Ecology, 85(11), 2004, pp. 2986–2996

PLASTICITY AND CANALIZATION OF INSECT REPRODUCTION: TESTING ALTERNATIVE MODELS OF LIFE HISTORY TRANSITIONS

STEVEN A. JULIANO,[1,3] JENNIFER R. OLSON,[1,2,4] EBONY G. MURRELL,[1,2,5] AND JOHN D. HATLE[1,6]

[1]*Department of Biological Sciences, Behavior, Ecology, Evolution, and Systematics Section, Illinois State University, Normal, Illinois 61790-4120 USA*
[2]*Department of Biology, Illinois Wesleyan University, 1312 Park Street, Bloomington, Illinois 61701 USA*

Abstract. Life histories may show phases of both plasticity and canalization in response to feeding rate. Models for life history canalization and plasticity postulate a threshold for initiation of canalized developmental events. Some models postulate adaptive plasticity, whereas others postulate nonadaptive plasticity that results from environmental modulation of fixed development. These models have been tested by changing feeding rate at various times and determining when timing of life history events becomes unresponsive to those changes. This approach has been criticized because putative thresholds are usually not known. We use an alternative experimental design to test models of reproductive plasticity and canalization, and to estimate thresholds, in the grasshopper *Romalea microptera*. We develop mathematical models for published verbal models that predict how life history timing changes with feeding rate. Alternative models predict distinct relationships of time to oviposition vs. mean food intake that we test via experimental manipulation of food intake and nonlinear regressions. Regressions yield estimates of both the threshold and the duration of post-threshold development. A model postulating a fixed threshold and canalized post-threshold development provides the best, most parsimonious fit to data for this grasshopper. Thus, the simplest model, postulating no adaptive variation in development, is supported, a result that is consistent with previous experiments on this system using changing feeding rates. We use the estimate of the threshold (in units of food eaten) and measurements of hemolymph protein content to estimate the threshold in units of physiologically relevant storage. These results elucidate the structure of reproductive plasticity in this system and how this alternative experimental approach can provide testable predictions of developmental thresholds for further experiments on life history plasticity and canalization.

Key words: fixed development model; grasshopper; nonlinear regression; oviposition; reproductive tactics; Romalea microptera; *Wilbur-Collins model.*

INTRODUCTION

Life history transitions, such as metamorphosis, maturation, and reproduction, can be characterized by the age and the condition of the organism (i.e., its size, nutritional status, or energy reserves) at which the transition occurs (Smith-Gill and Berven 1979, Travis 1984, Stearns and Koella 1986, Werner 1989, Rowe and Ludwig 1991, Nylin and Gotthard 1998, Hentschel 1999, Day and Rowe 2002). Empirical investigations of reaction norms for metamorphosis, maturation, and reproduction have shown that some form of life history plasticity in response to feeding rate is nearly ubiquitous (e.g., Travis 1984, Alford and Harris 1988, Gebhardt and Stearns 1988, Reznick 1990, Hensley 1993, Ebert 1994, Leips and Travis 1994, Bradshaw and Johnson 1995, Twombly 1996, Moehrlin and Juliano 1998, Flanagin et al. 2000, Hentschel and Emlet 2000, Morey and Reznick 2000, Shafiei et al. 2001). An important question concerning life histories is whether or not such plasticity is adaptive (Smith-Gill 1983, Reznick 1990, Day and Rowe 2002). Another important question concerns the biological mechanisms underlying a commonly observed pattern of plasticity of life history traits at some stages, but inflexibility of the same traits at other developmental stages (Leips and Travis 1994, Bradshaw and Johnson 1995, Hatle et al. 2000, 2001, 2003*a*, Hodin 2000). Such inflexibility (= canalization) is often interpreted as being a product of developmental or physiological constraints on the organism that are imposed because of the structure and control of developmental systems (Ricklefs and Wikelski 2002, Hatle et al. 2003*b*).

Most of the empirical and theoretical studies of life history plasticity in response to feeding or growth rate have been influenced by a seminal paper concerning metamorphosis of amphibians by Wilbur and Collins (1973). Wilbur and Collins (1973) postulated a lower

Manuscript received 5 December 2003; revised 15 March 2004; accepted 9 April 2004. Corresponding Editor: D. K. Skelly.
[3] E-mail: sajulia@mail.bio.ilstu.edu
[4] Present address: Department of Evolution, Ecology, and Organismal Biology, Ohio State University, 300 Aronoff Laboratory, 318 West 12th Avenue, Columbus, Ohio 43210 USA.
[5] Present address: P.O. Box 328, Swansea, South Carolina 29160 USA.
[6] Present address: Department of Biology, University of North Florida, 4567 St. John's Bluff Road, Jacksonville, Florida 32224 USA.

size threshold at which metamorphosis becomes possible and an upper size limit at which metamorphosis is certain. Their verbal model predicts that between these upper and lower size thresholds, the organism may show adaptive plasticity in response to growth rate. They proposed that when growth conditions are good, organisms should delay metamorphosis and continue to grow toward the maximum size. In contrast, when growth conditions are poor, organisms should undergo metamorphosis as soon as they attain the minimum size. The Wilbur-Collins model predicts that if growth conditions deteriorate after surpassing the lower threshold, organisms should accelerate metamorphosis and undergo this life history transition sooner than if conditions did not deteriorate. Thus, their model postulates adaptive post-threshold plasticity: metamorphosis is delayed in high-growth conditions because the resulting size increase has fecundity advantages (Wilbur and Collins 1973, Reznick 1990, Day and Rowe 2002).

