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Analysis of growth and development in the final instar of three species of predatory Coccinellidae under varying prey availability

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Abstract

For insects such as aphidophagous lady beetles, whose preferred food varies naturally in space and time, variation in adult body size is most likely a reflection of food acquired and allocated to growth by the final instar. We conducted a laboratory study to evaluate the nature of body size variation in *Coleomegilla maculata* (DeGeer), *Harmonia axyridis* (Pallas), and *Hippodamia convergens* Guerin-Meneville (all Coleoptera: Coccinellidae) by assessing how fourth instars respond developmentally to food deprivation. We also determined which growth functions best describe larval growth trajectories and evaluated whether these fourth instars regulate their growth based on initial body size. Access to food for a minimum of 1 day during the fourth stadium appeared to be a requirement for pupation in all three species. Putative critical weight for pupation falls within 10–14 mg for *C. maculata*, 13–16 mg for *H. convergens*, and 19–22 mg for *H. axyridis*, and development period after attaining the critical weight is not affected by food deprivation. The mixed-effect logistic function that accounted for individual and gender differences provided the best description of growth in the fourth instars of the three species. The reduced major axis regression revealed that growth in these lady beetles was convergent-regulated. The logistic growth trajectory, existence of critical weight, and determinate developmental response to food deprivation are traits that seem to suggest that fitness increase is attained by maximizing body size rather than survival in these beetles.

Introduction

An important challenge for many organisms from a physiological, ecological, and evolutionary perspective is to regulate growth and developmental processes that determine an optimal adult body size (Roff, 1992; Stearns, 1992). Body size is generally considered a key determinant of fitness of an organism, because of the size-mediated attributes such as fecundity, reproductive effort, competitive ability, and mating success (Roff, 1992; Stearns, 1992; Bernardo, 1993; Honěk, 1993). Both growth and developmental rates vary among individuals either due to genetic differences and/or variation in environmental conditions (Sebens, 1987). For genetically similar insects, variation in adult size is largely determined by variation in the amount of nutrient resources acquired and allocated to growth by the final instar (Nijhout et al., 2006; Berner & Blanckenhorn, 2007).

Variations in food availability mediate the connection between insect growth and development in two ways. First, there is a critical weight, defined as 'the minimal weight in which further feeding and growth are not required for a normal time course to metamorphosis and pupation' (Davidowitz et al., 2003), that must be attained during the last larval/nymphal stadium (Blakley, 1981; Bradshaw & Johnson, 1995; Nijhout, 2003). Second, development period after attaining the critical weight is flexible in some species but determinate in others (Blakley, 1981; Bradshaw & Johnson, 1995;

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Shafiei et al., 2001; Nijhout, 2003). In species with a flexible development period, individuals that experience food deprivation stop growing and pupate much sooner than those with continued access to food, e.g., *Onthophagus taurus* (Shreber) (Shafiei et al., 2001). For species with a determinate development period, food deprivation after attaining critical weight stops growth but has no effect on the stadium length, e.g., *Wyeomyia smithii* (Coquillet) (Bradshaw & Johnson, 1995) and *Manduca sexta* L. (Nijhout, 2003). The ability to respond developmentally to changes in food availability is an important attribute for organisms that rely on transient or unpredictable food resources (Bradshaw & Johnson, 1995; Shafiei et al., 2001; Phoofolo et al., 2008).

We conducted a laboratory study to evaluate the nature of body size variation in three species of aphidophagous lady beetles, *Coleomegilla maculata* (DeGeer), *Harmonia axyridis* (Pallas), and *Hippodamia convergens* Guerin-Meneville (all Coleoptera: Coccinellidae). The first goal of our study was to determine how the three species respond developmentally to changes in food availability. We evaluated both the existence of a critical weight for successful development to pupation and the level of flexibility in development period in response to food deprivation. We also developed a model that predicts the probability of pupation as a function of body weight attained by the fourth instars subjected to different food deprivation periods. The motivation for evaluating developmental responses to food deprivation in aphidophagous lady beetles was that aphids, their preferred and essential prey, vary widely both spatially and temporally in nature (Dixon, 1985, 2000; Hodek & Honěk, 1996). The abundance of aphids in a colony or a patch is mainly influenced by quality of host plants, mortality by natural enemies, and weather conditions. The combined effect of these factors is the creation of the ‘boom-and-bust’ dynamics of prey resources for aphid predators like lady beetles (Kindlmann & Dixon, 1999; Dixon, 2000).

The second goal was to describe and analyze growth trajectories during development in the fourth instars of these three species of lady beetles. Our hypothesis was that variation in adult size for a cohort of individuals of the same species that grow under the same environmental conditions may be attributable to the variation in certain components of individual growth trajectories such as initial weight and rate of growth. We approached this by determining which growth functions best described larval growth during the fourth stadium of the three species. The growth models used are exponential, monomolecular, logistic, Gompertz, and von Bertalanffy. These functions are commonly used to describe growth in fish, birds, and mammals (Lopez et al., 2000; Winship et al., 2001; Darmani Kuhi et al., 2003). We chose to model growth in the fourth instar because, as a final instar in coccinellids, the body size attained during this developmental stage determines adult body size (Hodek & Honěk, 1996; Dixon, 2000; Phoofolo et al., 2008).

The third goal was to determine whether lady beetle fourth instars regulate their growth rate on the basis of the initial size or weight. Growth in organisms can be characterized either as: (1) convergent-regulated, (2) divergent-regulated, or (3) non-regulated (Lytle, 2001). Convergent growth regulation occurs in organisms that adjust their growth trajectories to reach a targeted body size; i.e., for a given cohort experiencing uniform environmental conditions smaller individuals grow faster than larger individuals. Divergent growth regulation occurs in organisms where larger individuals grow proportionately more than smaller individuals, i.e., growth rate is proportional to body size. Organisms with non-regulated growth show no significant relationship between initial body size and growth rate.

The three species of aphidophagous lady beetles co-occur in many natural and crop habits throughout the Great Plains of the USA and generally vary in trophic specificity. Both *C. maculata* and *H. axyridis* are considered to be highly polyphagous because of their ability to use a wide variety of food, including soft-bodied insects and plant derived nutrients like pollen and nectar, for development and reproduction (Hodek & Honěk, 1996). Although *H. convergens* also accepts a wide range of food, it is considered to be more oligophagous as only aphids ensure its development and reproduction (Hodek & Honěk, 1996). Several studies have shown that the ability to use alternative food, including cannibalism, allows these beetles to survive when aphids are scarce (Hodek & Honěk, 1996; Berkvens et al., 2008). Our approach in this study did not consider the contribution of alternative food as we wanted to focus on a more simplified study system that would allow us to unravel growth and developmental responses to the availability of lady beetle essential prey.

Materials and methods

Source of aphids and lady beetles

The source of food provided to lady beetle larvae was the greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), an aphid species that is often an important food source for lady beetles in cereal farmlands in the USA Great Plains. Greenbug colonies were reared on sorghum plants in the greenhouse as described in Phoofolo et al. (2008).

