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INITIATION OF METAMORPHOSIS IN THE PITCHER-PLANT MOSQUITO: EFFECTS OF LARVAL GROWTH HISTORY[1]

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Abstract. Variable growth and development were imposed on the pitcher-plant mosquito, *Wyeomyia smithii*, to test whether the initiation of metamorphosis was determined early in larval life or remained flexible throughout development. Like anurans, larval *W. smithii* are specialized for a suspension-feeding way of life and they metamorphose into dispersing, reproductive adults. We therefore used models originally formulated in an amphibian context to examine the effects of modified larval growth on the triggering of metamorphosis and then used our results to reexamine metamorphosis in amphibians. Response to enhanced and decreased food regimens showed that larval growth during the first two (of four) instars affected both the time to and mass at metamorphosis. The major effects of developmental inertia are prominent for about one to two instars and then abate, but do not necessarily disappear, with each succeeding instar. Despite evidence for developmental inertia in *W. smithii*, experiments transferring larvae from high food to starvation show that the physiological commitment to undergo metamorphosis does not take place until the last (fourth) instar and that nonzero growth during the last instar is required to trigger metamorphosis.

All amphibian models for the initiation of metamorphosis involve the effects of rates: developmental rate and past or present growth rates. In *W. smithii*, there is no primacy of developmental rate and low growth rate does not stimulate metamorphosis of larvae having attained the minimum mass required for metamorphosis. An insect-derived model involving the effects of size-specific growth increments is more consistent than any amphibian model with our results in *W. smithii*. Testing the reciprocal ability of the insect model to predict amphibian metamorphosis is not possible with current data, because no study has considered zero growth in amphibians to identify the transition from growth-dependent to growth-independent development leading to metamorphosis. This transition marks the irrevocable commitment of the organism to an ontogenetic niche shift from an aquatic larva to a terrestrial, dispersing, and reproducing adult. Identifying the proximal causes of this mechanism is fundamental to understanding how flexible growth and ontogeny of complex life cycles have adapted to variable larval environments.

Key words: Amphibia; complex life cycles; life history; metamorphosis; plasticity; Wyeomyia smithii.

INTRODUCTION

Herein we investigate the role of growth and development rates in determining the timing of metamorphosis in the pitcher-plant mosquito, *Wyeomyia smithii* (Coq.). We define growth as increase in size, development as change in form or recognizable stage, and rate as either growth increment or developmental change per unit time. The interplay between growth and development rates controls age and size at maturity and, consequently, makes a major contribution to fitness. Growth and development rates can be modified by the environment; the plastic expression of these traits over an environmental gradient comprises their "norm of reaction." When the expression of growth or development rates is determined by different genes in different environments (genotype by environment interaction), then the shape of the reaction norm is itself a heritable trait able to respond to selection (Via and Lande 1985). There are now an abundance of papers, theoretical and empirical, that concern the optimization of reaction norms in growing or stationary populations and in constant and varying environments (Roff 1992: 179–241, Stearns 1992: 136–149, Kawecki and Stearns 1993, Kozlowski 1993, Berrigan and Koella 1994). All of these papers assume either explicitly or implicitly that the reaction norms are subject to optimizing selection. These studies are therefore concerned primarily with the genetics and evolution that mold reaction norms. In the present study, we assume that the reaction norms for growth and development have been optimized by selection and we examine the proximal physical determinants of metamorphosis in complex life cycles. We apply models originally proposed in an amphibian context (Wilbur and Collins 1973, Smith-Gill and Berven 1979, Travis 1984, Alford and Harris 1988) to examine the roles of growth and development in

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triggering metamorphosis in *W. smithii*. We then compare our results with a mechanistic model in an insect endocrine context to examine further questions about the initiation of metamorphosis in amphibians.

Complex life cycles typically involve an ontogenetic niche shift that occurs at the time of metamorphosis. The premetamorphic stages are committed to growth; the postmetamorphic stages are dedicated to reproduction and dispersal (Istock 1967, Wassersug 1975, Wilbur 1980). The physiological initiation of metamorphosis therefore heralds the individual's change in form and primary activity. This change is not revokable; there are no frogs that revert to suspension-feeding tadpoles, no moths that revert to chewing caterpillars, and no mosquitoes that revert to aquatic filter-feeding larvae. For all organisms, the accumulation of mass (growth) requires time. Larger individuals generally (but not universally) acquire higher fitness through enhanced survivorship, fecundity, or further acquisition of mass so that, in general, bigger is better. At the same time, larval safety lies in the rapid exploitation of resources; if fitness is approximated by the Malthusian parameter, those genotypes or species realizing a net shorter generation time will tend to increase in the population (Lewontin 1965). Hence, faster is also generally better and, in the real world, "a variety of (different) time constraints in complex life cycles leads to optimal habitat-shift sizes that vary with time" (Rowe and Ludwig 1991).

