Developmental response to a seasonal time constraint: the effects of photoperiod on reproduction in the grasshopper *Romalea microptera*

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Abstract. 1. Some organisms respond adaptively to seasonal time constraints by altering development time to life-history transitions (e.g. metamorphosis, oviposition). Such life-history changes may have costs (e.g. reduced fecundity, mass, offspring quality).

2. The hypothesis that a northern population of the grasshopper *Romalea microptera* (Beauvois) would show adaptive plasticity in oviposition timing in response to seasonal time constraints was tested by manipulating photoperiod to simulate the middle of the active season (Long photoperiod), the end of the active season (Short photoperiod), and seasonal change (photoperiod Declining from long to short). Females received either high or low food rations. Short or Declining photoperiod were predicted to induce early oviposition with costs of reduced egg number, post-oviposition mass, or egg size, particularly in low-food females.

3. Effects of food ration and photoperiod, but not interaction, were significant in failure time analysis of age at oviposition. MANCOVA on age at oviposition, egg number, and post-oviposition mass yielded similar effects. The multivariate effect of photoperiod resulted primarily from reduced time to oviposition in Short or Declining photoperiod. No costs in egg number or post-oviposition mass were associated with this photoperiod-induced reduction in time to oviposition. The multivariate effect of food ration resulted mainly from lower egg number with low food. Food ration affected egg size, but photoperiod and interaction did not. In all cases, Short and Declining photoperiod produced similar effects.

4. In its northern range, *R. microptera* accelerates reproduction in response to seasonal constraints, a response that may be adaptive. How *R. microptera* avoids costs associated with this reduced pre-oviposition period remains unknown.

Key words. Life history, lubber grasshopper, photoperiod, plasticity, reproduction, season, time constraint.

Introduction

Life-history traits – including an organism's maturation time, reproductive schedule, and longevity – are shaped by natural selection, may be constrained by physiology and development, and may be expressed differently in different environments (Stearns, 1992; Nylin & Gotthard, 1998). Such phenotypic plas-

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ticity of life-history traits can be adaptive, so that life-history phenotypes change in ways that increase fitness (Nylin & Gotthard, 1998). However, such phenotypic shifts have limitations, as the degree of flexibility is dictated by an organism's evolutionary history and physiological limits (Schlichting & Pigliucci, 1998). In addition, there are trade-offs associated with expression of plastic traits (Nylin & Gotthard, 1998; Schlichting & Pigliucci, 1998), so that potentially adaptive changes in one trait are associated with costs in other, correlated traits.

Reproduction is intimately tied to season in many insects (Tauber *et al.*, 1986), and limitations imposed by a seasonal change are called seasonal time constraints (Rowe & Ludwig, 1991; Abrams *et al.*, 1996). As the active season ends, plants

senesce, resulting in declining food resources for herbivores (Scriber & Slansky, 1981; English-Loeb *et al.*, 1997), which combine with seasonal decline in temperature to slow development (Whitman, 1988) and to increase the probability of mortality. Univoltine insects must lay eggs before the end of the active season or they will realise no reproductive success. Therefore, insects that can accelerate reproduction or maturation under seasonal time constraints and reach oviposition prior to weather-induced mortality may be favoured in environments with short growing seasons (Luker *et al.*, 2002). There are numerous insect populations capable of altering development toward metamorphosis or reproduction in response to seasonal cues that indicate approaching seasonal time constraints (Rowe & Ludwig, 1991; Tanaka *et al.*, 1993; Abrams *et al.*, 1996; Nakao, 1998; Olvido & Mousseau, 1998; Johansson & Rowe, 1999; Plaistow & Siva-Jothy, 1999; Johansson *et al.*, 2001; De Block & Stoks, 2004; Plaistow *et al.*, 2005; Stoks *et al.*, 2005, 2006b). Such plasticity of reproductive timing and investment can be an important determinant of fitness (e.g. Tanaka *et al.*, 1993; Moehrlin & Juliano, 1998; Nylin & Gotthard, 1998; Olvido & Mousseau, 1998; Ekesi *et al.*, 1999; Luker *et al.*, 2002).

Decreased time to oviposition in response to seasonal constraints is expected to come at a cost, as accelerated development to oviposition results in less time for accumulation of resources. The result may be some combination of reduced fecundity, reduced egg size, reduced immune function, reduced somatic storage, or increased risk of predation as foraging effort increases (Blanckenhorn, 2000; Hatle *et al.*, 2000; Luker *et al.*, 2002; Gunawardene *et al.*, 2004; Stoks *et al.*, 2005, 2006a,b). Despite the seemingly obvious association of costs with reduced development time, some investigations of plasticity in response to time constraints have failed to provide any evidence of costs (e.g. Plaistow *et al.*, 2005). Understanding these costs is a critical part of understanding how plastic responses to seasonal constraints may be adaptive.

There are probably multiple cues that signal the end of the active season but photoperiod is a reliable cue because it follows the same pattern every year (Tauber *et al.*, 1986). Effects of photoperiod on life-history traits vary (e.g. Leimar, 1996; Miles *et al.*, 1998; Olvido & Mousseau, 1998; Ekesi *et al.*, 1999; Johansson & Rowe, 1999; Johansson *et al.*, 2001; Luker *et al.*, 2002). Maturation times of damselfly nymphs are sometimes affected by photoperiod (Johansson & Rowe, 1999; Johansson *et al.*, 2001). Timing of reproduction often changes in response to photoperiod (e.g. Hewitt, 1985; Tanaka *et al.*, 1993; Miles *et al.*, 1998; Nakao, 1998; Ishihara, 2000; Zhou, 2001). This paper investigates the plasticity of reproductive timing to seasonal constraints and searches for costs of that response in an insect model system.

