Doak et al. 2006). The independent quality assessments of the rejected and accepted host plants in this study provide support for the second interpretation of the importance of female selectivity, as the host plants that were chosen for egg laying were more vigorous and displayed lower levels of deterioration even 3–4 weeks after the egg-laying event when compared with those plants that were rejected by the females.

The field study also showed that large females with larger egg loads were less selective than small females with smaller egg loads. Returning to our heavily courted outlier female xM1, being the least selective in choosing hosts for her offspring, it is perhaps not surprising to find that she carried 27 eggs at the start of the trial, the second-largest egg load of all of the females. This aspect of female egg laying seems to be highly state dependent and sensitive to the risk of egg versus time limitation, further implied by selectivity being dependent on the time of day the females were followed (fig. 2). In other words, large females are not inherently less selective than smaller females, because the significant relationship between body size and host selectivity is mediated by female egg load. Body size is, however, a very strong predictor of female egg load, meaning that behavioral plasticity in female selectivity is also important for the evolution of reproductive allometries (Berger et al. 2012). This prediction was also supported by our model simulations that showed that female selectivity and reproductive rate are co-optimized with female body reserves, leading to allometry in female reproductive behavior.

The responses of female reproductive effort and life span to a relieving of time limitation on egg laying were considerably weaker than the behavioral response in terms of host selectivity (fig. 4*d*–4*f*). The weak physiological response to ecological constraints in *L. sinapis* is largely ex-

plained by our model assumptions that defined variation in life span and reproductive effort within the empirically derived reproduction–life span trade-off, which means that large increases in reproductive rate are very costly in terms of mortality risk. It was beyond the scope of this study to investigate the genetic basis for this trade-off, and our simulations did not allow the trade-off function itself to evolve, which could be predicted given a change in the average opportunity for egg laying. In addition, in nature, high rates of reproduction may, for example, be facilitated by increased adult foraging (Boggs 1986, 2009), and thus it seems likely that the regulation of rates of female reproduction and life span is more flexible than we assumed in our simulations.

Phenotypic plasticity in reproductive rate is also commonly observed among adult life stages (Tatar and Yin 2001) or between the sexes (Clutton-Brock and Parker 1992). This implies substantial room for adaptive regulation of reproductive rates in response to predictable variation in conditions during adult reproduction. When variation in reproductive opportunities is highly stochastic, however, it is less clear whether plasticity in the reproductive machinery itself will evolve. There are limits to how fast eggs can mature and be reabsorbed (Rosenheim et al. 2000; Berger et al. 2008, 2012), because rates of reproduction are in part regulated by basal metabolic functions shared with the whole-organism physiology (Hochacka and Somero 2002; Brown et al. 2004). As is commonly observed for other traits with a highly integrated physiological basis (reviewed in Angilletta et al. 2003; Huey et al. 2003; Lind and Cresswell 2005; Dial et al. 2008), and as predicted by our simulations, it may well be that behavioral compensation (e.g., female selectivity) can alleviate putative costs associated with relatively inflexible metabolic and developmental processes governing reproductive rate and life span in organisms experiencing highly unpredictable environments during adult reproduction (Berger et al. 2012). Nevertheless, compensatory behaviors are likely to come with costs such as high predation risk associated with increased activity levels, and thus ultimately there must be limits to such compensation.

Summary

The relative roles of ecological constraints in shaping reproductive strategies are still poorly understood, as are the fitness consequences associated with variation in reproductive strategies in natural populations. We were able to partition the effects of different ecological constraints on female reproductive output and fitness in nature. Female fitness was strongly affected by temperature variation and access to host plants, and less so by a potential sexual conflict over optimal mating rates. The results of our sim-

ulations supported the hypothesis put forth previously, that the impact of time limitation on female egg laying can affect large females disproportionately because of their larger potential fecundities and higher demands on reproductive opportunities, thereby enforcing stabilizing selection on female body size (Gotthard et al. 2007; Berger et al. 2008). In addition, both field data and model simulations show that this size dependence translates into a body-size allometry of female parental investment behavior that is predicted to be co-optimized with respect to life-history variation (Berger et al. 2012). Thus, our study highlights the importance of understanding how the functional relationships between ecological variables and behavioral and life-history variation ultimately determine female fitness in natural populations.

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