In April and May 2004 we collected adult lady beetles of all three species from alfalfa and wheat fields in north central Oklahoma. Males and females of each species were paired and each breeding pair was individually maintained in half-pint (0.24 l) paper cartons (Neptune Paper Products, Jersey City, NJ, USA) covered with fine mesh in table-top environmental chambers at 22 °C and L16:D8 photoperiod. Pairs were provided daily with an unlimited supply of greenbugs, moist cotton, and a supplementary 1:1 mixture of honey and wheast (Product #176; Planet Natural, Bozeman, MT, USA). Egg masses from each pair were collected daily and reared separately in 10-ml glass vials stopped with cotton. Upon hatching larvae were transferred to rearing containers and provided with greenbugs ad libitum until they molted into third instars.

Modeling pupation success as a function of body weight

Monitoring larval development. The experiment was carried out at 22 °C and L16:D8 photoperiod. Within 24 h of molting to third instars, larvae were placed individually in vials stopped with cotton and were supplied with ad libitum greenbugs until molting to fourth instars. Fourth instars of each species were randomly assigned to one of six diet treatments: (1) larvae fed for the first 5 days, (2) larvae fed only for the 1st 4 days, (3) larvae fed only for the 1st 3 days, (4) larvae fed only for the 1st 2 days, (5) larvae fed only for the first day of the fourth stadium, and (6) individuals starved throughout the fourth stadium. Feeding larvae for the 1st 5 days of the stadium was equivalent to feeding until initiation of pupation because, in this treatment, fourth instars of all three species ceased feeding and entered the pre-pupal stage. The number of larvae assigned to each treatment ranged from 12 to 21 for each lady beetle species. In order to measure the effect of weight of fourth instars on induction of pupation, we measured weight [on a digital Sartorius M3P micro-balance (Sartorius, McGaw Park, IL, USA); resolution: 0.001 mg] of larvae daily throughout the fourth stadium (i.e., until a larva either pupated or died).

Estimates of critical weight. Three estimates of critical weight were determined. (1) The minimum critical weight, determined as the weight of fourth instars below which pupation was not possible. (2) The maximum critical weight, determined as the weight of fourth instars above which pupation success is 100% (except for mortality that occurs independent of body weight). (3) The median critical weight, estimated as the body weight at which presence or absence of pupation induction are equally likely (i.e., W_{50} is the weight of the fourth instar at which its probability, p, of pupating when deprived of food is 0.5). The logistic regression (SAS Proc LOGISTIC) (SAS Institute, 1999) was used to estimate the probability of pupation as a function of body weight of fourth instars of lady beetles. The relationship between W and p was modeled as

$$\ln\left(\frac{p}{1-p}\right) = b_0 + b_1 W,$$

where regression parameters b_0 and b_1 are the y-intercept and slope, respectively. This model relates the probability of pupation to body weight of fourth instars as

$$p = \frac{e^{(b_0+b_1 W)}}{1 + e^{(b_0+b_1 W)}}.$$

The median critical weight was obtained from the regression parameters as $W_{50} = -b_0/b_1$.

Determining level of flexibility in development period. The effects of food deprivation period on the length of development time between the day larvae reached peak weight and the day of pupation, hereafter referred to as the peak-weight-to-pupation period, were assessed by fitting linear mixed models (SAS Proc MIXED). Food deprivation period, gender, and their interaction were included in the models as fixed effects, whereas mating pairs, used as sources of larvae, were incorporated into the models as random effects. Effects of food deprivation period were further evaluated using orthogonal polynomial contrasts to determine linear and nonlinear trends in response variables. If polynomial contrasts were significant, analysis of covariance (ANCOVA) (Proc MIXED), with gender as the main effect, food deprivation period as the covariate, and mating pair as a random effect, was used to quantify the relationship between dependent variables and food deprivation period. The test for normality of data (Proc UNIVARIATE) performed prior to analyses by Proc MIXED indicated that no transformations were needed for any data.

Growth trajectory analysis

Data source. All experiments were carried out in a table-top environmental chamber at 22 °C and L16:D8 photoperiod. Within 24 h of molting to third instars, larvae were placed individually in vials stopped with cotton and were supplied with ad libitum greenbugs until molting to fourth instars. Fourth instars of each lady beetle species were weighed daily from the 1st day of the fourth stadium until they reached pre-pupal stage or died and

Table 1 Forms of mathematical growth functions used to describe the relationship between body weight and development time of lady beetle fourth instars

Function name	Equation	No. parameters	Differential form
Exponential	$W = W_0 e^{kt}$	2	$\frac{dW}{dt} = kW$
Monomolecular	$W = W_f - (W_f - W_0)e^{-kt}$	3	$\frac{dW}{dt} = k(W_f - W)$
Logistic	$W = \frac{W_0 W_f}{W_0 + (W_f - W_0)e^{-kt}}$	3	$\frac{dW}{dt} = kW\left(1 - \frac{W}{W_f}\right)$
Gompertz	$W = W_0 \exp\left[\left(\ln\frac{W_f}{W_0}\right)(1 - e^{-kt})\right]$	3	$\frac{dW}{dt} = kW\ln\left(\frac{W_f}{W}\right)$
von Bertalanffy	$W = [W_f^n - (W_f^n - W_0^n)e^{-kt}]^{1/n}$	4	$\frac{dW}{dt} = \frac{kW(W_f^n - W^n)}{nW^n}$

W is body weight (mg) at day t of the fourth stadium, W_0 is the initial body weight after molting to the fourth instar ($t = 0$), W_f is the final body weight that is approached asymptotically, k is an index of growth rate (day^{-1}), and n (dimensionless) is an allometric parameter.

were all fed ad libitum greenbugs immediately after weighing.

Growth functions. Formulas of the functions used in growth trajectory analysis are provided in Table 1. We included an exponential model because, in spite of its unrealistic assumption of maximum and unrestricted growth that only stops when food resources are exhausted, it has been used to describe growth in arthropods (Tessier & Goulden, 1987). The monomolecular model describes a growth rate that decreases continuously based on the assumption that the rate of growth is proportional to how close the weight of a larva at time t is to the maximum body weight as described by the differential equation in Table 1. There is, therefore, no point of inflection in the monomolecular curve. The logistic curve has a point of inflection when the current body weight, W, is at half of the maximum body weight, W_f (i.e., $W = W_f/2$). From its differential form, it can be seen that the logistic curve describes the rate of growth that is proportional to both W and a factor $(1 - W/W_f)$ that is the remaining room for growth $(W_f - W)$, divided by W_f. The Gompertz curve has a point of inflection when $W = W_f/e$ and, compared with the logistic model for the same initial and final weights and a similar time of inflection, it shows faster early growth, but a slower approach to the final weight. The von Bertalanffy curve has a variable point of inflection and is based on the assumptions that food is non-limiting and the growth process results from the difference between anabolism and catabolism, with both being related to weight allometrically and linearly, respectively (i.e., $dW/dt = aW^b - cW$, where a and c are anabolism and catabolism constants, respectively, and b is an allometric exponent) (Thornley & France, 2007).