Empirical tests of the Wilbur-Collins model for many taxa and life history transitions have typically failed to support one key prediction: most studies show that increasing or decreasing feeding rate late in development does not alter the timing of life history transitions (e.g., Alford and Harris 1988, Gebhardt and Stearns 1988, Hensley 1993, Leips and Travis 1994, Bradshaw and Johnson 1995, Twombly 1996, Moerhlin and Juliano 1998, Flanagin et al. 2000, Hentschel and Emlet 2000), suggesting that there is a canalized phase of development in many of these study organisms (see Reznick [1990], Morey and Reznick [2000], Shafiei et al. [2001] for exceptions). These empirical results have led several authors to postulate modified versions of the Wilbur-Collins model, incorporating a period of canalized (i.e., independent of feeding) development late in the life history stage (e.g., Reznick 1990, Hensley 1993, Leips and Travis 1994, Bradshaw and Johnson 1995, Twombly 1996, Hentschel 1999). The simplest of these alternative models incorporate no adaptive plasticity in development, so that plasticity of timing of life history events is solely a passive result of variation in feeding rate and the existence of a threshold that must be surpassed (e.g., Reznick 1990). Other models postulate alternatives to adaptive plasticity in timing after the threshold. For example, the threshold could be adjusted downward in response to poor feeding conditions, reducing costs of prolonged development, and upward in response to good feeding conditions, gaining the advantage of increased size and fecundity (Reznick 1990, Leips and Travis 1994, Bradshaw and Johnson 1995).

Most attempts at testing these sorts of life history models have used experimental switching of food rations from high to low or low to high at various times in order to probe the life cycle for phases of canalization. These food-switching experiments also test the Wilbur-Collins prediction that low growth after the threshold accelerates development. Day and Rowe (2002) criticized this approach because, under the Wilbur-Collins model, adaptive variation in development after the threshold can result in apparent unresponsiveness of development time to feeding rate, creating a false appearance of canalization late in development. That is, low feeding rate is expected to prolong the time to reach the threshold and also to reduce post-threshold development time, and the net result could be little or no change in the overall timing of a life history event. They point out that predictions of effects of food manipulations can only be evaluated accurately if the threshold is known, which has rarely been the case in empirical studies. Some authors have attempted to estimate the size or time associated with the threshold for initiation of a canalized phase (Reznick 1990, Hentschel 1999, Hentschel and Emlet 2000) or for the size at which the life history transition becomes possible (Morey and Reznick 2000). In most cases, knowledge of the physiological events associated with thresholds is lacking.

In this paper we use an alternative experimental approach to test models of life history plasticity in response to feeding rate and to estimate the threshold that we (Moehrlin and Juliano 1998, Hatle et al. 2000) have postulated is present in development toward oviposition in the grasshopper *Romalea microptera*. We use this alternative approach not as a replacement for food-switching experiments, which we have done with this system (Moehrlin and Juliano 1998, Hatle et al. 2000), but rather because it addresses the critique of Day and Rowe (2002), providing distinctive predictions for the Wilbur-Collins model. We believe the strongest inferences about mechanistic hypotheses can be drawn when several types of investigation are used to test those hypotheses. Further, the approach we take can yield estimates of relevant physiological parameters (e.g., the threshold, the duration of the canalized phase), which lead to additional testable hypotheses, and to better designed food-switching experiments. We develop models of development that include fixed thresholds and canalized post-threshold development, adaptive flexibility in the threshold itself, and adaptive flexibility in post-threshold development time. We then test alternative predicted relationships of development time to feeding rate. Based on parameter estimates of the preferred model and measures of physiologically relevant storage, we estimate the physiological threshold for reproduction in this grasshopper.

Model System

Based on food-switching experiments (Moehrlin and Juliano 1998, Hatle et al. 2000), the reproductive cycle of our model organism, the Eastern lubber grasshopper *Romalea microptera*, appears to include both an early plastic and a later canalized phase that begins between 7 and 14 d after the adult molt. Because these experiments did not include estimates of the threshold, the

apparent canalized phase of development may be an artifact (Day and Rowe 2002). Thus, for this system, alternative tests for a canalized phase, estimates of the threshold in relevant units, and tests for potentially adaptive plasticity in reproductive development could yield stronger inference of the organization of plasticity of reproductive tactics and generate hypotheses about physiological thresholds that can be tested in subsequent experiments.

The physiological control of this transition from plastic to canalized reproductive development is suggested by previous studies examining hemolymph titers of juvenile hormone (JH), vitellogenin (Vg), total hemolymph protein (TP), and ecdysteroids throughout the reproductive cycle (Hatle et al. 2000, 2001, 2003*a*). Although feeding rate can alter the time necessary to attain maximum levels of these hemolymph components, the times from these maxima to oviposition are not significantly affected by feeding rate (Hatle et al. 2000, 2002). This result suggests that these hemolymph components play a role in determining the threshold and the duration of the canalized phase. Three proteins that are members of the well-conserved family of insect storage proteins (J. D. Hatle and D. W. Borst, *unpublished data*) make up ~70% of total hemolymph protein in female *R. microptera* (Hatle et al. 2001). Oocyte development in *Romalea* is protein limited (T. J. Waskey, J. D. Hatle, and S. A. Juliano, *unpublished data*), so that these storage proteins (SPs) may be important reproductive resource pools. Maximum titers of hemolymph protein were strong predictors of clutch size (Hatle et al. 2001). Similar SPs serve as a general pool of amino acids during reproduction in the luna moth (Pan and Telfer 1996).

Romalea microptera is a large, univoltine, largely herbivorous grasshopper that occurs throughout the southeastern United States (Hatle et al. 2002). Eggs deposited in the soil have obligate winter diapause, and adults and nymphs do not overwinter (Chladny and Whitman 1997).