Model system

Romalea microptera (Beauvois) (Orthoptera: Acrididae) is a univoltine grasshopper from the southeastern U.S.A. Reproductive timing and investment in *R. microptera* are determined by resources acquired through feeding by adults, rather than reserves carried over from the nymphal stage (Moehrlin & Juliano, 1998; Borst *et al.*, 2000; Hatle *et al.*, 2004). Females mature in summer and lay multiple clutches of eggs (Gunawardene *et al.*,

2004) until the end of the active season some time in the autumn. Eggs are deposited in the soil and must go through obligate diapause before hatching in spring. Age at first oviposition differs among populations of *R. microptera*, with North Georgia ovipositing at the youngest age, South Florida ovipositing at the oldest age, and South Louisiana intermediate (Hatle *et al.*, 2002). Thus, the North Georgia population from a short-season environment is on a physiologically accelerated reproductive schedule relative to that of the other two populations (Hatle *et al.*, 2002, 2004), a pattern observed in other grasshoppers (Berner *et al.*, 2004; Berner & Blanckenhorn, 2006).

Seasonal photoperiod effects on time to oviposition for a South Florida population of *R. microptera* were not significant (Luker *et al.*, 2002). Paradoxically, there was a significant interaction of photoperiod and food affecting clutch size, with Short photoperiod and low food yielding a significantly reduced clutch size (Luker *et al.*, 2002). One interpretation of these results is that the subtropical climate of South Florida imposes only minor seasonal constraints and that selection has not favoured accelerated reproductive development at the end of the active season (Luker *et al.*, 2002). If this is so, then populations from higher latitudes, with more severe and earlier unfavourable seasons, may show accelerated oviposition in response to seasonal photoperiod. Therefore it is interesting to test for effects of seasonal photoperiod on reproduction of a northern population of *R. microptera*, which comes from an area with more severe seasonal constraints on life history than does the South Florida population.

Hypothesis and predictions

The hypothesis that *R. microptera* from North Georgia show adaptive acceleration of reproduction in response to seasonal time constraints associated with the end of the active season was tested experimentally. Photoperiod is the most likely cue for such a seasonal response. There were two major predictions from this hypothesis. First, time from adult eclosion to oviposition should be less in a Short or Declining photoperiod than in a Long photoperiod. Second, the cost of a shortened time to oviposition should be an associated reduction in egg number (Moehrlin & Juliano, 1998), mean egg mass, somatic mass of the female (Hatle *et al.*, 2002), or some other fitness-related trait, because accelerated reproduction limits the time available for accumulation of resources. Because accelerated reproduction should reduce resource accumulation, the hypothesis further predicts that any costs will be most evident when food availability is low, and minimal or absent when food availability approaches *ad libitum* feeding. Thus, high food should result in oviposition in nearly the minimum time with near maximal clutch size, so that a significant interaction of photoperiod and food ration is predicted.

Materials and methods

Experimental animals

Grasshoppers used in this experiment were obtained as field-collected first- and second-instar nymphs from Athens, Georgia

(longitude: 83.32°W, latitude: 33.90°N; elevation: 202 m), which is located close to the northern limit of *R. microptera*'s range. This was one of the populations investigated by Hatle *et al.* (2002, 2004) and Gunawardene *et al.* (2004). The number of days the temperature falls below freezing averages 116 and the frost season runs approximately from the first week in November to the end of February (Southeast Regional Climate Center, 2001). The median first freeze is the first week in November but may come as early as the second week of October (Southeast Regional Climate Center, 2001). Grasshoppers in this region may lay multiple clutches of eggs (Gunawardene *et al.*, 2004). After winter diapause, the first hatchlings emerge between 1 April and 1 May. However, by 21 September, most herbaceous vegetation is senescing and few lubbers remain alive (D. W. Whitman & M. R. Brown, pers. comm.) although mean high temperature is still 26 °C (Southeast Regional Climate Center, 2001). Nymphs were reared to adulthood on a diet of Romaine lettuce and oatmeal, supplemented with carrots, beans, and other vegetables. Upon moult to adulthood they entered the experiment.