The five growth models were fitted separately to the data sets for the three lady beetle species using the SAS Proc NLMIXED, which fits nonlinear mixed models by

Table 2 Mixed-effect growth models used to describe the relationship between body weight and development time of lady beetle fourth instars

Function name	Nonlinear mixed model
Exponential	$W_{ij} = (W_0 + b_{i1})\exp(kt) + e_{ij}$
Monomolecular	$W_{ij} = [(W_f + b_{i2}) - [(W_f + b_{i2}) - (W_0 + b_{i1})]\exp(-kt)] + e_{ij}$
Logistic	$W_{ij} = \left[\frac{(W_0 + b_{i1})(W_f + b_{i2})}{(W_0 + b_{i1}) + [(W_f + b_{i2}) - (W_0 + b_{i1})]\exp(-kt)}\right] + e_{ij}$
Gompertz	$W_{ij} = (W_0 + b_{i1})\exp\left[\left(\ln\frac{(W_f + b_{i2})}{(W_0 + b_{i1})}\right)(1 - \exp(-kt))\right] + e_{ij}$
von Bertalanffy	$W_{ij} = [(W_f + b_{i2})^n - [(W_f + b_{i2})^n - (W_0 + b_{i1})^n]\exp(-kt)]^{1/n} + e_{ij}$

W_{ij} represents the j-th weight measurement on the i-th larva, W_0 is initial body weight after molting to the fourth instar ($t = 0$), W_f is the final body weight that is approached asymptotically, b_{i1} and b_{i2} are random effects for W_0 and W_f, respectively; k is an index of growth rate (day^{-1}), n (dimensionless) is an allometric parameter for the von Bertalanffy function, and e_{ij} is the within-individual error.

an iterative maximum likelihood method integrated over the random effects. Information on the mixed models fitted to the data sets is provided in Table 2. The fixed effects, W_0 and W_f, represent the mean values of the parameters in the population of individuals, whereas the individual deviations from W_0 and W_f are represented by the random effects b_{i1} and b_{i2}, respectively, which are assumed to be independent and distributed normally with mean 0 and variance–covariance matrix Ψ, where

$$\Psi = \begin{pmatrix} \mathrm{var}(b_{i1}) & \\ \mathrm{cov}(b_{i1}, b_{i2}) & \mathrm{var}(b_{i2}) \end{pmatrix}.$$

The diagonal elements of Ψ are variances that summarize the extent to which the random effects cluster around zero and the off-diagonal element describes the degree to which pairs of random effects co-vary. The within-individual errors (random error for the j-th weight measurement of individual i), e_{ij}, are assumed to be independent and distributed normally with mean 0 and variance s^2 and to be independent of the random effects (Davidian & Giltiman, 2003).

Performance (goodness of fit) of the models was evaluated using values of the variance of error estimate, and its approximate standard error. We also compared the models by using Bayesian information criteria (BIC), which are model order selection criteria based on parsimony and impose a penalty on more complicated models for inclusion of additional parameters. The smaller the numerical value of BIC the better the model fits the data.

Because females are usually larger than males in these lady beetle species (Phoofolo et al., 2008) we assessed the relationship between the growth pattern of fourth instars and gender for the model that had the best fit to the data sets. This was achieved by modifying the mixed model to include gender-specific coefficients for initial weight W_0, maximum weight W_f, and index of growth rate k.

Growth regulation analysis

To determine whether growth was convergent-regulated, divergent-regulated, or non-regulated, we used the reduced major axis (RMA) regression to describe the relationship between initial and final body weight of *C. maculata*, *H. axyridis*, and *H. convergens* fourth instars. Reduced major axis regression is regarded as superior to, and therefore preferred over, ordinary least squares regression when describing a relationship between two variables or traits that are subject to measurement error (Warton et al., 2006). The reason lies in the fact that RMA minimizes the residual variances of both the X (e.g., initial weight) and Y (e.g., final weight) variables for each experimental unit. Furthermore, in RMA regression, the variables are standardized and the slope, b, of their major axis is computed as the ratio of the standard deviations, s_y/s_x. This allows the null hypothesis that b = 1 to be used to test for the presence of growth regulation. Convergent-regulated growth is inferred if b<1, because the expectation is that the variance in the final weight should be reduced relative to the variance in the initial weight. Similarly, divergent-regulated growth is inferred if b>1. We used SMATR 2.0 software (Falster et al., 2006) to perform the RMA regression relating (i) log (final weight of fourth instars) to log (initial weight of fourth instars), and (ii) log

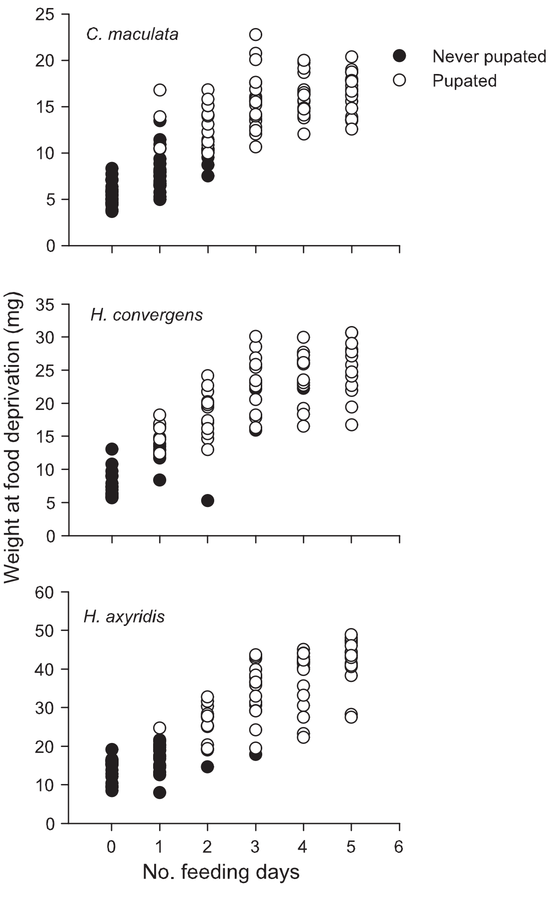

Figure 1 Minimum and maximum critical body weight necessary for successful development to pupation and adulthood in fourth instars of *Coleomegilla maculata*, *Hippodamia convergens*, and *Harmonia axyridis*.

(adult weight) to log (initial weight of fourth instars), and to statistically test for b = 1 for each species. We also tested for differences in the nature of the above relationships (i.e., between initial weight of fourth instars and final weight of fourth instars or adult weight) among species.

Results

Estimates of critical weight for pupation

None of the larvae that were denied food throughout the fourth stadium were able to pupate even though some of them weighed more than larvae that successfully pupated after being given access to food (Figure 1). Therefore, access to food for a minimum of 1 day during the fourth stadium appeared to be a requirement for pupation in all three species, with 14.3% pupation success in *C. maculata*, 35.7% in *H. convergens*, and 5.9% in *H. axyridis*. The estimated minimum and maximum critical body weights for *C. maculata* larvae were 10 and 13.5 mg, because none of fourth instars weighing <10 mg were able to pupate, whereas all larvae weighing >13.5 mg pupated. For *H. axyridis* the minimum and maximum critical body weights were 19.3 and 21.5 mg. Although it is clear that all *H. convergens* fourth instars weighing <12.5 mg did not pupate, it is not so clear where the maximum critical weight lies as some larvae weighing up to 22 mg did not pupate because of unknown mortality factors. However, it is possible that mortality among fourth instars weighing >15.9 mg was independent of larval weight.