Relationships of Development Time and Feeding Rate

Our hypothesis is that reproductive investment is initiated by exceeding a threshold of some storage product, probably SPs. Whatever the identity of the storage product, it is ultimately derived from feeding, and we expect a direct relationship of cumulative feeding to storage. The hypothesis of a storage threshold thus predicts that consumption of a specific amount of food is necessary to reach this threshold, providing us with an operational way of estimating the threshold, in units of cumulative food consumed. We have posed four alternative hypotheses for how this threshold may fit into the reproductive cycle. (1) The threshold is fixed, and there is a canalized (= unresponsive to feeding rate) post-threshold development period. (2) The threshold is fixed, and the post-threshold development period responds to feeding rate as described by Wilbur and Collins (1973). (3) The threshold decreases with decreasing feeding, and post-threshold development is canalized. (4) The threshold decreases with age, and post-threshold development is canalized. These hypotheses are derived from verbal models for developmental plasticity from the literature (Wilbur and Collins 1973, Reznick 1990, Leips and Travis 1994, Bradshaw and Johnson 1995, Moehrlin and Juliano 1998, Hentschel 1999). Hypotheses 2–4 postulate potentially adaptive responses to feeding rate (Reznick 1990). Note that the Wilbur-Collins threshold in hypothesis 2 is fundamentally different from those postulated in hypotheses 3 and 4. The former is a fixed minimum value, whereas the latter two are variable functions of the environment. These hypotheses yield different predicted relationships of age at oviposition and feeding rate, and we test these hypotheses by fitting different nonlinear regression functions to data relating age at oviposition to feeding rate. Because the nonlinear models we use are derived from previous hypotheses about mechanisms, we do not search for the best fitting polynomial, but instead assess the fit of specific models and test hypotheses of nonzero values for parameters. Our regressions yield estimates of both the duration of the post-threshold, potentially canalized phase of the oviposition cycle and the threshold, estimated in units of mass of food consumed. We use our best estimates of these parameters in conjunction with measurements of hemolymph titers of protein to estimate the threshold (in milligrams per milliliter of hemolymph protein) for initiation of the canalized phase of development.

Threshold is constant, post-threshold time is canalized

The simplest model of plasticity postulates that the threshold is independent of both feeding rate and age and that post-threshold development time is independent of feeding rate (i.e., represents a canalized phase). In this model, plasticity of time to oviposition is solely a product of variation in the time required to attain the fixed threshold and represents expression of a fixed pattern of development in different environments (Reznick 1990, Moehrlin and Juliano 1998, Nylin and Gotthard 1998). Time from adult molt to oviposition (T_o) can be divided into two stages: time from adult molt to the threshold (T_H; the duration of the plastic phase) and time from the threshold to oviposition (T_p; post-threshold development time). In this model, post-threshold development time is presumed to be independent of the environment (i.e., a canalized phase):

$$T_o = T_H + T_p. \quad (1)$$

Although physiologically the threshold (H) may depend on SP or some other storage product, this threshold could also be represented by the cumulative amount of food necessary to attain that SP threshold. Thus, H could operationally be measured as mass of food con-

sumed from adult molt until the attainment of H, which would be determined by the rate of consumption, F (in grams per day). These values are related as follows:

$$T_H = H(1/F). \quad (2)$$

By substituting Eq. 2 for T_H in Eq. 1,

$$T_o = H(1/F) + T_p. \quad (3)$$

Thus, the hypothesis of constant threshold and canalized post-threshold development time predicts a *linear* relationship of T_o with $1/F$, with H = slope (in units of grams of food) and T_p = y-intercept (in units of days) (Fig. 1). We will refer to this as the ''Constant/Canalized'' model.

Threshold is constant, post-threshold time is flexible

Analogous to Wilbur and Collins (1973), the second model postulates a fixed storage threshold, H, at which initiation of reproduction (vitellogenesis and provisioning of eggs) is possible. Further, we postulate a post-threshold development time, T_p, that is an asymptotically increasing function of feeding rate (F). Wilbur and Collins (1973) postulated that upon attaining the threshold, animals would immediately initiate the life history transition if feeding rate was low. As F increases, Wilbur and Collins (1973) postulated that post-threshold development time would increase up to some maximum (T_{pmax}) associated with attainment of some maximum of the relevant storage variable (e.g., size, protein store, or in this case cumulative food eaten). We presume that as F approaches 0, T_p approaches 0 so that this asymptotic relationship is

$$T_p = T_{pmax} F/(K + F) \quad (4)$$

where T_{pmax} is the maximum post-threshold development time and K is the value of feeding rate resulting in post-threshold development time of $T_{pmax}/2$. Thus, Eq. 4 describes a hyperbolic increase in T_p analogous to the Michaelis-Menten equation for substrate-dependent reaction rates (Juliano 2001). Time to reach the threshold (T_H) is as in Eq. 2 and total time to oviposition is as in Eq. 1. Substituting Eqs. 2 and 4 into Eq. 1 we obtain the predicted relationship:

$$T_o = H(1/F) + [T_{pmax} F/(K + F)]. \quad (5)$$

Thus, the hypothesis of constant threshold and adaptive variation in post-threshold time predicts a *hyperbolic* relationship of T_o with the inverse of food eaten per day, $1/F$ (Fig. 1). Eq. 5 is particularly useful as a regression model because if K is equal to 0, the model reduces to Eq. 3, with $T_{pmax} = T_p$. We will refer to this as the ''Constant/Flexible'' model.

Threshold decreases with decreasing feeding rate, post-threshold time is canalized

Analogous to verbal models by Reznick (1990) and Bradshaw and Johnson (1995), the third model postulates that the threshold for vitellogenesis decreases linearly as the inverse of food eaten per day increases. This may be advantageous when feeding rate is low (and the inverse is high), because it results in a shorter time to oviposition than with the constant threshold model. The threshold for an individual can be defined by the following, in which H_0 is the maximal threshold and b_1 is the slope of the relationship of H and $1/F$:

$$H = H_0 + b_1(1/F) \quad (6)$$

with $b_1 < 0$, because $b_1 > 0$ implies that the threshold (H) increases with the inverse amount of food per day ($1/F$), which would seem to be disadvantageous. Substituting Eq. 6 into Eq. 2,

$$T_H = [H_0 + b_1(1/F)](1/F). \quad (7)$$

Substituting Eq. 7 into Eq. 1 yields the final equation:

$$T_o = b_1(1/F)^2 + H_0(1/F) + T_p. \quad (8)$$

This model also postulates that the post-threshold time (T_p) is independent of feeding. With a threshold that decreases linearly with $1/F$, this model predicts a *quadratic* relationship of T_o and $1/F$, rather than the linear relationship of Eq. 3. The predicted quadratic has the second-order coefficient $b_1 < 0$ (Fig. 1). We will refer to this as the ''Food-dependent/Canalized'' model.