As in the previous amphibian models, we assume explicitly the repeated empirical observation that there are upper and lower limits to size at metamorphosis. When larval growth rate is high, there is little advantage in extending a minimal development time to increase an already large mass; when larval growth rate is low, there is an increasing advantage to extending an already long development time to increase minimal mass (Istock 1967, Wilbur and Collins 1973, Wilbur 1980, Werner 1986, Reznick 1990). Consequently, when resources are scarce, organisms are expected to prolong the growth phase of their development but time constraints on realized fitness will impose metamorphosis at smaller than potential size (Rowe and Ludwig 1991). Exceptions to this generalization do occur, usually in ephemeral habitats (Taylor 1988, So and Dudgeon 1989, Goodbrod and Goff 1990, Newman 1994) or in environments where the risk of predation (Werner 1986) is extremely high. Most larvae develop in less extreme habitats, where variable larval resources lead to an inverse relationship between time to and size at metamorphosis (fish: McKenzie et al. 1983, Policansky 1983, Reznick 1990; amphibians: Wilbur and Collins 1973, Wilbur 1980; cladocerans: Lynch and Ennis 1983, Lynch 1989; and insects: Beddington et al. 1976, Scriber and Feeny 1979, Blakely 1981, Hard et al. 1989, Büns and Ratte 1991).

Important factors influencing the evolutionary optimal size–time at metamorphosis include risk of continuing as a larva, size-dependent opportunities for growth and survivorship of the juveniles and for growth (among fish and amphibians), survivorship, and reproduction of the adults (Wilbur and Collins 1973, Wilbur 1980, Werner 1986, Conover 1992, Berrigan and Koella 1994, Leips and Travis 1994, Newman 1994), and the adaptive significance of phenotypic plasticity in general (Blakely 1981, Stearns and Koella 1986, Gebhardt and Stearns 1988, Newman 1988, Reznick 1990, Blouin 1992). Three basic mechanistic arguments have been applied specifically to the timing of amphibian metamorphosis, but apply to all metamorphosing animals.

1) Wilbur and Collins (1973) argue that metamorphosis is a continually flexible trait that varies according to past and current growth. Metamorphosis is then initiated when size is above some evolutionary determined minimum, and current growth rate indicates poor future expectation of growth.

2) Smith-Gill and Berven (1979) argue that the timing of metamorphosis "is dependent primarily on differentiation (= development) rates, while body size at any particular state of metamorphosis is a function of both growth and differentiation rates." Their arguments have served to increase awareness that development may have inertia and that development rate may contribute to the timing of metamorphosis.

3) Travis (1984) argues that both time to and size at metamorphosis are plastic traits but that the development rate, and therefore development time, is set early in larval life. Size at metamorphosis is then simply a consequence of nutrient conditions later in life. Travis (1984) fed individual *Hyla gratiosa* a high and a low food ration and, in a third experiment, simulated competitive release by supplementing a low food regimen. He observed that size at metamorphosis but not length of the larval period responded to competitive release: "Developmental rate appears to have been set early in the larval period, and subsequent growth can be explained by postulating body size to be merely a highly plastic trait."

Alford and Harris (1988) identified the fundamental difference between the flexible Wilbur–Collins model and the more deterministic Travis model as being the proportion of the larval period during which individuals can respond to changes in growth rate to alter development rate. They pointed out that neither Wilbur and Collins (1973) nor Travis (1984) had examined the effects of diminishing resources over time as might occur in real larval habitats. Consequently, Alford and Harris (1988) exposed individual *Bufo woodhousei fowleri* to constant low food, constant high food, or changing food levels. For the latter, growth was enhanced by switching developing individuals from low to high food at three different times during development, or growth was depressed by switching developing individuals from high to low food at three different times during development. Their results indi-

cated that development rate responded to alterations in resource availability throughout the larval period. Subsequently, Leips and Travis (1994) used the same approach as Alford and Harris (1988) to compare developmental plasticity in *Hyla gratiosa* and *H. cinerea*. Their results indicated that the switch from resource-dependent to resource-independent rate of development was made earlier in larval development either than predicted by the Wilbur–Collins model or than was determined by Alford and Harris (1988). Finally, Reznick (1990) used the Alford–Harris approach to examine flexibility in the development of the guppy *Poecilia reticulata*. Reznick demonstrated that both age and size at which metamorphosis was initiated, as well as completed, responded to food level up until the point of initiation. Hence, as Alford and Harris (1988) proposed, the transition from resource-dependent to resource-independent rate of development may occur at species-specific times or stages of development.

To our knowledge, Alford and Harris' (1988) approach has not been used to examine the effects of larval growth history on the timing of and size at metamorphosis in any insect. We used this approach to evaluate developmental flexibility and developmental determinism in the pitcher-plant mosquito, *Wyeomyia smithii*.