Experimental design

Three different photoperiod treatments were used: Short, Long, and Declining, chosen based on astronomical information for Athens, Georgia. To estimate the time of moult to adulthood, the median hatching date (15 April) was added to the approximate nymphal development period (approximately 60 days, R. H. Homeny and S. A. Juliano, unpublished data), yielding a date of 15 June. Day length on this date, 14.5 h (U.S. Naval Observatory, 2003), was used as the Long photoperiod treatment. By 21 September most grasshoppers have disappeared. On this date, day length is 12 h (U.S. Naval Observatory, 2003), and this value was used as the Short photoperiod. The Declining photoperiod treatment began with the Long photoperiod (L:D 14.5:9.5 h) and ended with the Short photoperiod (L:D 12:12 h), and was designed to simulate photoperiod change across the active season. Facilities to produce continuous changes in photoperiod were unavailable, hence photoperiod was reduced in a stepwise fashion, using two intermediate photoperiods: L:D 13.75:10.25 h and L:D 13:11 h (Fig. 1). Grasshoppers spent 20 days in each of the first three photoperiods, beginning with the longest, and moving toward the shortest. Once they reached the shortest photoperiod, they remained there until oviposition or death. Each photoperiod was implemented in a separate environmental chamber, with all chambers set to the same temperature and thermoperiod for all treatment groups. The same thermoperiod and day and night temperatures used by Luker *et al.* (2002) were used in this study: 12:12 h, 32:24 °C, with the high-temperature phase symmetrically situated within the light phase (Fig. 1). Within each photoperiod grasshoppers were placed on either a high- (Mean ± SD 7.00 ± 0.02 g of lettuce and 0.10 ± 0.01 g of dry oatmeal) or low food ration (1.00 ± 0.02 g of lettuce and 0.02 ± 0.01 g of dry oatmeal). The amount in the high food ration was in excess of the consumption possible for a female, whereas the amount in the low food ration was sufficiently low that virtually all females consumed the entire ration each day.
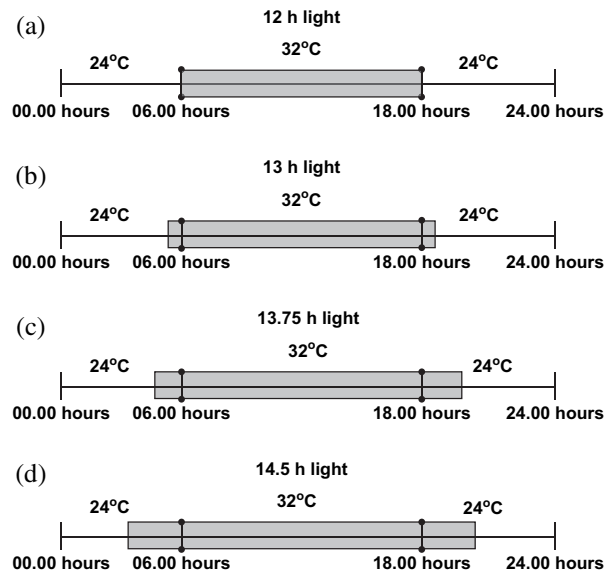

Fig. 1. Photoperiod (indicated by grey bars) and thermoperiods (indicated by vertical lines with filled circles) for Short (a), two intermediate (b, c), and Long (d) photoperiod treatments.

Each day, newly moulted female grasshoppers were chosen at random and assigned in sequence to the six treatment combinations, so that all combinations were approximately equally replicated (see Fig. 2 for sample sizes). Grasshoppers were housed in individual, ventilated plastic containers in their respective environmental chambers. For simplicity, grasshoppers were unmated, as they will oviposit without mating, although lack of mating delays oviposition by about 2 days (Walker *et al.*, 1999). Grasshoppers were placed in an individual container with sand, a suitable substrate for oviposition, for 1 h each day, beginning 21 days after adult eclosion. The experiment continued until all grasshoppers either oviposited (52 grasshoppers) or died (15 grasshoppers). Mortality was independent of treatment (data not shown).

Each grasshopper's mass at adult eclosion was recorded as a measure of overall size. When grasshoppers oviposited, their age at oviposition and their post-oviposition fresh mass were recorded. Post-oviposition fresh mass, relative to initial mass, is an indicator of reserves remaining after the first clutch is produced, and thus may be an indicator of any costs (e.g. reduced reserves) due to accelerated oviposition (Hatle *et al.*, 2002). Eggs were removed from the sand and counted. A sample of 10 eggs was dried (60 °C, 24 h) and weighed to the nearest 0.1 μg using a Cahn microbalance (Cahn Instruments, Cerritos, California). After oviposition, grasshoppers were dissected and full size (>9 mm) unlaid eggs were counted. The unlaid eggs were included in estimated clutch size because they represent material investment in reproduction up to the time of egg laying.

Statistical analysis

Failure-time analysis (also called survival analysis) is the method of choice for comparing times to events for different
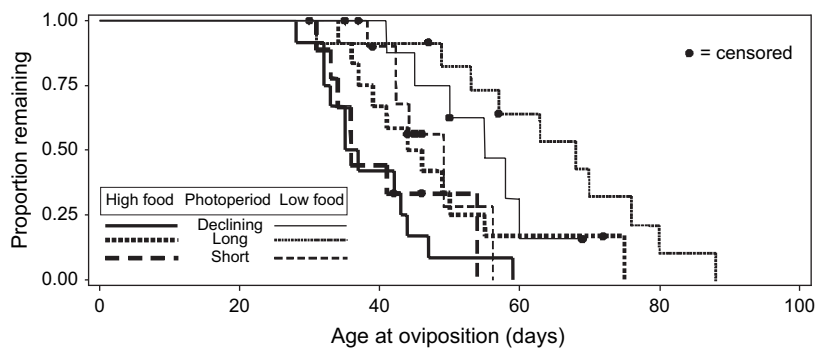
Fig. 2. Survivorship for all combinations of photoperiod treatment and food ration. Statistical tests comparing survivorship distributions in each of the six combinations are given in Table 1. Sample sizes for each group are: high food ration–Declining $n = 12$, Long $n = 12$, Short $n = 10$; low food ration–Declining $n = 10$, Long $n = 12$, Short $n = 11$.