Threshold decreases with age, post-threshold time is canalized

A fourth model postulates an alternative mechanism for adaptive change in the threshold described by Rez-

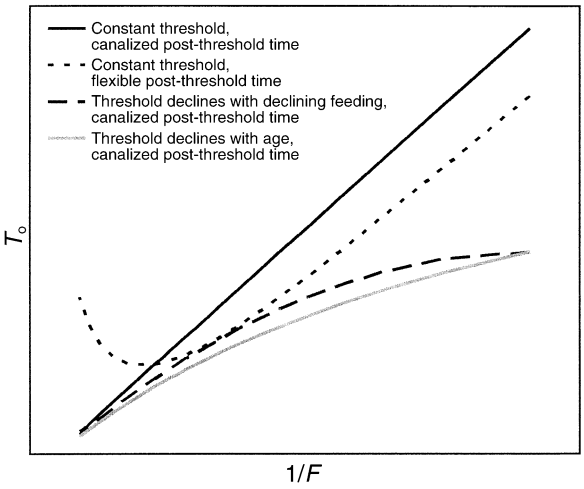

FIG. 1. Predicted relationships of time to oviposition (T_o) and inverse of daily food ration ($1/F$) for four different models of development toward oviposition. All models postulate a threshold for initiation of the process of vitellogenesis and a post-threshold phase of development. The threshold may be fixed, as in hypotheses 1 and 2, or may show adaptive flexibility in response to food intake, as in hypotheses 3 and 4. Post-threshold development may be canalized and unresponsive to food intake, as in hypotheses 1, 3, and 4, or may show adaptive flexibility in response to food intake, as in hypothesis 2. See *Relationships of development time and feeding rate* for details.

TABLE 1. Models for time to oviposition (T_o) and the regression model used to fit regressions of T_o vs. $1/F$.

Verbal model (reference examples)	Post-threshold plasticity	Storage or food threshold	Predicted relationship of T_o vs. $1/F$	Transformed regression model
Constant/Canalized (Reznick 1990)	none, canalized development	constant, unaffected by food or age	$T_o = H(1/F) + T_p$	$1/T_o = 1/(H(1/F) + T_p)$
Constant/Flexible (Wilbur and Collins 1973)	high food prolongs development	constant, unaffected by food or age	$T_o = H(1/F) + [T_{pmax}F/(K + F)]$	$1/T_o = 1/(H(1/F) + [T_{pmax}F/(K + F)])$
Food-dependent/Canalized (Reznick 1990, Bradshaw and Johnson 1995)	none, canalized development	declines linearly as $1/F$ increases	$T_o = b_1(1/F)^2 + H_0(1/F) + T_p$	$1/T_o = 1/(b_1(1/F)^2 + H_0(1/F) + T_p)$
Age-dependent/Canalized (Reznick 1990, Bradshaw and Johnson 1995)	none, canalized development	declines linearly with age	$T_o = [H_0/(F - b_2)] + T_p$	$1/T_o = 1/([H_0/(F - b_2)] + T_p)$

Notes: For all equations, units for T_o are days, and units for F are grams dry mass per day. T_p is post-threshold development time; H is threshold in units of grams dry mass consumed; T_{pmax} is maximum post-threshold development time attained with maximal feeding rate; K is the value of feeding rate resulting in post-threshold development time of $T_{pmax}/2$; H_0 is the maximal threshold (in dry grams consumed) attained when fed ad libitum; b_1 is the slope relating threshold (in grams dry mass consumed) to $1/F$; and b_2 is the slope relating threshold (in grams dry mass consumed) to age. Both b_1 and b_2 are expected to be negative, whereas K is expected to be positive.

nick (1990) and Bradshaw and Johnson (1995). A threshold that decreases linearly as the animal's age increases would have the same effect on life histories as a threshold that declines with increasing inverse feeding rate. Which of these two models is mechanistically more realistic remains unknown; however, an age-dependent threshold is in a sense simpler as it does not require the animal to perceive its feeding rate. Whereas the model of a threshold that declines with decreasing feeding rate may require a regulated response (developmental conversion, Smith-Gill [1983], Schlichting and Pigliucci [1998]), the age-dependent threshold may represent a fixed pattern of development expressed in a different environment (phenotypic modulation, Smith-Gill [1983], Reznick [1990], Schlichting and Pigliucci [1998]). The threshold for an individual can be defined by the following equation in which H_0 is the maximal threshold, T_H is the time to the threshold (i.e., the animal's age), and b_2 is the slope of the relationship of H and T_H:

$$H = H_0 + b_2(T_H). \quad (9)$$

As in the previous model, $b_2 < 0$. Substituting Eq. 9 into Eq. 2, we have

$$T_H = H_0(1/F) + b_2(T_H)(1/F). \quad (10)$$

Solving for T_H and substituting Eq. 10 into Eq. 1 yields the final equation:

$$T_o = H_0[1/(F - b_2)] + T_p. \quad (11)$$

This model postulates that post-threshold development time is canalized and yields a *hyperbolic* function (Fig. 1). We will refer to this as the ''Age-dependent/Canalized'' model.

Thus, these four different models of plasticity of reproduction (Table 1) make distinct predictions about the relationship of T_o to $1/F$. For the three models that postulate adaptive variation in H or T_p in response to food availability, low feeding rates (i.e., greater $1/F$) yield lower T_o than does the model postulating no adaptive variation (Fig. 1). For high feeding rate (i.e., lower $1/F$), the model postulating adaptive variation in post-threshold development time yields greater T_o than does the model postulating no adaptive variation (Fig. 1). We tested these models by determining which one best fits data from an experiment manipulating feeding rate.

EXPERIMENTAL METHODS

We used female *Romalea microptera* from a laboratory colony established in 1996 from animals collected near Copeland, Florida, USA. We used only grasshoppers with femur lengths between 27 and 33 mm. We collected females on the day of their adult molt and assigned them serially to one of eight feeding treatments consisting of 1.00, 1.50, 1.82, 2.00, 2.22, 2.50, 2.86, and 3.33 g (fresh mass) Romaine lettuce per day. We chose these values to produce approximately evenly spaced values on the inverse scale (i.e., $1/F$). We also gave each female two medium-sized flakes of rolled oats each day. For each new box of lettuce, we collected samples equal in mass to each feeding treatment and dried them to obtain a fresh-to-dry mass conversion factor.