Wyeomyia smithii *as an analogue of amphibian development*

Individuals of *Wyeomyia smithii* complete their entire preadult development within water-filled leaves of the insectivorous pitcher plant, *Sarracenia purpurea* L. Larval growth and development depend upon prey captured by their host leaf (Bradshaw 1983, Farkas and Brust 1985, Bradshaw and Holzapfel 1986, Heard 1994). Per capita resources determine larval survivorship, time to metamorphosis (pupation), and size at metamorphosis (Istock et al. 1975, 1976, Bradshaw and Holzapfel 1990, 1992). Size at metamorphosis (pupal mass) is the main determinant of female longevity and fecundity (Bradshaw and Holzapfel 1992). Egg size does not vary with size at metamorphosis or adult age so that reproductive success of females is a direct consequence of their adult size (Bradshaw et al. 1993). Prey capture by host leaves is not constant. When leaves open, they are soft and do not attract prey. Upon hardening, the young leaves are maximally effective at capturing prey and prey capture then declines exponentially with time (Bradshaw 1983, Bradshaw and Holzapfel 1986). Mosquitoes oviposit in the youngest leaves and complete their development in synchrony with maximum prey capture by their host leaf. The ecology of *W. smithii* development in leaves of *S. purpurea* is then analogous to that of amphibian development in bodies of water where the persistence of the habitat or resource has a duration approximating that of individual larval development. Like anurans (Wassersug 1975), larval *W. smithii* are specialized for a suspension-feeding way of life and they metamorphose into dispersing, reproductive adults. Development of adult structures in mosquitoes begins early in larval development (White 1961, 1963) and increases in both the number of different structures and rate of development as the larval instars progress (Christophers 1960: 242, 345–351, Vongtangswad and Trpis 1980). Similarly, the differentiation of adult structures begins early in amphibian larval life and continues up until the time of metamorphosis (Taylor and Kollros 1946, Gosner 1960). With an internal skeleton, amphibian larvae and adults are capable of continuous growth and there may be little correlation between size at metamorphosis and size at reproduction in anurans (Werner 1986). Insects have a rigid exoskeleton but much of the body surface of holometabolous insects is unsclerotized, placing little limit on larval growth (Nijhout 1981). In adult insects, the rigid exoskeleton imposes a tight correlation between size at metamorphosis and adult size and, consequently, size-dependent survivorship and reproduction (Blakely 1981, Bradshaw and Holzapfel 1992). Hence, in both anurans and holometabolous insects, metamorphosis involves an irrevocable niche shift but the life history consequences of this niche shift are more rigidly determined in insects than in amphibians.

Determinate vs. facultative metamorphosis

Following Alford and Harris (1988), we tested for the effects of altered growth on the size at and time to metamorphosis. We altered growth rate by enhancing or depressing growth through transfer of larvae from a low to a high food regimen and vice versa. The predictions of these treatments are illustrated in Fig. 1. In Fig. 1, we use straight lines to illustrate growth trajectories. In actuality, these trajectories may be curvilinear with the precise shape depending upon the availability of food and the plasticity of mosquito growth and development.

The responses to continual high food (HHHH) and continual low food (LLLL) set the respective boundary conditions for the expected minimum development time (vertical dashed line) and minimum mass at pupation (horizontal dashed line). If development rate responds continuously to changes in growth rate enforced through decreased (Fig. 1A, B) or increased (Fig. 1C, D) food availability, then mass–time combinations should fall approximately on a straight line connecting mass–time combinations of consistently high and low food (Fig. 1A, C). If developmental rate is fixed early in development, then decreasing food during development (Fig. 1B) will retard growth but not development. Individuals will exceed the minimum mass necessary to trigger metamorphosis and will pupate at low mass. If development time is fixed early in development, then increasing food during development (Fig. 1D) will accelerate growth but not development and larvae will acquire excess mass before metamor-

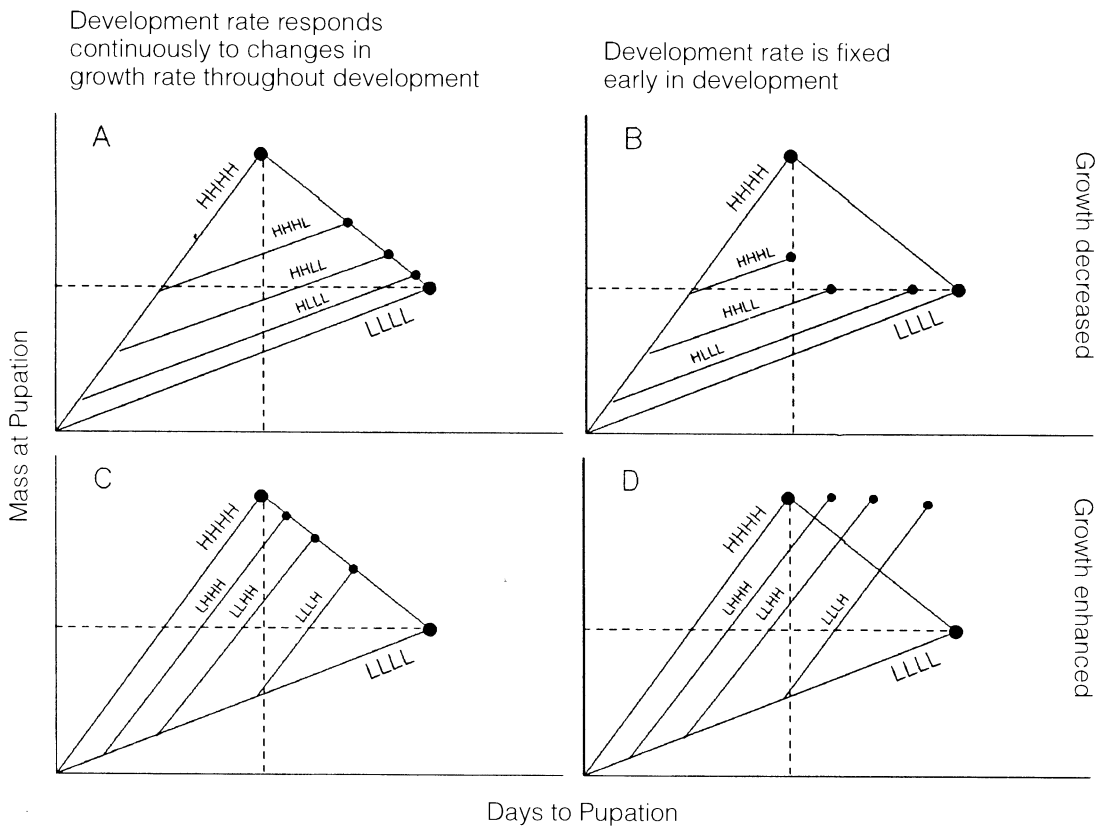

FIG. 1. Predicted growth trajectories of *W. smithii* in response to varying food during development. Each line is labelled according to its instar-specific food level during each of four consecutive instars; for example, HHLL represents high food during the first and second instars followed by low food during the third and fourth instars.

phosis is triggered. The basic predictions then relate to the plot of mass–time under different food regimens: (1) If developmental rate responds continuously to changes in growth rate throughout development, mass–times should plot as a straight line between the extreme points; or, the mass–times resulting from decreased growth should at least fall on the same line as mass–times resulting from increased growth. (2) If developmental rate is fixed early in development, mass–times should plot as a concave line when growth is decreased and a convex line when growth is enhanced; or, the mass–times resulting from decreased growth should fall below the line resulting from increased growth.