experimental groups because this approach can take into account censored observations (i.e. females that died prior to oviposition) and a variety of non-normal distributions of times (Allison, 1995; Fox, 2001). Thus, analysis of the effects of seasonal constraints on *R. microptera* reproduction began with failure-time analysis testing for effects of food ration (low vs high), photoperiod (Long, Short, Declining), and interaction. Effects of two covariates (initial mass and date of adult eclosion of the female) were also tested using PROC LIFEREG (SAS Institute Inc., 2004) with a Weibull distribution of error. That distribution adequately described the apparently increasing hazard function evident in these data (see Allison, 1995 for details). Choosing Weibull, gamma, and log-logistic distributions (Allison, 1995) did not alter the statistical conclusions.

In order to understand costs of changes in reproductive timing, age at oviposition, egg number, and post-oviposition somatic mass (i.e. subtracting estimated mass of any unlaid eggs) were analysed by multivariate analysis of covariance (MANCOVA), using initial mass at adult eclosion as a covariate. This approach considers these response variables as a correlated set and is thus ideally suited for identifying costs, but cannot include censored observations. Collectively these dependent variables are referred to as *reproductive output*. A separate ANOVA on egg mass (the mean dry mass of 10 eggs) was also carried out. Egg masses were unavailable for some females; hence this variable was not included in the MANCOVA. Both MANCOVA and ANOVA tested effects of photoperiod, food, and interaction. Age at oviposition, post-oviposition somatic mass, and egg number were $\log_{10}$ transformed to meet assumptions of homogeneity of variances and normality. Mean egg mass required no transformation. Multivariate equality of slopes was tested and verified prior to employing MANCOVA. Multivariate contrasts (Scheiner, 2001; Hatle *et al.*, 2002), with a Bonferroni adjustment for an experiment-wise $\alpha = 0.05$, were used to compare effects of photoperiod treatments, comparing first Long versus Short and Declining photoperiods, then comparing Short versus Declining photoperiods. Standardised canonical coefficients (Scheiner, 2001; Hatle *et al.*, 2002; SAS Institute Inc., 2004) were used to evaluate the contributions of each dependent variable to significant MANCOVA effects.

Results

Age at oviposition

Both photoperiod ($\chi^2 = 16.84$, d.f. $= 2$, $P = 0.0002$) and food ration ($\chi^2 = 14.93$, d.f. $= 1$, $P = 0.0001$) were significant in failure time analysis, but interaction was not ($\chi^2 = 1.89$, d.f. $= 1$, $P = 0.3886$). Covariates of date of eclosion and mass at eclosion were not significant ($P > 0.05$) and were removed from the analysis. As expected, high food ration animals oviposited earlier than did low food ration animals (Fig. 2). Also as predicted, Long photoperiod yielded later age at oviposition than did Short or Declining photoperiods (contrast $\chi^2 = 6.21$, d.f. $= 1$, $P = 0.0127$), which did not differ significantly (contrast $\chi^2 = 1.74$, d.f. $= 1$, $P = 0.1871$, Fig. 2). Short or Declining photoperiod resulted in median age at oviposition anywhere from 8 to 19 days earlier than did Long photoperiod under the same feeding conditions (Fig. 2), representing an approximately 20% reduction in the time to first oviposition.

Reproductive output

There was no multivariate interaction between food ration and photoperiod. A significant multivariate food ration effect (Table 1) resulted primarily from effects on egg number (see standardised canonical coefficients in Table 1). Age at oviposition and mass after oviposition contributed relatively little to the food ration effect, in part because of the considerable variation in age at oviposition within a food ration (Fig. 3). Means (Fig. 3) showed the typical food-dependent pattern of high food producing large mean egg number and low age at oviposition. A significant photoperiod effect resulted primarily from differences in age at oviposition, with a much lower contribution from differences in number of eggs or post-oviposition mass (Table 1, Fig. 3). All canonical coefficients are positive, indicating that means are positively associated (i.e. certain treatments lead to lower time to oviposition, and to a lesser extent lower egg number and post-oviposition mass, see Fig. 3). Combined Short and Declining photoperiod treatments differed significantly from Long photoperiod (Table 1), with Short or Declining photoperiod resulting in earlier oviposition

Table 1. MANCOVA results and canonical coefficients for age at oviposition, number of eggs, and post-reproductive mass. Significant effects are highlighted in bold face. Multivariate contrasts for photoperiod effects are included.

				Standardised canonical coefficients		
Source	Pillai's trace (*F*)	d.f.	*P*	Age at oviposition	Number of eggs	Mass after oviposition
Food ration	**31.82**	**3,43**	**0.0001**	−0.10	**1.63**	**0.13**
Photoperiod	**2.40**	**6,88**	**0.0341**	1.35	**0.43**	**0.10**
Long vs Declining and Short	**3.68**	**3,43**	**0.0192**	1.31	**0.45**	**0.02**
Declining vs Short	1.25	3,43	0.3046	0.92	−0.23	1.24
Food ration × Photoperiod	0.49	6,88	0.8121	0.73	−1.26	0.27
Eclosion mass	**3.44**	**3,43**	**0.0249**	−0.59	**0.79**	**0.87**

(Fig. 3). Short and Declining photoperiod did not differ significantly (Table 1). Eclosion mass had a significant effect, resulting to some extent from trends in all three variables (Table 1). As eclosion mass increased, mass after oviposition and egg number increased, and age at oviposition decreased (see canonical coefficients, Table 1).