Logistic regression indicated that the probability of pupation success was significantly related to the peak weight of fourth instars in all three species (Figure 2, Table 3). For *C. maculata* fourth instars, the regression model, $p = e^{(-15.788\ +\ 1.421W)}/(1 + e^{(-15.788\ +\ 1.421W)})$, correctly predicted which larvae were successful in pupating based on their weight 99% of the time. From the model the median critical weight (W_{50}) for *C. maculata* is calculated as 11.11 mg and the odds of successful pupation by a larva with such a peak weight is about four times that of a larva with a peak weight of 10.11 mg. The regression model for *H. axyridis* fourth instars, $p = e^{(-20.088\ +\ 0.956W)}/(1 + e^{(-20.088\ +\ 0.956W)})$, also correctly predicted which larvae were successful in pupating 99.4% of the time. The median critical weight was estimated as 21.01 mg with the odds of pupation by a larva with such a peak weight being about three times that of a larva with a peak weight of 20.01 mg. Although the model for *H. convergens* fourth instars, $p = e^{(-6.934\ +\ 0.474W)}/(1 + e^{(-6.934\ +\ 0.474W)})$, correctly predicted pupation 94.8% of the time, a significant Hosmer and Lemeshow Goodness-of-Fit Test indicated that the model was not an adequate fit to the data (Table 3).

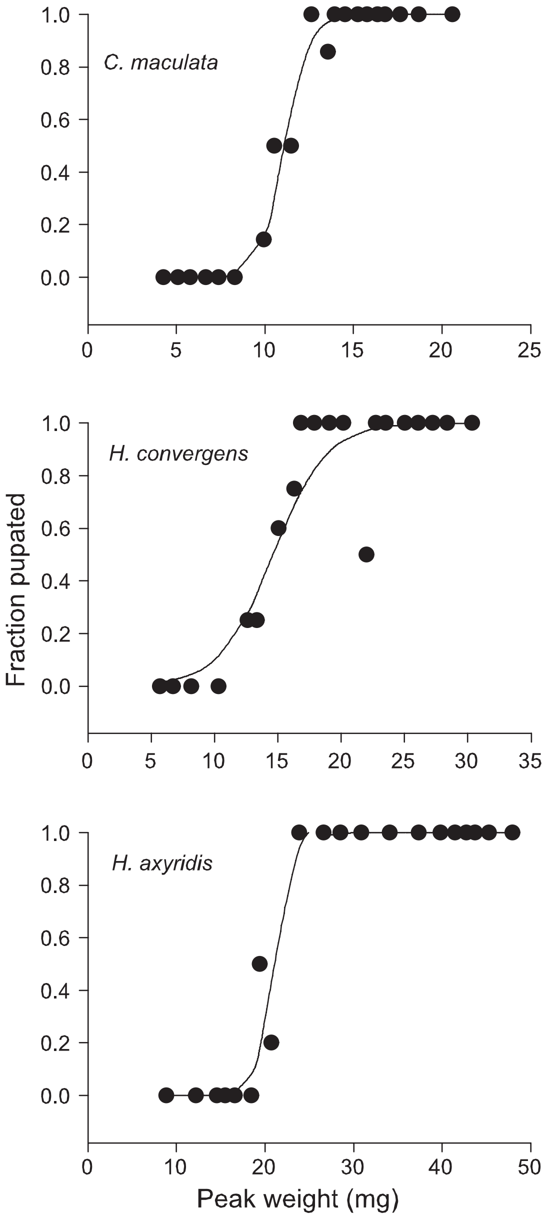

Figure 2 Relationship between peak weight of fourth instars and pupation induction in *Coleomegilla maculata*, *Hippodamia convergens*, and *Harmonia axyridis*. The peak weights were binned into 20 groups so that each point is the percentage of larvae that were able to successfully pupate in a group. Solid lines indicate the probability that a fourth instar would pupate when deprived of food based on logistic regression: logit(p) = −15.788 + 1.421W for *C. maculata*, logit(p) = −20.088 + 0.956W for *H. axyridis*, and logit(p) = −6.934 + 0.474W for *H. convergens*.

Table 3 Results of the logistic regression analysis (SAS Proc LOGISTIC) to evaluate the relationship between probability of pupation success and peak weight of fourth instars of *Coleomegilla maculata*, *Hippodamia convergens*, and *Harmonia axyridis*

Statistics	*C. maculata*	*H. convergens*[1] Full data	*H. convergens*[1] Reduced data	*H. axyridis*
Likelihood ratio test				
χ^2	134.002	59.775	74.364	108.378
P	<0.0001	<0.0001	<0.0001	<0.0001
d.f.	1	1	1	1
Hosmer & Lemeshow test				
χ^2	2.032	16.653	0.512	1.039
P	0.980	0.034	0.999	0.984
d.f.	8	8	8	6
Odds ratio	4.141	1.606	2.407	2.602
% concordance	99.0	94.8	98.2	99.4

[1] Full data refers to analysis results from the complete data set and reduced data to analysis results on the data that excluded the two outliers. See text for details.

In order to identify the outlier and influential observations on the values of the estimated parameters, we reanalyzed the *H. convergens* data with Proc LOGISTIC that included the INFLUENCE and IPLOTS options in the MODEL statement. The resulting diagnostic statistics indicated the two observations that were outliers as larvae that had peak weight of 22.20 and 22.21 mg but failed to pupate (Figures 1 and 2). Deletion of these observations resulted in a new model, $p = e^{(-12.601 + 0.878W)}/(1 + e^{(-12.601 + 0.878W)})$, that had both improved predictive power and fit to the data (Table 3). The median critical weight was estimated from the new model as 14.35 mg with the odds of pupation by a larva with such a peak weight being 2.4 times that of a larva with a peak weight of 13.35 mg.

Determining level of flexibility in development period

Fourth instars of each lady beetle species reached their peak weights on the day they were denied food. Gender and its interaction with food deprivation period did not significantly affect the peak-weight-to-pupation period indicating a similar relationship between peak-weight-to-pupation period in both males and females of all three species (Table 4). The relationship between food deprivation period and peak-weight-to-pupation period was significantly linear in all species ($P<0.0001$) with a significant quadratic trend ($0.01 \leq P < 0.05$) in *H. axyridis* indicating an accelerating increase in peak-weight-to-pupation period with an increase in food deprivation period (Table 4, Figure 3).