MATERIALS AND METHODS

Larvae of *Wyeomyia smithii* were collected from a bog near Forge Village, Massachusetts (42.5° N) and reared in the laboratory for ≈14 generations before the start of experiments. The laboratory population had been maintained as an unselected line with alternating diapause and developing generations as described in Hard et al. (1993). We maintained populations and ran experiments in a controlled environment room programmed with a smooth, sine-wave thermoperiod (mean = 21°C) with daily high and low of 28° and 13°C. Mosquitoes received constant 85% relative humidity and an L:D = 17:7 photoperiod that led the thermoperiod by 3 h plus two 0.5-h transitory twilights. These conditions provided a long-day, diapause-averting environment to avoid a food by photoperiod interaction (Istock et al. 1975).

Larval food consisted of 3:1 mix by volume of ground guinea-pig chow and freeze-dried brine shrimp. We ground the food, sifted it through an 0.25-mm mesh, mixed it, and reground it in bulk prior to the start of experiments and maintained it as a single dry source of food throughout the duration of the study. We prepared stock food suspension by adding the specified amount (weighed to the nearest 0.01 g) to 1.00 L distilled water containing Photo-Flo 200 (Eastman Kodak, Rochester, New York) to act as a wetting agent. We maintained the stock suspension in an ordinary refrigerator ≤10 d until needed for experiments. Photo-Flo itself, or the bacteria that feed on it, acts as a nutrient source for *W. smithii* (W. E. Bradshaw and P. Fernando, *unpublished data*) and 50–100 μL/L Photo-Flo alone will support individual mosquito development through the first instar. Consequently, we reared first instars in 100 μL/L Photo-Flo with (high) or without (low) par-

TABLE 1. High, low, enhanced, and decreased food regime.

A) Food provided each instar (mg/larva) in 10 μL/L Photo-Flo*

Instar	Low food	High food
I	0.00	0.50
II	0.05	0.50
III	0.30	1.50
IV	2.50	3.00

B) Total food (mg) provided by each regimen and treatment sample sizes

Regimen	Food	N_0†	♂‡	♀
LLLL§	2.85	288	16	8
LLLH	3.35	192	10	8
HLLL	3.35	96	41	27
HHLL	3.80	96	43	40
LLHH	4.55	192	19	18
HHHL	5.00	96	32	33
LHHH	5.00	192	38	37
HHHH	5.50	96	30	37

* Except for I: in 100 mL/L Photo-Flo.
† Initial number of first instars.
‡ Number of each sex that survived to pupation.
§ Food regime, high or low, is listed for first through fourth instar as H or L, respectively.

ticulate food and all subsequent instars on particulate food suspended in 10 μL/L Photo-Flo. We ran a series of preliminary experiments to determine (1) the highest food levels for each consecutive instar that would sustain maximum growth and development without producing toxic conditions due to rotting food and (2) the lowest food level that would just sustain development through each consecutive instar. These food levels became the high and low food treatments for each instar (Table 1A). During experiments, we added food once on the first day of each instar, commencing with the day of hatch and continuing until the day of ecdysis of the fourth instar. We mixed the food suspension at moderate speed in a kitchen food blender and, while the blender was operating, we decanted 1 mL of suspension in a volumetric pipette and added the suspension to larval containers. All experiments were run with individual larvae reared in 3 mL wells of 24-well tissue-culture plates. We fed the first instar 1.0 mL of food suspension on the day of hatch. On the day of ecdysis, we transferred the second instar to a new well and provided 1.0 mL of new food suspension. On the day of ecdysis to the third instar, we added a second 1.0 mL of food suspension and on the day of ecdysis to the fourth instar, we added the final 1.0 mL of food suspension. Hence, we reared larvae in 1.0, 1.0, 2.0, and 3.0 mL during the first to fourth instars, respectively. We checked larvae for development daily throughout the duration of the experiment. Upon pupal ecdysis, we sexed, blotted, and weighed the pupae wet to the nearest 0.01 mg on a microbalance. We recorded instar durations, days to pupation, sex, and pupal mass for each individual.

Rather than feeding larvae on a time- or growth-dependent basis, we fed them on a development-dependent basis: once on the first day of each instar. We established the boundary conditions from continual high and low food regimens (HHHH and LLLL in the first to fourth instars, respectively). Treatments analogous to Alford and Harris' (1988) enhanced food and decreased food consisted of low to high transitions on the first day of the second, third, or fourth instar (LHHH, LLHH, or LLLH) or high to low transitions on the first day of the second, third, or fourth instar (HLLL, HHLL, or HHHL), respectively. Early instar survivorship was lower in treatments receiving low food levels in the first instar and initial sample sizes were higher for these treatments (Table 1B). We observed individual larvae until they ecdysed to the next instar, died, or had spent 60 d in a given instar. We scored the latter larvae as nondevelopers.