Egg mass

There was no significant interaction between food ration and photoperiod ($F_{2,42} = 0.74$, $P = 0.4819$), and no effect of eclosion mass ($F_{1,40} = 0.04$, $P = 0.8520$) on egg mass. Food ration had a significant effect on egg mass ($F_{1,42} = 6.63$, $P = 0.0136$), with high food ration yielding slightly, but significantly larger eggs than did low food ration (Fig. 4). Photoperiod did not have a significant effect on egg mass ($F_{2,42} = 2.34$, $P = 0.1086$, Fig. 4).

Discussion

Differences due to photoperiod resulted mainly from effects on age at oviposition. Age at oviposition for females on both high and low rations was reduced by Short or Declining photoperiod. This is consistent with the hypothesis of accelerated development in response to seasonal time constraints, with photoperiod as the seasonal cue, and this acceleration of timing of oviposition could represent adaptive plasticity in response to season. The absence of significant food ration–photoperiod interaction effects indicates that even under high-food conditions, these grasshoppers accelerate reproduction in response to late-season photoperiods, implying that development toward oviposition is not necessarily proceeding at its maximum rate, even with *ad libitum* food availability. Accelerated development under simulated seasonal time constraints resulted in relatively large decreases in median age at first oviposition, ranging from about 8–9 days for high food ration females to 13–19 days for low food ration females, representing approximately 19% and 23% reductions respectively of the time to reach oviposition relative to the Long photoperiod conditions that simulate no seasonal constraint.

For *R. microptera* that become adults on 21 September, when natural photoperiod would be as in the Short treatment, it is interesting to ask: What would be the effects of the changes in

age at oviposition in nature? For poorly fed animals, as in the low food treatment, short photoperiod induced oviposition after a mean of 45 days, equivalent to a mean oviposition date of 5 November. In contrast, if such poorly fed animals oviposited at the age associated with a Long photoperiod (mean = 62 days), mean oviposition date would be delayed until 22 November, well after median first freeze date at Athens (Southeast Regional Climate Center, 2001). This estimate is based on the laboratory animals that were kept at warm summer-like temperatures of 32 °C by day. In fact, mean high temperatures in Athens over this
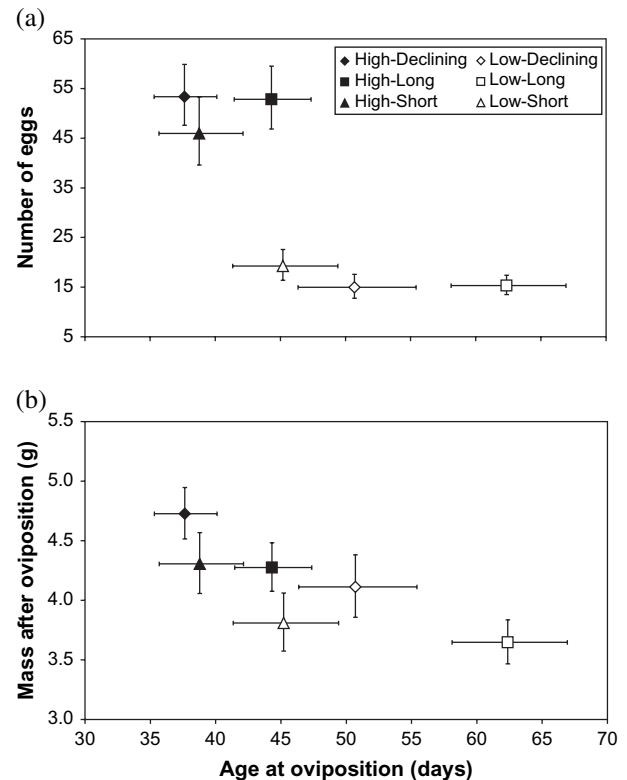

Fig. 3. Multivariate means ± SE for reproductive output under six combinations of photoperiod and food ration. Statistical tests are reported in Table 1. (a) Bivariate means for clutch size and age at oviposition. (b) Bivariate means for post-oviposition somatic mass and age at oviposition.
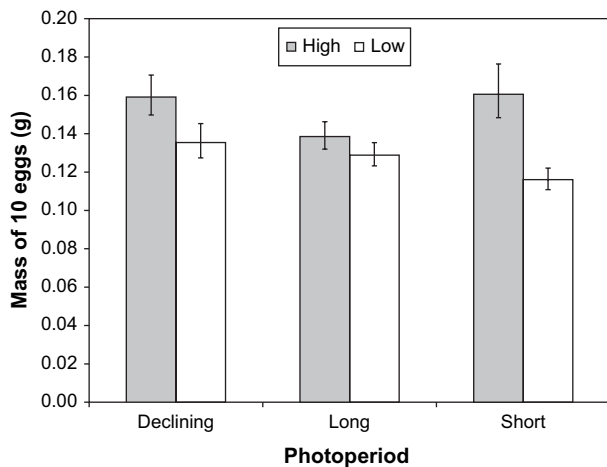
Fig. 4. Mean masses of 10 eggs (±SE) for each combination of photoperiod and food ration. Statistical tests are reported in the text.

period are much lower (21 September = 26 °C; mid November = 17 °C; Southeast Regional Climate Center, 2001), and probably would produce a longer time to oviposition, as reproductive development in grasshoppers is highly temperature dependent (Whitman, 1988; Blanford & Thomas, 2000). For well fed animals the effect would be similar, but less dramatic. Nevertheless, it seems likely that the acceleration of oviposition observed in the present study could increase the probability of successful oviposition before freezing conditions are likely.