Analysis of growth trajectories

Comparison of the growth models on the basis of BIC values indicated that both the exponential and monomolecular functions did not provide a good fit to the data relating changes in body weight as a function of age in fourth instars of *C. maculata*, *H. convergens*, and *H. axyridis* (Table 5). This lack of fit was also supported by the large residual variance associated with both functions. The three sigmoidal models (logistic, von Bertalanffy, and Gompertz) performed better than the non-sigmoidal models. A comparison among the three sigmoidal models based on their residual variances (s^2) and fit statistics showed that the logistic model provided the best fit for fourth instars of all three species (Figure 4). Both the Gompertz and von Bertalanffy models provided similar fit and estimates of growth parameters (Figure 4); but because the von Bertalanffy model has one more parameter its BIC values were larger (Table 5). We decided to focus the growth trajectory analysis on the logistic model because it provided the best fit for all three lady beetle species.

None of the three species had a significant estimated variance of the random effect associated with

Table 4 Results of analysis of variance (SAS Proc Mixed) to evaluate effects of food deprivation period on fourth instar peak-weight-to-pupation period in *Coleomegilla maculata*, *Hippodamia convergens*, and *Harmonia axyridis*

Source of variation	*C. maculata* d.f.	*C. maculata* F	*C. maculata* P	*H. convergens* d.f.	*H. convergens* F	*H. convergens* P	*H. axyridis* d.f.	*H. axyridis* F	*H. axyridis* P
Treatment	4,59	10.55	<0.0001	4,39	12.30	<0.0001	3,43	20.15	<0.0001
Linear	1,59	27.88	<0.0001	1,39	38.24	<0.0001	1,43	54.78	<0.0001
Quadratic	1,59	0.79	0.38	1,39	0.69	0.41	1,43	5.87	0.02
Cubic	1,59	3.19	0.079	1,39	2.66	0.11	1,43	0.12	0.73
Quartic	1,59	2.25	0.14	1,39	0.76	0.39			
Gender	1,59	0.52	0.47	1,39	0.00	0.96	1,43	0.15	0.70
Treatment*gender	4,59	0.84	0.50	4,39	1.33	0.28	3,43	0.67	0.57

the growth rate parameter, k, implying that, for each species, growth rate may be relatively constant among individuals. Both of the estimated variance components, s^2_{b1} and s^2_{b2}, quantifying the variability among individual random effects associated with the initial and final weight, were significantly different from zero only in *C. maculata* ($t = 2.85$, d.f. = 18, $P = 0.011$ and $t = 2.84$, d.f. = 18, $P = 0.011$, respectively). In both *H. convergens* and *H. axyririds*, only the estimates of variance components, s^2_{b2}, of the random effects associated with the final weight were significantly greater than zero ($t = 2.02$, d.f. = 8, $P = 0.078$ and $t = 2.02$, d.f. = 12, $P = 0.066$, respectively) (Table 5). No species had a significant covariance estimate between random effects associated with initial and final weight (Table 5). Although these results imply that the initial weight may not vary significantly among fourth instars of *H. convergens* and *H. axyririds*, including random effects associated with initial weight in the logistic model resulted in a better description for the data structure as indicated by the smaller and better fit statistics compared to when initial and final weight were modeled as fixed effects (*H. axyridis* BIC = 503.5, *H. convergens* BIC = 365.2). Also, the mixed-effects model provides individual-specific functions that describe the growth trajectory for each individual and thus illustrating the extent of the heterogeneity of the response between subjects.

The following modified mixed logistic model was used to test the dependence of the parameters on gender in lady beetle species:

$$W_{ij} = \frac{(W_f + g_2.S + b_{i2})(W_0 + g_1.S + b_{i1})}{(W_0 + g_1.S + b_{i1}) + [(W_f + g_2.S + b_{i2}) - (W_0 + g_1.S + b_{i1})]\exp(-(k + g_k.S)t)} + e_{ij}.$$

In this model W_0, W_f, k, b_{i1}, and b_{i2} are as defined above, g_1, g_2, and g_k are gender coefficients associated with W_0, W_f, and k, respectively, and $S = 1$ if gender was female and 0 if male. The logistic model that accounted for gender effects improved the fit to the data for both *C. maculata* and *H. convergens* but not for *H. axyridis* (Table 6), indicating that the growth patterns of males and females are not different in *H. axyridis*. Gender differences in growth patterns for *C. maculata* and *H. convergens* were mainly due to the final weight being significantly larger in females than males (significant gender coefficient for final weight, g_2), whereas there were no significant gender effects on initial weight and the index of growth rate (Table 6).

Growth regulation analysis

We present only the results of the RMA regression between initial weight of fourth instars and adult weight as this relationship was similar to that between initial and final weight of fourth instars across species. The slope, b, of the RMA regression relating weight of adults to the initial weight of fourth instars was significantly less than 1.0 for all three species (Table 7). This suggested that (a) adult weight was not directly proportional to initial weight of fourth instars, and (b) growth in these beetles was regulated such that individuals that were small at the beginning of the fourth stadium grew more than individuals that were large. Comparison of the slopes among the species also showed no significant differences in their magnitudes (test statistic = 0.18, d.f. = 2, $P = 0.90$), with a common fitted slope of 0.60 (95% confidence interval: 0.50–0.72) (Figure 5, Table 7).

There were also significant elevation shifts in the regression lines (i.e., significantly different y-intercepts) among species (test statistic = 49.72, d.f. = 2, $P<0.0001$), with the largest difference being that between *C. maculata* and *H. axyridis* (Figure 5). This means that fourth instars with similar initial weight across species developed into adults that differed in weight, with *C. maculata* < *H. convergens* < *H. axyridis*. Conversely, adults with similar weight across species were likely to have developed from fourth instars that differed in initial weight, with *H. axyridis* < *H. convergens* < *C. maculata*.

Discussion

Our data indicate that body weight is an important factor in determining whether lady beetle fourth instars can successfully complete development under starvation conditions. We have identified the minimum critical weight that has to be attained during growth in the fourth stadium of *C. maculata*, *H. convergens*, and *H. axyridis* in order for successful pupation to occur. We have also identified the maximum critical weight that, when attained by fourth instars of these species, ensures invariable pupation success. Logistic regression models that predict the probability of pupation based on fourth instar peak weight were developed for each species. Our results are consistent with the hypothesis