To determine whether high food for the first three instars alone was sufficient to support metamorphosis, we provided 48 larvae with high food for the first three instars. On the day of ecdysis to the fourth instar, we weighed each larva wet and placed it in 3 mL of 10 μL/L Photo-Flo but with no other food (HHH0). We observed all larvae until they developed or died.

All statistical analyses used procedures in SAS (SAS 1985), using Type III estimable functions for unbalanced designs. Positive skew was corrected by $\log_e$ transformation. $\log_e$ transformation was also used to correct for a positive correlation between the standard deviations and means. If the preliminary ANOVA indicated significant treatment effects, we used Tukey–Kramer pairwise comparisons to identify significant ($P < 0.05$) differences between specific means because of unequal sample sizes (Day and Quinn 1989).

RESULTS

Residual effects of decreased or enhanced growth

Fig. 2 shows the growth and development of *W. smithii* when subjected to low, high, decreased, or enhanced food regimens. The mass–time responses to consistent high food (HHHH) and consistent low food (LLLL) provide the end points for the linear expectation for plots of decreased (HxxL) or enhanced(LxxH) food regimens. The response to decreased food was concave in both sexes: two mean mass–times (HHLL and HLLL in ♂♂; HHHL and HHLL in ♀♀) fell >2 SE below the linear expectation and no mean fell above it. The response to increased food was convex in both sexes: two mean mass–times (LHHH and LLHH in ♂♂ and ♀♀) fell >2 SE above the linear expectation and no mean fell below it.

The differences in response to decreased and enhanced growth could reflect differences in total food made available to developing larvae (Table 1B). Previous studies on amphibians provided food continually at high or low rates; we provided food in finite amounts and were able to calculate mass yield of pupae (mil-

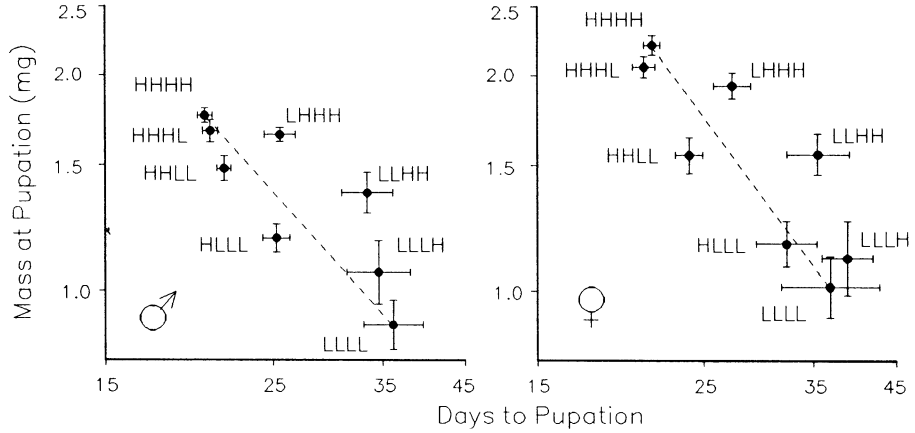

FIG. 2. Size at and time to pupation of *W. smithii* larvae reared at high (HHHH), low (LLLL), enhanced (LxxH), or decreased (HxxL) food levels. The error bars show ±2 SE. Labels designate high (H) or low (L) food levels (Table 1) during the first through fourth instars. The dashed line shows the linear expectation connecting continuous high and low food levels.

ligrams pupal mass per milligram total food). One-way ANOVA showed that the yield of pupal mass was affected by treatment in both males ($F_{5, 177} = 17.67$; $P < 0.001$) and females ($F_{5, 156} = 8.70$; $P < 0.001$) (Fig. 3). Among males, high food in the first two instars (HHLL) resulted in greater yield than any other treatment followed by high food in the first instar (HLLL) which produced a greater yield than low food during the first two instars (LLHH). Among females, high food in the first and second (HHLL) or first through third (HHHL) instars sustained a higher yield than high food in the first instar alone (HLLL) or low food in the first and second (LLHH) or first through third (LLLH) instars. When significant differences in mass yield occurred, high food early in larval life tended to sustain a higher yield than low food early in larval life.

When combined with the data in Fig. 2, these results show that larval growth early in development affected both the time to and mass at metamorphosis.

Because we provided a fixed amount of food for each instar, we are able to examine the instar-specific response to decreased or enhanced food. The plots in Fig. 4 show the mean durations of each instar and sex under each food regimen. These plots do not reveal anything about growth, but they do reflect development rates (inverse of duration) for each instar. Log-transformed durations of each instar and sex were subjected to one-way ANOVA across treatments within decreased or enhanced growth. Of the 16 ANOVAs, 15 indicated significant ($P < 0.05$) treatment effects. The data in Fig. 4 reveal at least four patterns.

1) Food level had no consistent effect on the duration of the first instar.