We housed grasshoppers individually in 500-mL plastic containers in environmental chambers (14 h light:10 h dark photoperiod; corresponding 32°:24°C thermoperiod). Each day we collected each grasshopper's uneaten food and determined its dry mass (dried at 60°C for ≥48 h). We subtracted dry mass of remaining food from the dry mass of food offered (based on our conversion factor; see previous paragraph) and determined dry mass of food eaten per day. Previous

experiments (Moehrlin and Juliano 1998, Hatle et al. 2002) suggested that grasshoppers on these diets would consume all offered food each day. We chose rations that the grasshoppers would likely consume completely to increase the accuracy of our estimates of food eaten each day.

Beginning at 27 d after adult molt, we placed females into 1-L cups containing moist sand for at least 1 h every day until oviposition (methods used by Hatle et al. [2000, 2001]). For each female we determined the number of days from adult molt to oviposition (T_o). We summed the dry masses of food consumed each day for each individual grasshopper and determined the mean dry mass consumed per day by dividing by T_o. This yielded F, the mean daily feeding rate.

Nonlinear regressions

We ran regressions of T_o vs. $1/F$ using nonlinear curve-fitting procedures (PROC NLIN, SAS Institute 1998, Juliano 2001) to determine which of our four models provided the best description of the relationship between T_o and $1/F$. To meet assumptions of normality and homogeneity of variances, we used an inverse transformation of both sides of Eqs. 3, 5, 8, and 11. By using the nonlinear curve fitting, we were able to fit the transformed models described in Table 1 and thus to estimate the parameters of interest in the appropriate units. We determined the best fit among these four functions based on three criteria: (1) all parameters in the best fit model must be statistically significant; if a model yields nonsignificant parameters, a more parsimonious model is preferred; (2) significant parameters must differ from 0 in the predicted direction (i.e., $b_1 < 0$, $b_2 < 0$, $K > 0$); if this criterion is not met, the associated model is rejected; (3) for models with all parameters significant, greater R^2 is preferred. By these criteria, we could reject all four of these models and conclude that none of these hypotheses are consistent with our data, which may suggest that thresholds are not involved in reproduction.

Hemolymph protein titer at the threshold

Every third day, we collected a 5-μL hemolymph sample from each grasshopper. Each hemolymph sample was combined with 250 μL of hemolymph buffer (100 mmol/L NaCl; 1 mmol/L ethylenediamine tetraacetic acid [EDTA]; 0.1 mmol/L dithiothreitol [DTT]; 0.15% Tween 20; 10 μg/mL leupeptin; 10 μg/mL phenylmethylsulfonyl fluoride [PMSF] in propanol; 50 mmol/L Tris buffer; pH 7.5) and stored at −20°C. After all the hemolymph samples had been collected for an individual grasshopper, total hemolymph protein values were determined using the Bradford (1976) assay, with bovine serum albumin standards.

We used the developmental profile of total hemolymph total protein for each grasshopper and estimates of H (in units of cumulative food eaten), T_p, and related parameters from the best fit regression of T_o vs. $1/F$ (see *Nonlinear regressions*, above) to estimate hemolymph protein levels in each grasshopper at the time the apparent threshold was reached. We used two methods to estimate the grasshopper's age at the threshold

$$\text{based on } T_p\text{: age at threshold } T_H = T_o - T_p$$

$$\text{based on } H\text{: age at threshold } T_H = (1/F) \times H$$

where T_p and H are estimated from the best-fit function (see *Nonlinear regressions*). These two estimates are related, in that both are derived from parameters estimated by the same regression. They are not identical, however (see *Results*), because they make use of relationships in Eq. 1 or Eq. 2, respectively, and it was not clear which one would be preferable. We estimated the hemolymph protein concentration at the apparent threshold for each grasshopper from the hemolymph sample taken closest to age at the threshold. We excluded from this process any grasshopper that did not have a hemolymph sample available within 1 d of the age at the threshold. We tested whether estimated hemolymph protein thresholds were related to feeding treatments (one-way ANOVA) or to feeding rate (F) or apparent age at the threshold (T_H) (by regressions).

Egg number and resorption bodies

We counted the number of eggs laid by each grasshopper, selected 10 eggs from each clutch, dried them overnight at 60°C, and determined their aggregate mass. We dissected each grasshopper and counted any unlaid eggs, the number of ovarioles, and the number of resorption bodies (indicating aborted oocytes; Moehrlin and Juliano [1998], Sundberg et al. [2001]). For egg number (laid + retained after oviposition), mean egg dry mass, and number of resorption bodies, we tested for relationships to mean feeding rate (F) via linear and polynomial regressions.

RESULTS

Of 104 grasshoppers in this experiment, only 5 (4.8%) died prior to oviposition. One grasshopper failed to oviposit by age 92 d, and dissection revealed that this female had an abnormal reproductive tract. Thus, 94.2% of the grasshoppers in the experiment oviposited.

Regressions of T_o vs. 1/F

All four of the regression models accounted for at least 62% of the variation in T_o (Table 2). Three of the four models produced at least some parameter estimates that were significantly different from 0 (Table 2). The Constant/Canalized model, which was the simplest, with only two parameters and a prediction of a linear relationship of T_o and $1/F$ (Table 1, Fig. 2A), had all parameters significant ($P < 0.0001$) and an $R^2 = 0.621$. For the Constant/Flexible model, the third parameter (K) was not significantly different from 0 and $R^2 = 0.621$ (Table 2). Thus, this model converged to the same

TABLE 2. Nonlinear curve fitting results.