The significant food ration effect, which resulted primarily from effects on clutch mass, was expected. Poorly fed females would not be expected to have the same reproductive output as well-fed females, unless they also reduced somatic mass retained after oviposition, but there was no significant difference between high and low food ration animals in post-reproductive somatic mass. Age at oviposition also contributed to the significant food ration effect, as expected, with females on low rations requiring a longer feeding period to acquire the resources needed to reproduce.

Although time to oviposition was significantly shorter for Short and Declining photoperiods, there was no associated cost in egg number, post-reproductive mass, or mean egg mass. Because low ration animals had limited resources, they should show a trade-off with early oviposition (i.e. in Short or Declining photoperiod) associated with reduced clutch mass or post-oviposition mass. That no such costs can be detected is surprising, and suggests that any costs are not apparent in measures as crude as egg number, post-oviposition fresh mass, or mean egg mass. The product of mean egg dry mass and egg number (= clutch mass) also was not affected by photoperiod (data not shown). Clutch mass is an underestimate of investment in clutches for *R. microptera*, which deposits eggs in a mass of proteinaceous foam (Stauffer & Whitman, 1997; Stauffer *et al.*, 1998). If early-ovipositing females reduced the amount of foam surrounding the egg clutch, this effect would not have detected. The consequences of any such a reduction in foam are unknown. Post-oviposition somatic mass was measured as fresh mass of

the female after depositing the eggs, and thus would include the loss of mass associated with foam. However, the cost of early oviposition may be expressed in reductions of particular body components (e.g. protein or fat storage) that may be obscured by correlated increases in other body components (e.g. carbohydrate). Thus, costs of accelerated development to oviposition may be expressed in individual condition and not readily detectable in gross body mass. In some insects accelerated life-history transitions are associated with costs in energy storage (Stoks *et al.*, 2006a) and immune function (e.g. Koella & Boëte, 2002; Rantala & Roff, 2005; Stoks *et al.*, 2006b) that ultimately may translate in reduced lifetime mating success (De Block & Stoks, 2005). These other costs were not assessed in this experiment. Costs of accelerated development may be minimised by behavioural changes, particularly increased foraging effort (Johansson *et al.*, 2001). Under seasonal constraints, as development accelerates, time spent foraging may also increase, resulting in greater food intake and growth in the shorter time leading up to oviposition. Such change in behaviour could explain the lack of detectable costs in the high-food-ration females (which had *ad libitum* food, and thus could have increased intake), but this explanation is unlikely for the low-food-ration females, which typically consumed all the food that they were given each day. The general lack of interaction of photoperiod and food ration in all these analyses is surprising in light of the similar study conducted on a Florida population of *R. microptera* (Luker *et al.*, 2002), which showed interacting effects of photoperiod and food ration. The limited photoperiodic response, particularly in timing of oviposition, documented for south Florida *R. microptera* by Luker *et al.* (2002, see Fig. 2) is understandable given the limited seasonal constraint at this subtropical latitude. What remains unresolved are the mechanisms yielding no detectable costs to photoperiod-induced plasticity in the North Georgia population, and the presence of a photoperiod–diet interaction on clutch size in the absence of an effect on reproductive timing in the south Florida population.

In all cases, effects of Short and Declining photoperiod on timing of oviposition, clutch size, post-oviposition mass, and egg number were similar, and differed from the effects of Long photoperiod. This result suggests that reproductive tactics are sensitive to photoperiod during the mid- to late-oviposition cycle. Females in the Declining photoperiod groups experienced long photoperiod for the first 20 days of the experiment. If they were sensitive to photoperiod only during this early part of their oviposition cycle, no difference between Long and Declining photoperiods would be expected. Females on the high food ration oviposited between days 30 and 60 of the experiment (Fig. 2), and even among these females, timing of oviposition under Declining photoperiod was more similar to that of females on Short photoperiod (Fig. 2). This observation suggests that *R. microptera* is sensitive to photoperiod between 20 and 40 days after eclosion, and that a relatively small decline of photoperiod from 14.5 to 13.75 h (i.e. the first incremental reduction in photoperiod – see Fig. 1) is sufficient to induce earlier reproduction. Such a photoperiod corresponds to that in Athens Georgia on about 1 August (U.S. Naval Observatory, 2003), and suggests accelerated oviposition responses to season by *R. microptera* could occur rather early in the season.

Egg number and egg mass were positively affected by food ration but not by simulated seasonal constraints. Previous experiments have indicated little plasticity in egg size in response to food ration for female for *R. microptera* (Moehrlin & Juliano, 1998; Hatle *et al.*, 2002; Luker *et al.*, 2002), and thus contrast with the present results. However, these previous experiments showed that *R. microptera* has considerable plasticity of egg number in response to food ration of the female, and the current experiment is consistent with those observations.