2) Compared with low food (LLLL), high food in the first instar (HLLL) shortened the duration of subsequent instars, most notably the second instar, but the effects of low food persisted even through the fourth instar in males (Fig. 4A). Among females, the duration

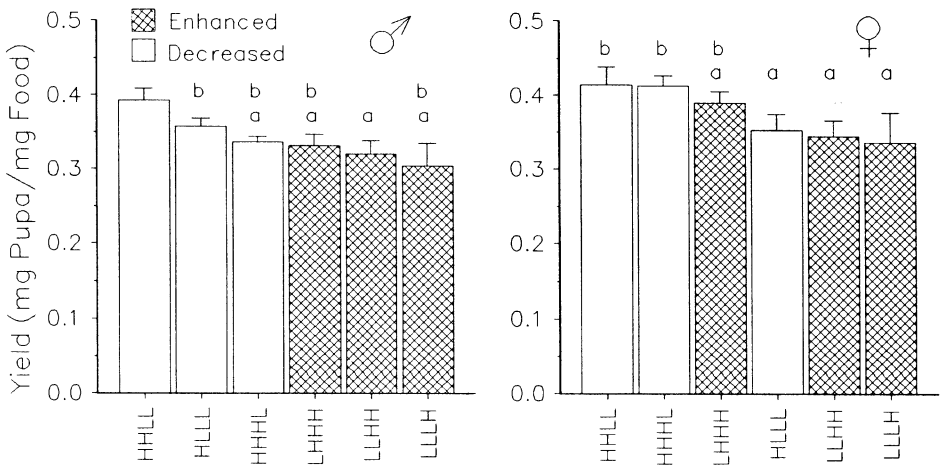

FIG. 3. Yield of pupal mass (mean ±2 SE mg pupal mass per mg total food) in treatments where food was enhanced (LxxH) or decreased (HxxL). Bars with the same letter above them are not significantly different (Tukey-Kramer pairwise comparisons: $P < 0.05$).

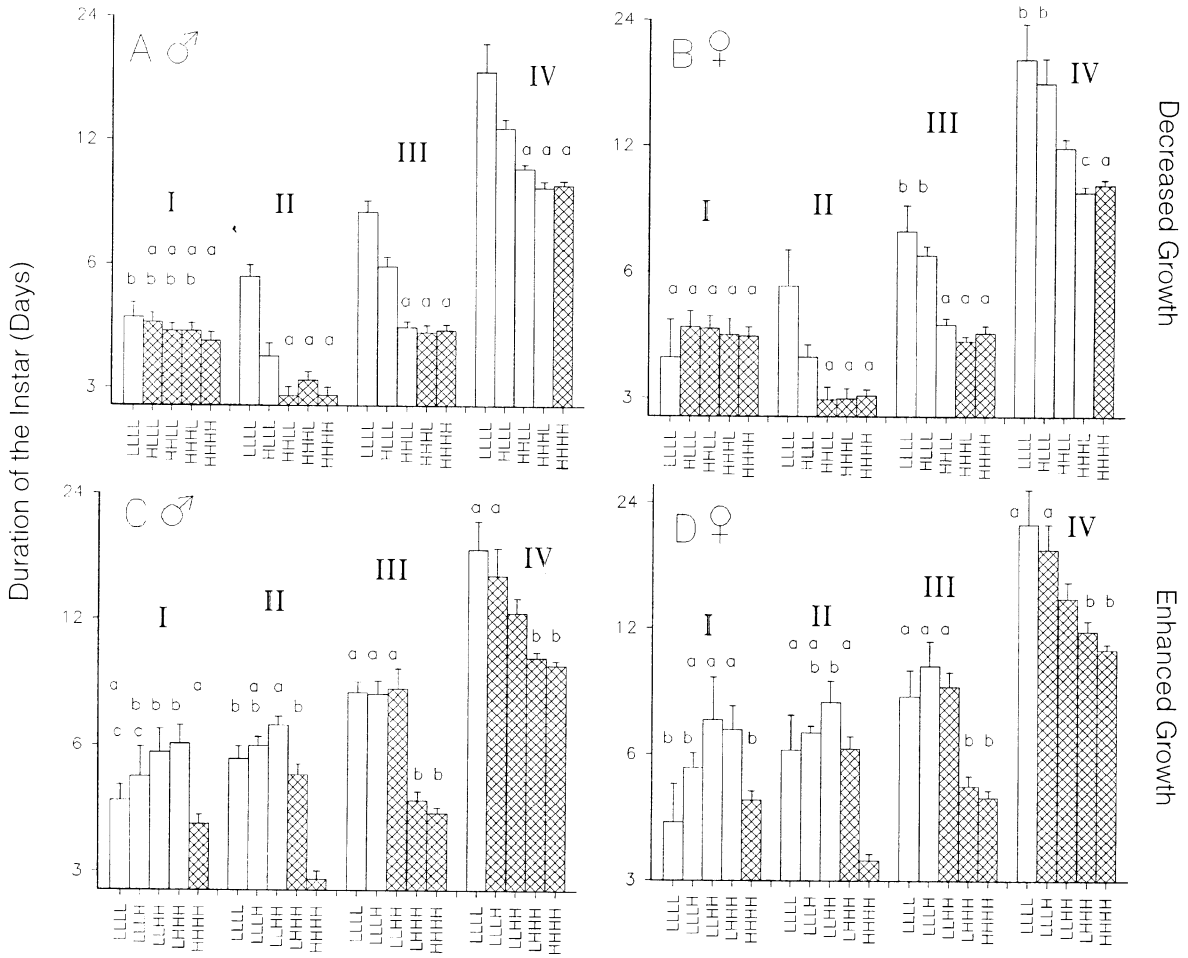

FIG. 4. Duration of each instar [mean ± 2 SE log(duration)] when provided various food regimens (shown as in Fig. 1). Roman numerals designate the instar whose duration is plotted. Open bars indicate that low food was experienced during the designated instar; cross-hatched bars indicate that high food was experienced during the designated instar. Bars with the same letter above them are not significantly different (Tukey–Kramer pairwise comparisons: $P < 0.05$).

of the fourth instar was shorter if the first two instars had received high food (HHLL) than if the first instar had received low (LLLL) or high (HLLL) food. These results show that the effects of high nutrition early in larval life persisted throughout male development regardless of subsequent resource levels but could be overcome by consistently low nutrition late in female development.