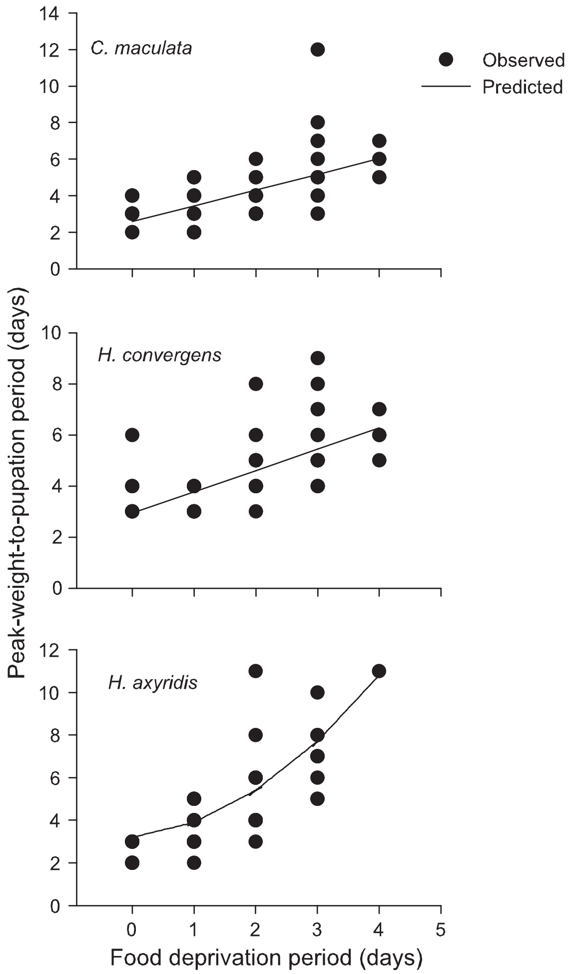

Figure 3 Relationship between the developmental period from peak weight attainment to pupation and food deprivation period. The relationship is described by the following equations: $P = 2.576\ (\pm\ 0.352) + 0.863\ (\pm\ 0.129)D$ (*Coleomegilla maculata*), $P = 2.941\ (\pm\ 0.375) + 0.833\ (\pm\ 0.120)D$ (*Hippodamia convergens*), and $P = 3.170\ (\pm\ 0.569) + 0.384\ (\pm\ 0.476)D + 0.367\ (\pm\ 0.157)D^2$ (*Harmonia axyridis*), where P is peak-weight-to-pupation period (days) and D is food deprivation period (days).

that fourth instars of lady beetles are able to 'assess' their body size so that when a critical weight is attained, physiological processes are initiated which lead to successful development to pupation and adulthood. This biological attribute appears to be common in the final instars of many other holometabolous insects (Bradshaw & Johnson, 1995; Shafiei et al., 2001; Davidowitz et al., 2003; Nijhout et al., 2006). Although we did not study the nature of the physiological processes that underlie the relationship between weight and pupation success in lady beetles, it is quite plausible that they are mediated by hormonal mechanisms similar to those found in other insects (Nijhout, 2003; Mirth et al., 2005).

Food availability is one of the several environmental factors that impose time constraints on insect development. When food availability varies seasonally, insects cope by engaging in diapause-mediated responses such as dormancy or migration (Tauber & Tauber, 1993). For sudden changes in food availability, which are typical in ephemeral habitats, there appear to be several ways in which insects developmentally respond. If food scarcity occurs before attaining a critical weight, many species extend the last larval stadium beyond normal lengths, e.g., *M. sexta* (Nijhout et al., 2006) and *W. smithi* (Bradshaw & Johnson, 1995), whereas others undergo supernumerary larval molting, e.g., *Trogoderma glabrum* (Herbst) (Beck, 1971). When food scarcity occurs after attaining the critical weight, many insects respond by only ceasing growth without changes in development period to pupation (i.e., determinate development), e.g., *M. sexta* (Nijhout et al., 2006) and *W. smithi* (Bradshaw & Johnson, 1995). Other species respond by both cessation of growth followed by immediate pupation, thus shortening the development period (i.e., flexible development), e.g., *O. taurus* (Shafiei et al., 2001). Our finding that the peak-weight-to-pupation period increased significantly with an increase in food deprivation period in *C. maculata*, *H. axyiridis*, and *H. convergens* indicates that the relationship between growth and development in these beetles fits the determinate developmental pattern. If they had the flexible developmental response, the peak-weight-to-pupation period would have been similar across food deprivation periods, with individuals subjected to food deprivation early in the stadium having shorter development periods. Further evidence for determinate developmental response to starvation in these lady beetles was reported by Phoofolo et al. (2008) showing lack of significant relationships between food deprivation period and the stadium length.

Higher relative fitness is generally associated with larger individuals because, among other things, they tend to be more fecund and competitive. Higher fitness is also generally associated with individuals that have faster development rate because they tend to have higher survival rate to adulthood. Therefore, there is a trade-off between attaining a large body size, which requires longer development time and therefore subjects immature stages to greater mortality risk from intra-guild predation and cannibalism, and developing rapidly, which results in smaller adult size but reduces immature mortality risk. The determinate developmental response to food deprivation in the three lady beetle species can be

Table 5 Parameter estimates of the various growth models relating body weight to developmental time of fourth instars of *Coleomegilla maculata*, *Hippodamia convergens*, and *Harmonia axyridis* (SE in parentheses)

Function[1]	W_0	W_f	k	n	s^2	s^2_{b1}	s^2_{b2}	cov	BIC
C. maculata									
Exponential	6.68 (0.39)		0.17 (0.01)		4.06 (0.61)	1.51 (0.56)			524.9
Monomolecular	0.26 (0.80)	20.53 (1.21)	0.30 (0.04)		1.14 (0.19)	4.56 (2.21)	9.11 (3.50)	1.46 (1.87)	431.8
Logistic	2.45 (0.32)	17.25 (0.65)	0.84 (0.03)		0.52 (0.09)	1.59 (0.56)	5.84 (2.06)	0.26 (0.92)	387.5
Gompertz	1.95 (0.43)	18.21 (0.74)	0.56 (0.04)		0.78 (0.13)	1.74 (0.65)	6.74 (2.43)	0.80 (0.93)	410.5
von Bertalanffy	1.95 (0.42)	18.21 (0.74)	0.56 (0.40)	0.00001	0.78 (0.13)	1.74 (0.65)	6.74 (2.43)	0.80 (0.93)	413.4
H. convergens									
Exponential	11.09 (0.90)		0.15 (0.01)		12.71 (2.49)	3.78 (2.06)			368.0
Monomolecular	0.02 (0.05)	30.81 (2.01)	0.33 (0.03)		4.35 (0.93)	0.76 (3.25)	28.11 (14.48)	2.74 (4.74)	316.3
Logistic	4.37 (0.61)	27.01 (1.54)	0.83 (0.06)		2.94 (0.63)	1.36 (0.84)	19.98 (9.88)	4.28 (2.35)	301.5
Gompertz	3.19 (0.71)	28.10 (1.73)	0.58 (0.06)		3.55 (0.76)	0.90 (0.88)	22.45 (11.31)	3.79 (2.38)	308.6
von Bertalanffy	3.19 (0.71)	28.10 (1.74)	0.58 (0.06)	0.00002	3.55 (0.76)	0.90 (0.88)	22.46 (11.31)	3.79 (2.38)	310.9
H. axyridis									
Exponential	13.80 (0.85)		0.21 (0.01)		21.77 (3.80)	3.02 (1.45)			506.8
Monomolecular	2.13 (1.98)	74.39 (13.10)	0.15 (0.04)		9.98 (1.96)	10.57 (9.67)	116.36 (72.29)	−16.56 (18.38)	469.7
Logistic	7.15 (0.79)	47.93 (2.60)	0.70 (0.06)		9.38 (1.85)	1.18 (0.97)	42.21 (20.86)	3.22 (3.26)	462.7
Gompertz	5.64 (0.97)	52.97 (3.76)	0.43 (0.05)		9.57 (1.90)	1.85 (1.76)	50.39 (24.03)	0.35 (4.66)	465.5
von Bertalanffy	5.64 (0.98)	53.06 (3.81)	0.43 (0.05)	0.0048	9.59 (1.90)	1.83 (1.75)	50.43 (24.08)	0.43 (4.66)	468.1