Romalea microptera from this northern population shows potentially adaptive life-history responses to seasonal time constraints. This population responds to seasonal constraints quite differently from a conspecific population from the southern part of its range (Luker *et al.*, 2002), suggesting local adaptation to seasonality. As yet, there is no evidence for costs of this response to season. Resolving the actual costs (if any) of that response, or how any costs are avoided, will be the next challenge for continuing research on responses of *R. microptera* to seasonal constraints.

Acknowledgements

References

Abrams, P.A., Leimar, O., Nylin, S. & Wiklund, C. (1996) The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. *American Naturalist*, **147**, 381–395.

Allison, P.D. (1995) *Survival Analysis Using the SAS® System: A Practical Guide*. SAS Institute Inc., Cary, North Carolina.

Berner, D. & Blanckenhorn, W.U. (2006) Grasshopper ontogeny in relation to time constraints: adaptive divergence and stasis. *Journal of Animal Ecology*, **75**, 130–139.

Berner, D., Körner, C. & Blanckenhorn, W.U. (2004) Grasshopper populations across 2000 m of altitude: is there life history adaptation? *Ecography*, **27**, 733–740.

Blanckenhorn, W.U. (2000) The evolution of body size: what keeps organisms small? *Quarterly Review of Biology*, **75**, 385–407.

Blanford, S. & Thomas, M.B. (2000) Thermal behavior of two acridid species: effects of habitat and season on body temperature and the potential impact on biocontrol with pathogens. *Environmental Entomology*, **29**, 1060–1069.

Borst, D.W., Eskew, M.R., Wagner, S.J., Shores, K., Hunter, J., Luker, L. *et al.* (2000) Quantification of juvenile hormone III, vitellogenin, and vitellogenin-mRNA during the oviposition cycle of the lubber grasshopper. *Insect Biochemistry and Molecular Biology*, **30**, 813–819.

De Block, M. & Stoks, R. (2004) Life-history variation in relation to time constraints in a damselfly. *Oecologia*, **140**, 68–75.

De Block, M. & Stoks, R. (2005) Fitness effects from egg to reproduction: bridging the life history transition. *Ecology*, **86**, 185–197.

Ekesi, S., Maniania, N.K. & Onu, I. (1999) Effects of temperature and photoperiod on development and oviposition of the legume flower thrips, *Megalurothrips sjostedti*. *Entomologia Experimentalis et Applicata*, **93**, 149–155.

English-Loeb, G., Stout, M.J. & Duffey, S.S. (1997) Drought stress in tomatoes – changes in plant chemistry and potential nonlinear consequences for insect herbivores. *Oikos*, **79**, 456–468.

Fox, G. (2001) Failure-time analysis: studying times to events and rates at which events occur. *Design and Analysis of Ecological Experiments* (ed. by S. M. Scheiner and J. Gurevitch), 2nd edn, pp. 235–266. Oxford University Press, Oxford.

Gunawardene, E.U., Stephenson, R.E., Hatle, J.D. & Juliano, S.A. (2004) Are reproductive tactics determined by local ecology in *Romalea microptera* (Orthoptera: Acrididae)? *Florida Entomologist*, **87**, 119–123.

Hatle, J.D., Andrews, A.L., Crowley, M.C. & Juliano, S.A. (2004) Interpopulation variation in developmental titers of vitellogenin, but not storage proteins, in lubber grasshoppers. *Physiological and Biochemical Zoology*, **77**, 631–640.

Hatle, J.D., Crowley, M.C., Andrews, A.L. & Juliano, S.A. (2002) Geographic variation of reproductive tactics in lubber grasshoppers. *Oecologia*, **132**, 517–523.

Hatle, J.D., Juliano, S.A. & Borst, D.W. (2000) Juvenile hormone is a marker of the onset of reproductive canalization in lubber grasshoppers. *Insect Biochemistry and Molecular Biology*, **30**, 821–827.

Hewitt, G. (1985) *Review of Factors Affecting Fecundity, Oviposition, and Egg Survival of Grasshoppers in North America*. United States Department of Agriculture, Agricultural Research Service 36, Washington, D.C.

Ishihara, M. (2000) Effect of variation in photoperiodic response on diapause induction and developmental time in willow leaf beetle, *Plagiodera versicolora*. *Entomologia Experimentalis et Applicata*, **96**, 27–32.

Johansson, F. & Rowe, L. (1999) Life history and behavioral responses to time constraints in a damselfly. *Ecology*, **80**, 1242–1252.

Johansson, F., Stoks, R., Rowe, L. & DeBlock, M. (2001) Life history plasticity in a damselfly: effects of combined time and biotic constraints. *Ecology*, **82**, 1857–1869.

Koella, J.C. & Boëte, C. (2002) A genetic correlation between age at pupation and melanization immune response in the yellow fever mosquito *Aedes aegypti*. *Evolution*, **56**, 1074–1079.

Leimar, O. (1996) Life history plasticity: influence of photoperiod on growth and development of the common blue butterfly. *Oikos*, **76**, 228–234.

Luker, L., Hatle, J.D. & Juliano, S.A. (2002) Reproductive responses to photoperiod by the grasshopper, *Romalea microptera* (Orthoptera: Romaleidae) from south Florida. *Environmental Entomology*, **31**, 702–707.