Model	df	H or H_0 ± 95% CI (g)	P
Constant/Canalized	96	$H = 4.0 \pm 0.8$	<0.0001
Constant/Flexible	95	$H = 4.0 \pm 0.8$	<0.0001
Food-dependent/Canalized	95	$H_0 = 6.9 \pm 4.4$	0.0024
Age-dependent/Canalized	95	$H_0 = 9.9 \pm 16.8$	0.2440

Notes: Significance levels for regression parameters (see Table 1 for definitions) are from two-tailed t tests. Calculation of R^2 is relative to the corrected total sum of squares (i.e., after adjusting for the overall mean). Estimates of the threshold (H) and maximum threshold (H_0) are cumulative dry mass of food eaten in grams. Estimates of post-threshold time (T_p) and maximum post-threshold time (T_{pmax}) are in days. The Constant/Canalized model is deemed the best with respect to parsimony, significance, and fit. Parameter definitions are as follows: K, value of feeding rate resulting in post-threshold development time of $T_{pmax}/2$; b_1, slope of the relationship of H and $1/F$; b_2, slope of the relationship of H and T_H.

solution as the Constant/Canalized model (compare estimates in Table 2 and Fig. 2A and B). Therefore, this model of plastic post-threshold development was rejected. The two models postulating plasticity of the threshold, the Food-dependent/Canalized and Age-dependent/Canalized models, both yielded a slightly greater R^2 than the Constant/Canalized model (Table 2, Fig. 2C and D). However, for the Food-dependent/Canalized model, the coefficient associated with $(1/F)^2(b_1)$ was not significantly different from 0, although it was <0, as predicted (see *Relationships of development time and feeding rate,* above, Table 2, and Fig. 2C). The Age-dependent/Canalized model yielded all three parameters not significantly different from 0, although the coefficient b_2 was <0, as predicted (Tables 1 and 2, Fig. 2D). Because these two models did not meet the criterion of all parameters significant, they too were rejected. Thus, the simplest model, with a constant threshold and canalized post-threshold development, yields the most parsimonious description of development toward oviposition.

Based on the Constant/Canalized model, estimated duration of the canalized post-threshold phase (±95% confidence interval) was 23.5 ± 3.8 d (Table 2). Estimated threshold for initiation of this canalized phase was 4.0 ± 0.8 g dry mass of food consumed (Table 2).

Hemolymph protein concentration at the threshold

Using the preferred Constant/Canalized model, estimates of the hemolymph protein threshold for initiating the canalized phase of reproduction were 101.2 ± 9.0 mg/mL (mean ± 95% confidence interval) based on the food intake threshold (H) and 101.8 ± 9.0 mg/mL based on the post-threshold canalized time (T_p). Estimates via these methods did not differ significantly (paired t test, $t = 0.01$, df = 82, $P = 0.98$) and were significantly correlated ($r = 0.67$, $P = 0.0001$, $N = 83$). Hemolymph threshold estimates based on T_p or H were not significantly related to mean food intake ($F_{1,86} = 1.35$, $P = 0.25$, $r^2 = 0.015$; $F_{1,85} = 0.49$, $P = 0.49$, $r^2 = 0.006$, respectively) and not significantly related to age of the female at the apparent initiation of the canalized phase ($F_{1,86} = 0.05$, $P = 0.82$, $r^2 = 0$; $F_{1,85} = 0.49$, $P = 0.49$, $r^2 = 0.006$, respectively).

Egg production and egg resorption

Both total eggs produced and the number of eggs resorbed were significantly related to mean of food eaten per day (Fig. 3). Quadratic regressions (Fig. 3) produced significantly better fits than did linear regressions, and third order parameters were, in all cases, not significantly different from 0 ($P \gg 0.10$). Total number of eggs produced or number of eggs resorbed were not significantly related to initial eclosion mass ($P \gg 0.10$, $N = 83$, in both cases). Egg mass was not significantly related to either feeding rate or eclosion mass ($P \gg 0.10$ in both cases).

DISCUSSION

Based upon our criteria for the best model describing the relationship of time to oviposition and feeding rate, the simplest model, the Constant threshold/Canalized post-threshold time model, is the preferred model. This model includes only two parameters, H, the threshold, and T_p, the post-threshold canalized time. For all three parameter models, the third parameter was not significant, indicating that its contribution to the description of this relationship was negligible. The Constant threshold/Flexible post-threshold time model, which was derived from the verbal model of Wilbur and Collins (1973), was perhaps the poorest at describing the data, failing to produce even a trivial increase in R^2 over that of the linear model. The general shape of the observed relationship (Fig. 2A) is, indeed, inconsistent with the concave upward shape predicted by this model (Fig. 1), without any suggestion of the increase in T_o at high F (i.e., at low $1/F$ in the plots, Fig. 1). One explanation for the lack of the concave upward shape predicted by the Constant threshold/Flexible post-threshold time model is that our values of $1/F$ were not sufficiently low to reach the portion of the curve where T_o declines with increasing $1/F$. This appears to be unlikely. Low values of $1/F$ correspond to high values of food eaten (F), and under this model, greater T_o occurs at high feeding rate because the animal gains an advantage of greater growth. Such prolongation should be most likely when food consumption rate is maximal and growth is high. The absence of such a prolongation (i.e., the absence of the ''up-turn'' shown

TABLE 2. Extended.

T_p or T_{pmax} ± 95% CI (d)	P	Other parameters ± 95% CI	P	R^2
$T_p = 23.5 \pm 3.8$	<0.0001			0.621
$T_{pmax} = 23.5 \pm 3.8$	<0.0001	$K = 0 \pm 0$	1.000	0.621
$T_p = 15.2 \pm 13.0$	0.0230	$b_1 = -0.2 \pm 0.3$	0.1810	0.628
$T_p = 10.2 \pm 30.9$	0.5126	$b_2 = -0.1 \pm 0.2$	0.4054	0.628

in Fig. 1) would likely be an artifact if the food treatments were insufficient to yield maximum consumption rate. One-way ANOVA of food intake (in grams dry mass) showed significant differences among treatments ($F_{7,90} = 316.3$, $P < 0.0001$), but means for the three highest food rations were not significantly different (Ryan-Einot-Gabriel-Welsh test; SAS Institute 1998), all averaging 0.24–0.25 g dry mass ingested per day (range of SE = 0.01–0.02). Thus, for these treatments, *R. microptera* appear to have reached maximum daily consumption, and prolonging development ought to be advantageous. Thus, we believe our data span the part of the curve where the concavity should be obvious, yet there is not even a hint of such a shape (Fig. 1). It is clear, however, that the nonlinear curve-fitting approach we take requires that experimental feeding treatments span as wide a range as possible.