3) Compared with high food (HHHH), low food in the first instar (LHHH) lengthened the duration of the subsequent second but not third or fourth instar in both sexes (Fig. 4C, D); low food in the first two instars (LLHH) lengthened the duration of both the subsequent third and fourth instars. These results show that the retarding effects of low nutrition during the first instar but not both the first and second instars can be overcome by consistently high nutrition later in the development of either sex.

4) The duration of fourth instars experiencing high food was more prolonged if the preceding third instar had received low (IV: LLLH) than high (IV: LLHH) food (Fig. 4C, D). In females but not males, the duration of fourth instars experiencing low food was shortened if the preceding third instar had received high (IV: HHHL) rather than low (IV: HHLL) food (Fig. 4A, B). These results show that variation in food level in the penultimate instar alone was capable of affecting variable development time during the last instar and, consequently, altering the timing of female metamorphosis.

Developmental inertia

When a separate set of larvae was reared under the high food regimen for the first three instars and then completely deprived of food (HHH0), not one of the 46 larvae pupated. Since the sex of these starved larvae could not be ascertained, we compared their mass on day zero of the fourth instar with pupal mass on the low food treatment (LLLL) after pooling male and female pupae. In this case, the mean mass ± 2 SE of day

zero fourth instars from the high food regimen (1.04 ± 0.04 mg) was higher than the mean mass of pupae from the low food regimen (0.95 ± 0.06 mg)(ANOVA: $F_{1,68} = 7.73$; $P < 0.01$).

Discussion

The response of *W. smithii* to decreased or enhanced food (Fig. 2) shows that there is developmental inertia in this species. The major effects of developmental inertia are prominent for about one to two instars and then abate, but do not necessarily disappear, with each succeeding instar (Fig. 4). Although developmental inertia affects the duration of individual instars, developmental inertia alone does not determine the timing of metamorphosis. The availability of abundant food early in larval life promotes more rapid development and higher mass at metamorphosis even when total food availability is held constant (Fig. 3). Larvae exposed to the highest food regimen achieved the highest developmental rates through the first three instars (HHHL and HHHH in Fig. 4A, B) and, upon entering the fourth instar should have the highest developmental inertia. In a separate experiment, larvae fed the same high food regimen through the first three instars, and then starved, had attained by day zero of the fourth instar greater mass than required to sustain poorly fed larvae through to metamorphosis. Still, no larva pupated after having been starved in the fourth instar, despite high previous food. Hence, high developmental inertia and the attainment of minimum pupal mass by fourth instars are not sufficient alone or in combination to trigger metamorphosis. However, even low food levels during the fourth instar promote rapid completion of that instar (HHHL in Fig. 4A, B) and the attainment of near-maximum mass at pupation (HHHL in Fig. 2). We conclude that the physiological commitment to undergo metamorphosis does not take place until the last instar and that nonzero growth during the last instar is required to trigger metamorphosis.

To what extent can models of amphibian metamorphosis be applied to *W. smithii* and vice versa? Despite the differences in response to enhanced and decreased growth (Fig. 2), there is flexible growth and development in *W. smithii* (Figs. 3–4) and metamorphosis itself requires growth during the last instar. This pattern of flexible growth and development in *W. smithii* is more consistent with the Wilbur and Collins (1973) concept of flexible metamorphosis than with the more deterministic concepts of Travis (1984) or Smith-Gill and Berven (1979). Developmental inertia does exist in *W. smithii* but its ability to sustain metamorphosis in the absence of growth is restricted to some time during the last instar. There is no primacy of developmental rate (Smith-Gill and Berven 1979) in the initiation of metamorphosis in *W. smithii*.

Leips and Travis (1994) propose a hybrid concept where allocation of incremental resources changes from primarily development early in larval life to primarily growth later in larval life. Once larvae pass a "certain temporal boundary . . . the development trajectory is fixed, and . . . changes in food levels after that point affect only final body size." This temporal boundary occurs during the last instar in Lepidoptera (Nijhout 1975, but see Allegret 1964), Hemiptera (Wigglesworth 1934, Nijhout 1979, Blakely 1981), and Diptera (Robertson 1963, Lounibos 1979, this study) and exists at a clearly defined morphological landmark in guppies (Reznick 1990). In insects, the physiological event demarcating the transition from flexible to determinate development is probably the activation (or release from inhibition) of neurosecretory cells in the brain to produce a peptide hormone (PTTH = prothoracicotropic hormone) that, in turn, stimulates the prothoracic glands to produce ecdysteroid, the immediate cause of metamorphosis (Nijhout 1981, Raabe 1989, Riddiford 1994). The interplay between the brain and a third hormone (JH = juvenile hormone) varies among insects (Riddiford 1994); but in all insects, once the prothoracic glands of the final instar have been stimulated to produce ecdysteroid, the individual is committed to metamorphosis. Subsequent development can proceed without further growth but additional growth is possible if the nutritional resources are available (Robertson 1963, Lounibos 1979). Although Leips and Travis' (1994) "temporal boundary" is an endocrine event in insects, their verbal model is, like the Wilbur–Collins model, more consistent with the initiation of metamorphosis in *W. smithii* than with the deterministic models of Travis (1984) or Smith-Gill and Berven (1979).