Miles, J.E., Bale, J.S. & Hodkinson, I.D. (1998) Life cycle regulation in the heather psyllid *Strophingia ericae*: responses to temperature and photoperiod. *Physiological Entomology*, **23**, 376–381.

Moehrlin, G.S. & Juliano, S.A. (1998) Plasticity of insect reproduction: testing models of flexible and fixed development in response to different growth rates. *Oecologia*, **115**, 492–500.

Nakao, S. (1998) Effects of photoperiod and temperature on induction and termination of reproductive diapause of *Thrips setosus* Moulton (Thysanoptera: Thripidae). *Japanese Journal of Applied Entomology and Zoology*, **42**, 172–173.

Nylin, S. & Gotthard, K. (1998) Plasticity in life-history traits. *Annual Review of Entomology*, **43**, 63–83.

Olvido, A.E. & Mousseau, T.A. (1998) Seasonal effects on oviposition behavior in *Allonemobius socius* (Orthoptera: Gryllidae), test of the sense of malaise hypothesis. *Annals of the Entomological Society of America*, **91**, 488–492.

Plaistow, S.J. & Siva-Jothy, M.T. (1999) The ontogenetic switch between odonate life-history stages: effects on fitness when time and food are limited. *Animal Behaviour*, **58**, 659–667.

Plaistow, S.J., Tsuchida, K., Tsubaki, Y. & Setsuda, K. (2005) The effect of a seasonal time constraint on development time, body size, condition, and morph determination in the horned beetle *Allomyrina dichotoma* L. (Coleoptera: Scarabaeidae). *Ecological Entomology*, **30**, 692–699.

Rantala, M.J. & Roff, D.A. (2005) An analysis of trade-offs in immune function, body size and development time in the Mediterranean Field Cricket, *Gryllus bimaculatus*. *Functional Ecology*, **19**, 323–330.

Rowe, L. & Ludwig, D. (1991) Size and timing of metamorphosis in complex life cycles: time constraints and variation. *Ecology*, **72**, 413–427.

SAS Institute Inc. (2004) *SAS/STAT® 9.1 User's Guide*. SAS Institute Inc., Cary, North Carolina.

Scheiner, S.M. (2001) MANOVA: multiple response variables and multispecies interactions. *Design and Analysis of Ecological Experiments* (ed. by S. M. Scheiner and J. Gurevitch), 2nd edn, pp. 99–115. Oxford University Press, Oxford, U.K.

Schlichting, C.D. & Pigliucci, M. (1998) *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer Associates, Sunderland, Massachusetts.

Scriber, J.M. & Slansky, F. Jr (1981) The nutritional ecology of immature insects. *Annual Review of Entomology*, **26**, 183–211.

Southeast Regional Climate Center (2001) *Historical Climate Summaries* [WWW document]. URL http://www.sercc.com/climateinfo/historical/historical.html, accessed May 2001.

Stauffer, T.W., Hegrenes, S.G. & Whitman, D.W. (1998) A laboratory study of oviposition site preferences in the lubber grasshopper, *Romalea guttata* (Houttuyn). *Journal of Orthoptera Research*, **7**, 217–221.

Stauffer, T.W. & Whitman, D.W. (1997) Grasshopper oviposition. *The Bionomics of Grasshoppers, Katydids, and their Kin* (ed. by S. K.

Gangwere, M. C. Muralirangan and M. Muralirangan), pp. 231–280. CAB International, Wallingford, U.K.

Stearns, S.C. (1992) *Evolution of Life Histories*. Oxford University Press, New York.

Stoks, R., De Block, M., Van de Meutter, F. & Johansson, F. (2005) Predation cost of rapid growth: behavioural coupling and physiological decoupling. *Journal of Animal Ecology*, **74**, 708–715.

Stoks, R., De Block, M. & McPeek, M. (2006a) Physiological costs of compensatory growth in a damselfly. *Ecology*, **87**, 1566–1574.

Stoks, R., De Block, M., Slos, S., Van Doorslaer, W. & Rolff, J. (2006b) Time constraints mediate predator-induced plasticity in immune function, condition, and life history. *Ecology*, **87**, 809–815.

Tanaka, S., Hakomori, T. & Hasegawa, E. (1993) Effects of day length and hopper density on reproductive traits in a Japanese population of the migratory locust, *Locusta migratoria* L. *Journal of Insect Physiology*, **39**, 571–580.

Tauber, M.J., Tauber, C.A. & Masaki, S. (1986) *Seasonal Adaptations of Insects*. Oxford University Press, New York.

U.S. Naval Observatory (2003) *Astronomic Applications Department* [WWW document]. URL http://aa.usno.navy.mil/, accessed June 2003.

Walker, M.P., Lewis, C.J. & Whitman, D.W. (1999) Effects of males on the fecundity and fertility of female *Romalea microptera* grasshoppers. *Journal of Orthoptera Research*, **8**, 277–283.

Whitman, D.W. (1988) Function and evolution of thermoregulation in the desert grasshopper *Taeniopoda eques*. *Journal of Animal Ecology*, **57**, 369–383.

Zhou, H.-Z. (2001) Reproduction of *Lagria hirta* (Coleoptera: Lagriidae) and its life-history trait correlation. *Environmental Entomology*, **30**, 686–691.