It may be argued that the two remaining three-parameter models improve the fit of the regression over that of the linear relationship derived from the Constant/Canalized model, however R^2 necessarily increases as the number of regression parameters increases, and the actual gain in R^2 is very small in both cases (Table 2). A further objection to these analyses may be that there was insufficient statistical power to declare significant parameters that lead to a relatively small degree of curvature. Although the value of post hoc power analysis has been questioned (Steidl and Thomas 2001), it is useful to assess the power to declare significant higher-order parameters in the model. For the

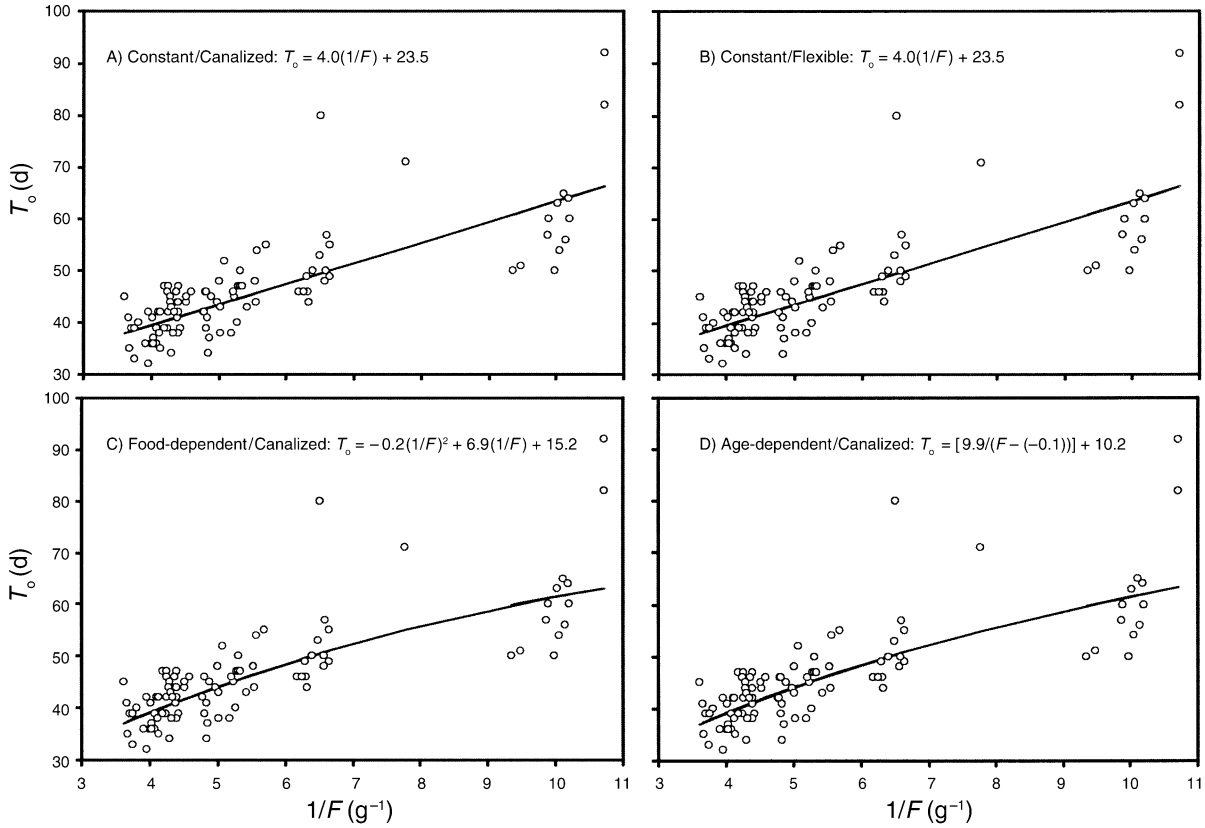

FIG. 2. Observed relationships of time to oviposition (T_o) and inverse of mean daily food intake ($1/F$) and fitted nonlinear regressions. (A) Constant threshold/Canalized post-threshold time model; (B) Constant threshold/Flexible post-threshold time model; (C) Threshold declines with declining feeding (Food-dependent/Canalized post-threshold time model); (D) Threshold declines with age (Age-dependent/Canalized post-threshold time model). Regression models are found in Table 1; parameter estimates and R^2 are found in Table 2.

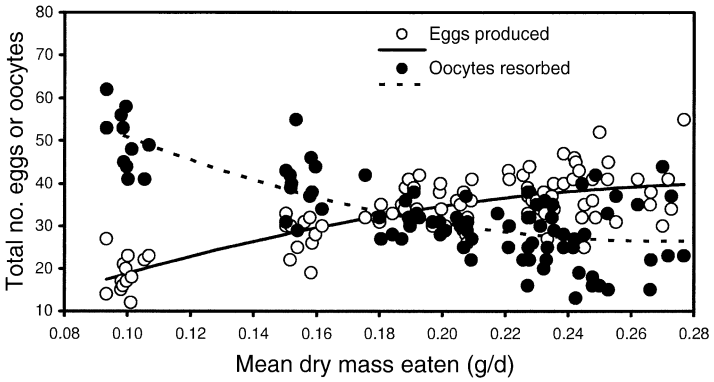

FIG. 3. Relationships of total number of eggs produced (laid + mature eggs retained) and oocytes resorbed to mean daily food intake. Quadratic regressions were significant for both total eggs (eggs = $-7.3 + 314.1(F) - 519.3(F^2)$; $R^2 = 0.62$, $P < 0.0001$, $N = 95$) and oocytes resorbed (resorbed = $87.5 - 449.6(F) + 827.2(F^2)$; $R^2 = 0.56$, $P < 0.0001$, $N = 88$).