[1] W_0 is the initial body weight after molting to the fourth instar ($t = 0$), W_f is the final body weight that is approached asymptotically, k is an index of growth rate (day^{-1}), n (dimensionless) is an allometric parameter, s^2 is the residual variance, s^2_{b1} and $s^2_{b2}$are variance components for individual random effects associated with the initial and final weight, respectively and cov is an estimate of the degree to which pairs of random effects co-vary. BIC, Bayesian information criterion (see Materials and methods)

considered an adaptation that emphasizes fitness increase by maximizing body size, which results in more fecund adults and individuals that are likely to win in intra-guild interactions, whereas fitness increase in insects such as *O. taurus* with the flexible developmental response is a result of faster development and higher survival. This difference in developmental responses to food deprivation between lady beetles and the scarab beetle is most likely related to their nutritional ecology. Although the abundance of the preferred prey (aphids) for larvae of lady beetles varies both spatially and temporally, their generalist feeding habits enable them to rely on other types of food, most of which are adequate for survival and some for growth (Hodek & Honěk, 1996). It is therefore likely that, because of fitness benefits of larger individuals, lady beetle developmental period after attaining the critical weight tends to be determinate in order to optimize the possibility of encountering alternative or additional prey. In *O. taurus*, individual larvae feed on an underground dung ball that varies both in nutrition size and quality; and once their food is exhausted, there is no opportunity for encountering alternative nutrition, hence the probable adaptive significance of having a flexible developmental rate (Shafiei et al., 2001).

The superior performance of sigmoid models in describing growth trajectories in the ad libitum fed fourth instars of *C. maculata*, *H. convergens*, and *H. axyridis* indicates that growth in this developmental stage is a two-phased process with an inflection point separating the phases. The first phase is characterized by a slow initial rate of growth that, as time progresses, increases to a maximum rate. During the second phase, growth rate begins to decline and to approach zero. Sigmoid models are widely used in describing growth trajectories in many organisms including plants (Yin et al., 2003), insects (Gordon, 1999; Teuschl et al., 2007), and vertebrates (Lopez et al., 2000; Moscarella et al., 2001; Winship et al., 2001; Darmani Kuhi et al., 2003). The statistical evaluation of the three sigmoid models used in this study pointed toward the logistic model as one that offered the best description of growth pattern in the fourth instars of the three lady beetle species. Several attributes of the logistic model make it biologically plausible (Thornley & France, 2007). First, all the parameters of the model have biological connotations. For lady beetle fourth instars, W_0 is equivalent to their weight immediately after ecdysis, W_f is equivalent to the maximum weight attained just before pupation, and k is a growth rate parameter. Second, an exponential absolute growth

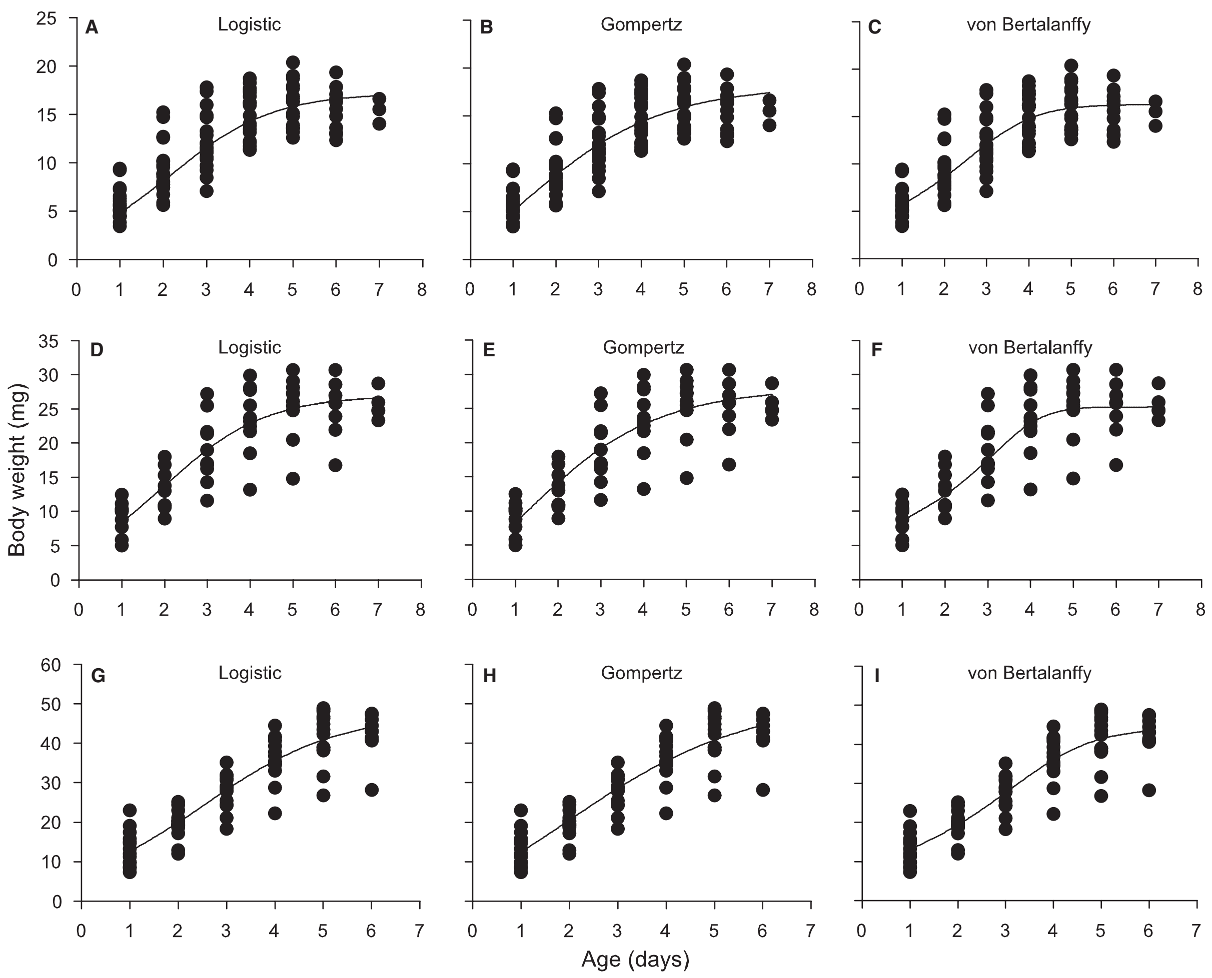

Figure 4 Growth trajectories of fourth instars based on the estimated mean parameters of the logistic, Gompertz, and von Bertalanffy models for *Coleomegilla maculata* (A–C), *Hippodamia convergens* (D–F), and *Harmonia axyridis* (G–I).

rate is predicted for young fourth instars by the model (i.e., as $W \rightarrow 0$, $dW/dt = kW(1\text{-}W/W_f) \rightarrow kW$ and $W = W_0e^{kt}$). Third, the model predicts that the absolute growth rate for mature fourth instars will tend to zero as their body weight, W, tends towards its upper limit, W_f [i.e., $dW/dt = kW(1\text{-}W/W_f) \rightarrow kW(1\text{-}W_f/W_f)$]. Fourth, the model predicts that maximum absolute growth rate (i.e., point of inflection) occurs when the weight of an individual, W, is at half its maturity weight, $W_f/2$. It is quite possible that the weight of fourth instars at the point of inflection is related to the critical or threshold weight for pupation, which as determined for *M. sexta*, is a key developmental stage because of its tight association with the onset of hormonal regulation of metarmophosis (Nijhout et al., 2006).