The picture that is emerging is that in amphibians as well as in arthropods, there exists a hormonally mediated transition between flexible, growth-dependent development and deterministic, growth-independent development. This transition signals an irrevocable commitment to metamorphosis. Also common to amphibians and insects is the concept of a lower size threshold below which metamorphosis is never initiated and an upper size threshold above which metamorphosis is always initiated. Most of the differences in viewpoints involve the initiation of metamorphosis at sizes intermediate between these upper and lower thresholds.

At intermediate sizes, the explicit Wilbur–Collins amphibian model (Fig. 5A) argues that slowing of the growth rate below a current mass-specific threshold [$dM/dt < g(M)$] triggers metamorphosis. We have used Wilbur and Collins' mechanistic diagram (Fig. 5A) relating to amphibians and the verbal description of Nijhout (1981) to construct an analogous mechanistic insect model (Fig. 5B). The insect model would argue that attaining some percentage increment in mass above a previous landmark triggers metamorphosis rather than a change in growth rate. This landmark mass (M_0) is established earlier at the start of the fourth instar and when the increment in mass ($M - M_0$) exceeds some

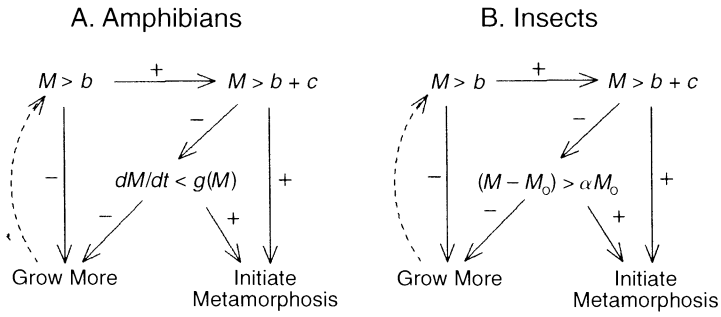

FIG. 5. Hypothetical control processes determining the stimulation of neurohormone processes that initiate metamorphosis. Arrows indicate direction of the process depending upon whether the inequality is true (+) or false (−). Constants b and (b + c) are the species- or genotype-specific lower and upper limits, respectively, of larval mass M at which metamorphosis will be initiated. Between these limits, metamorphosis will be initiated in amphibians (Wilbur and Collins 1973) only if recent growth rate dM/dt is less than $g(M)$, some function of current mass, and in insects (based on Nijhout 1981) only if current mass M exceeds the size M_0 at the start of the metamorphosis-determining stage by some percentage a of M_0.

fraction a of M_0, i.e., $(M - M_0) > aM_0$, metamorphosis is initiated. The specific predictions of the Wilbur–Collins model do not account for the initiation of metamorphosis in *W. smithii* where attainment of minimum mass ($M > b$) is not sufficient to sustain metamorphosis even though further growth is prevented by starvation and, therefore, $dM/dt < gM$. These same results are consistent with the insect model because, upon molting to the fourth instar, $M > b$ but $(M - M_0) < aM_0$ and metamorphosis should be delayed. Previous experiments concerning the initiation of metamorphosis in amphibians were not designed to test for consistency with such an insect model but such tests raise questions for future research.

We conclude that the major remaining questions concerning the initiation of metamorphosis in complex life cycles are not specific to frogs, mosquitoes, or fish. These questions all revolve around the transition from growth-dependent to growth-independent development because it is at this point that the individual becomes irrevocably committed to metamorphosis. Is there a point of no return in anuran development when the individual is committed to and capable of metamorphosis to a functional frog in the absence of food (zero growth)? Does this transition occur at a specific developmental stage? Development may be monitored by progressive stages in amphibians (Taylor and Kollros 1946, Gosner 1960) and by internal growth and development of imaginal tissue in insects (Christophers 1960: 242, 345–351, Vongtangswad and Trpis 1980, Neumann 1986). When animals are transferred from food levels sustaining growth and development to starvation, does development halt at a specific internal stage? The imaginal discs in the marine midge *Clunio marinus* cease developing at the same size and stage for the externally cued hibernal diapause as they do for the internally cued circasemilunar eclosion rhythm (Neumann 1986). Would amphibians, if transferred from ample food to starvation also cease developing at a consistent species- or genotype-specific stage? Would this stage be independent of recent growth history or larval size? Nijhout (1981) reported that the critical size for the initiation of metamorphosis in the tobacco hornworm, *Manduca sexta*, was dependent upon the size of the hard parts (head) of the final instar. Larger last instars required the attainment of higher larval mass before making the transition from growth-dependent to growth-independent development. In the milkweed bug, *Oncopeltus fasciatus*, metamorphosis is initiated by critical stretch of abdominal nerve clusters that are "calibrated" at the time of prior larval ecdysis and measure a critical percentage increase in larval length (Nijhout 1979). The important point is that flexible size–time initiation of metamorphosis involves a factor or structure whose dimension had been established at a previous time. Do such reference landmarks also exist in amphibian development? These questions need to be answered before the commonality among animals with complex life cycles can be determined. More importantly, the answers to these sorts of questions will be fundamental to understanding the interplay between the individual's evolutionary and genetic background and its immediate environment, i.e., how flexible growth and ontogeny of complex life cycles have adapted to variable larval environments.

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