Food-dependent/Canalized model, which appears to be the higher-order model that best fits the data, the quadratic parameter b_1 is the slope of the relationship of the threshold to inverse feeding rate (see Eq. 6). Our model estimates the intercept of that relationship (H_0), and we can assess the power of our regressions to declare significant a slope (b_1) with a value of 10% of that intercept (i.e., the threshold declines by 10% for each unit on the $1/F$ scale of Fig. 2). Neter and Wasserman (1974) provide methods and charts for estimating power of t tests such as this. Our estimate of H_0 is 6.9, so that we are interested in the power to declare significant a b_1 value of 0.69. The method of Neter and Wasserman (1974) yields a noncentrality parameter of 4.1, and with 95 degrees of freedom and $\alpha = 0.05$, an estimated power of 98%. Thus, given our sample size and the variability of our data, we should have had a high likelihood of finding a significant quadratic relationship if the quadratic parameter (b_1) was only 10% of the value estimated for the parameter H_0. An alternative power analysis for regression parameters of medium effect size (accounting for 15% of the total sum of squares; Cohen 1988) yielded an estimated power of 90%. We thus feel we have adequate power to detect biologically meaningful curvilinearity and conclude that our failure to detect such an effect results from that effect being absent or trivial.

In summary, all three of the more complex models postulating potentially adaptive plasticity in reproductive development predict some kind of nonlinear relationship of T_o and $1/F$, and we find no evidence for a significant departure of the observed relationship from linearity. Thus, in this system, we find no evidence for the potentially adaptive plasticity of development that has been postulated in previous life history models.

Previous experiments on *R. microptera* reproduction using food-switching designs (Moehrlin and Juliano 1998, Hatle et al. 2000) have also been consistent with models incorporating a fixed threshold and canalized post-threshold development (Travis 1984, Leips and Travis 1994, Bradshaw and Johnson 1995) and so would predict a linear relationship of T_o and $1/F$. Because we obtain similar conclusions with two alternative methods, we feel this conclusion is correct despite the criticisms of food-switching designs (Day and Rowe 2002). Indeed, we have compared the predictive abilities of the model Day and Rowe (2002) suggest would be inadequately tested by food-switching designs (Wilbur-Collins type models) and the Constant threshold/Canalized post-threshold model and found the latter to provide the best prediction of reproductive timing. The Wilbur-Collins model gives perhaps the poorest fit to the data. Our results suggest that if there is any potentially adaptive plasticity in this system, it is more likely to take the form of adjustments of the threshold itself, rather than adjustments of the post-threshold development time (Reznick 1990).

Although we find no evidence for any of the models proposed by Wilbur and Collins (1973) and Reznick (1990) as descriptions of adaptive life history plasticity, we cannot prove that reproductive plasticity in this grasshopper is nonadaptive. Our data are consistent with the hypothesis that plasticity is simply a manifestation of the presence of a threshold for feeding or storage that must be met before reproduction can proceed. This response has been described by Reznick (1990:188) as ''. . . a fixed pattern of development in different environments.'' That is, plasticity of timing of oviposition is not a result of regulated change in development pattern, but rather is imposed by the environment (the feeding environment, in this case). Our data suggest that plasticity in timing of oviposition in this grasshopper represents phenotypic modulation rather than developmental conversion (Smith-Gill 1983, Schlichting and Pigliucci 1998).

Even if variation in reproductive timing of *R. microptera* in response to feeding rates is not a product of regulated developmental flexibility, the possibility of developmental flexibility in other reproductive responses to feeding environments cannot yet be ruled out. We have shown that egg production and egg resorption are significantly affected by feeding rate, suggesting developmental flexibility in these reproductive tactics. Indeed, egg number and egg resorption in *R. microptera* remained flexible in response to changing

feeding rate until very late in the oviposition cycle, after the grasshoppers had apparently entered the canalized phase of reproduction in which timing of egg production was no longer flexible (Moehrlin and Juliano 1998). Because egg number appears to be regulated via egg resorption (Moerhlin and Juliano 1998, Sundberg et al. 2001) this response may represent developmental conversion.

Our experimental approach to evaluating different models of life history plasticity is distinct from previous experimental approaches to the subject (e.g., Travis 1984, Alford and Harris 1988, Hensley 1993, Leips and Travis 1994, Bradshaw and Johnson 1995, Moehrlin and Juliano 1998, Flanagin et al. 2000, Hentschel and Emlet 2000, Morey and Reznick 2000, Shafiei et al. 2001) in that we have not used food-switching manipulations to probe the life history for plasticity. Instead we developed explicit models of developmental events and evaluated their predicted relationships of reproductive timing to feeding rate. We suggest that this approach is a valuable addition to the array of experimental tools for investigating life history plasticity and canalization and should be used for other life history transitions that have been investigated by food-switching experiments, such as amphibian or arthropod metamorphosis. It would be particularly interesting to use this approach in one of the systems for which previous experiments lead to the prediction of nonlinear relationships of time vs. inverse feeding rate (e.g., Reznick 1990, Morey and Reznick 2000, Shafiei et al. 2001).

Our approach can yield estimates of postulated thresholds for developmental events. In most experiments it is feeding rate of the organism that is easily manipulated and quantified, hence thresholds are initially estimated in units of food intake. However, because investigators often hypothesize that some measure of condition (protein storage in our case, but body mass or fat storage are other possibilities) constitutes the threshold, such experiments will benefit from associated measurements of condition variables. Estimates of thresholds in any relevant unit may be very valuable for designing food-switching experiments (Day and Rowe 2002) and may provide testable hypotheses in their own right (e.g., we predict that vitellogenesis in our grasshopper should begin when cumulative food eaten has reached 4.0 g and hemolymph total protein has reached 101 mg/mL). In the case of a physiological variable like hemolymph total protein, the threshold estimate provided by our approach could serve as a prediction for experiments that manipulate directly the physiological variable or alternatively manipulate hormones postulated to control life history to test the effects of decoupling hormonal signals from physiological storage. Developing physiological methods for such experiments could provide a powerful tool for testing mechanistic hypotheses about life history plasticity.

Acknowledgments

We thank our colleagues in the NSF Collaborative Research at Undergraduate Institutions program at Illinois State University, particularly D. W. Whitman and D. W. Borst, for discussion during development of these ideas, D. W. Borst for technical and laboratory support for protein assays, and V. A. Borowicz, D. N. Reznick, L. Rowe, and an anonymous referee for helpful comments that greatly improved the manuscript. This research was supported by NSF grants DBI-9978810 and BIR-9510979 to S. A. Juliano, D. W. Borst, and D. W. Whitman.

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