Our results also showed that nonlinear mixed-effects modeling provided a better fit for each lady beetle species compared with a similar model without random effects. This is because the mixed-effects model accounted for the data structure more completely by analyzing inter-individual differences in model parameters and thus defining the process of growth for each individual, as well as providing the population mean trajectory that characterizes a typical or average individual. The ability of nonlinear mixed models to characterize subject-specific growth trajectories is particularly important as individual profiles are likely to vary on particular features, e.g., asymptotic weights may be somewhat higher or lower than the average weight and points of inflection may be reached earlier or later (Pinheiro & Bates, 2000). The results obtained from the logistic model that accounted for gender effects are consistent with what we have previously reported regarding significant sexual dimorphism in body weight in *C. maculata* and *H. convergens* (Phoofolo et al., 2008).

Table 6 Parameter estimates (± SE) for the logistic growth model with sex as a covariate relating body weight to developmental time of fourth instars of *Coleomegilla maculata*, *Hippodamia convergens*, and *Harmonia axyridis*

Parameter	*C. maculata*	*H. convergens*	*H. axyridis*
W_0	1.99 ± 0.36***	3.96 ± 0.80**	6.20 ± 1.47**
W_f	14.87 ± 0.80***	23.63 ± 1.43***	44.63 ± 4.30***
k	0.88 ± 0.06***	0.82 ± 0.10***	0.77 ± 0.13***
g_1	0.67 ± 0.57	0.96 ± 1.19	1.28 ± 1.73
g_2	3.84 ± 1.05**	7.07 ± 2.19*	4.59 ± 5.32
g_k	−0.06 ± 0.07	0.0009 ± 0.1299	−0.09 ± 0.14
s^2	0.51 ± 0.09***	2.94 ± 0.63**	9.18 ± 1.81***
s_{i1}^2	1.79 ± 0.67*	1.10 ± 0.73	1.26 ± 0.97
s_{i2}^2	3.33 ± 1.31*	7.67 ± 4.26	41.87 ± 20.89
$\mathrm{cov}(b_{i1},b_{12})$	1.13 ± 0.88	2.62 ± 1.41	3.29 ± 3.28
BIC	385.3	299.0	469.8

Parameter values significantly different from zero are indicated as *0.01<P<0.05, **0.001<P≤0.01 and ***P≤0.001.

Table 7 Results of the reduced major axis regression relating adult weight to the initial weight of fourth instars in *Coleomegilla maculata*, *Hippodamia convergens*, and *Harmonia axyridis*

Species	n	Slope	95% confidence interval	F	P
C. maculata	39	0.57	0.42–0.76	16.00	<0.001
H. axyridis	30	0.66	0.45–0.96	5.19	0.031
H. convergens	22	0.63	0.44–0.91	7.32	0.014

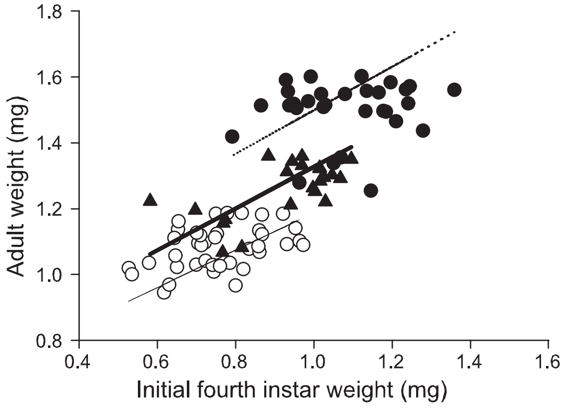

Figure 5 Relationship between adult weight and initial weight of fourth instars in *Coleomegilla maculata* (open circles and thin line), *Hippodamia convergens* (closed triangles and thick line), and *Harmonia axyridis* (closed circles and dotted line).

If the initial exponential rate of growth inherent in the logistic growth trajectory accurately characterizes growth in lady beetle fourth instars, then we believe that this attribute, in combination with the determinate developmental response when deprived of food after attaining the critical weight, are part of a set of traits that enable these species to deal with their varying prey abundance in nature. However, this observed set of traits could be a reflection of phylogenetic history rather than a physiological adaptation to nutritional variability or stress, especially given the different trophic relationships of the three lady beetle species. But because our study was not set-up to separate the effects of phylogenetic constraints from those of adaptations for feeding on narrow (e.g., *H. convergens*) vs. wide food resources (e.g., *C. maculata* and *H. axyridis*), we are unable to speculate as to what extent these closely related species are constrained by their phylogenetic history to respond to a particular selection regime in a narrowly defined way. Furthermore, it is possible that a combination of abiotic (e.g., moisture and temperature) and biotic factors (i.e., food) interact to form microclimatic conditions that influence life cycles of these lady beetle species more than a single factor such as food fluctuations.

Modeling growth and development for predator species may also be useful in biological control programs. A conservation biological control program that is based on increased plant diversity usually leads to the occurrence of a higher diversity of insect herbivores within the agroecosystem, some of which may be alternative prey during aphid scarcity. Although female lady beetles tend to lay eggs in the vicinity of aphid aggregations that are likely to sustain larval development (Dixon, 2000), the terminal developmental position and high consumption rate of fourth instars likely subject them to localized aphid depletion. Such individuals are therefore more likely to benefit from alternative prey, leading to the development of larger and fitter beetles, as well as rapid build-up of the predator populations. Another potential use of modeling growth and development in predators like lady beetles is in identifying optimum habitat manipulation strategies for conservation biological control, where plants grown in an agroecosystem can be selected based on their potential to harbor nutrient resources that maximize predator growth and development. Modeling growth in lady beetles also has a potential utility in identifying optimum mass rearing strategies for augmentative biological control. Although augmentation biological control based on mass-reared lady beetles is not commonly practiced (mainly because of relatively high rearing costs) (Obrycki & Kring, 1998), different elements of mass rearing programs (e.g., rearing diet or temperature) can be evaluated based on their effects on growth function parameters like growth rate and asymptotic weight of fourth instars. Similarly, different genotypes, populations or species can be compared with respect to how long it takes for their development to reach critical weights, at which point cost-based adjustments in food supplies can be made.

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