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Developmental Arrest During Larval Life and Life-Span Extension in a Marine Mollusc

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The length of larval life in the nudibranch *Phestilla sibogae* is determined by a chance encounter with a specific metamorphic stimulus associated with the post-larval benthic habitat. A developmental hiatus begins at the onset of larval metamorphic competence and ends at metamorphosis; aging is suspended during this hiatus. Because the duration of post-larval life is unaffected by the duration of larval life, total life-span varies with the length of the larval period. Developmental control of the timing of expression of life-history stages is an important factor regulating aging and senescence in animals with complex life cycles.

ORGANISMS WITH COMPLEX LIFE cycles undergo a metamorphic transition that is prerequisite for post-larval growth and development (*1*). For many marine invertebrate species with planktonic larvae, metamorphosis is induced by environmental cues associated with the benthic habitat, and this helps place juveniles in appropriate post-larval settings (*2*). The reliance of metamorphic induction on chance encounters with environmental cues imparts variation to the duration of the larval period [that is, the time between hatching and metamorphosis (*3*)] and, consequently, to the temporal age at which post-larval life begins. The effects of larval duration on post-larval life reveals developmental relations among life-history stages that influence aging and senescence and the evolution of complex life cycles in benthic marine invertebrates.

Evolutionary theories of life-span determination (*4*) propose that deleterious genetic effects expressed late in life result in aging and senescence. These effects may arise pleiotropically from genes that are favored early in life or from the accumulation of mutations in genes that are expressed during post-reproductive life. From these theories it follows that an extension of the larval period should extend life-span by delaying subsequent life-history stages in which these deleterious genetic effects are expressed.

The effects of extended larval life on durations of the juvenile (metamorphosis to first egg laying) and adult (first egg laying to death) periods and on reproductive output were assessed in the aeolid nudibranch *Phestilla sibogae*. Veliger larvae from each of 35 egg masses hatched 5 days after fertilization and were fed phytoplankton freely for the duration of the larval period (*5*). Larvae from each egg mass were sampled 7, 14, 21, and 28 days after hatching. A subset of larvae from each sample was induced to metamorphose by exposure to a fragment of the nudibranch's coral prey (*5*). The remaining sampled larvae and some of the 24-hour post-metamorphic juveniles were used for weight determinations (*6*). Post-larval life was followed in juveniles and adults raised as isolated pairs on their coral prey (*7*). At first reproduction, some adult pairs were sacrificed for weight determinations, and egg masses produced by the remaining pairs were collected daily and weighed (*6*).

Larval weights remained the same throughout all larval periods, and the weights of 24-hour-old juveniles were not significantly affected by the duration of the larval period (Table 1). The maximum experimental larval period, 28 days, represented a more than threefold increase over the minimum developmental time needed to attain the morphology and physiology necessary for successful metamorphosis (that is, 5 days of embryonic development plus 3 days of precompetent larval development). In spite of this great increase in larval period, durations of the juvenile and adult periods were essentially unchanged (Fig. 1). Consequently, both the average life-span and the maximum achievable life-span (oldest individual) increased by the number of days that the larval period was extended. Extending the larval period to 28 days increased average life-span 20 days (26%) over that obtained when an individual metamorphosed soon after it achieved metamorphic competence (that is, 7-day larval period).

The data presented here demonstrate that extension of the planktotrophic larval period beyond the time required to attain metamorphic competence does not significantly affect the duration of post-larval life. Because the timing of expression of post-larval life-history stages is influenced by development (*8*), a hiatus in development must occur during the competence phase of the larval period in order to account for the unchanged post-larval duration. This hiatus begins with the onset of metamorphic competence and ends with the induction of metamorphosis. That a developmental hiatus does occur is further supported by two additional observations: larvae of *P. sibogae* show no signs of morphological differentiation or growth during the competence phase of the larval period (*9*); and the metamorphic transition from larva to juvenile requires induction by a chemical signal from the nudibranch's coral prey. Until this signal is encountered, larvae do not undergo juvenile development (*10*).

Delay or arrest of larval development has been experimentally induced in a variety of organisms by changes in nutrition and temperature regimes (*11*). However, these experimental regimes invariably affect metabolism as well (*12*), complicating the assessment of developmental effects on post-larval life history. Insect diapause also alters developmental and metabolic rates in response to seasonal environmental changes (*13*). In contrast, the experiments on *P. sibogae* were conducted at normal (ambient seawater)

Table 1. Mean (± SE) ash-free dry weights of larvae, 24-hour-old juveniles, and adults at first reproduction for individuals that were held for increasing durations as larvae. Sample sizes (n) are the number of weight determinations used to estimate larval or juvenile weights (*10*), and the number of adults used to estimate adult weight. Regressions were not statistically significant for larval, juvenile, or adult weights.

Larval period	Larval weight (μg)	Juvenile weight (μg)	Adult weight (mg)
At hatching	0.76 ± 0.02 (n = 6)		
7 days	0.70 ± 0.03 (n = 11)	0.62 ± 0.05 (n = 5)	32.8 ± 1.4 (n = 20)
14 days	0.72 ± 0.04 (n = 9)	0.67 ± 0.06 (n = 5)	33.4 ± 1.2 (n = 20)
21 days	0.77 ± 0.03 (n = 4)	0.66 ± 0.04 (n = 5)	30.7 ± 1.0 (n = 37)
28 days	0.73 ± 0.04 (n = 5)	0.63 ± 0.06 (n = 4)	31.6 ± 1.2 (n = 33)

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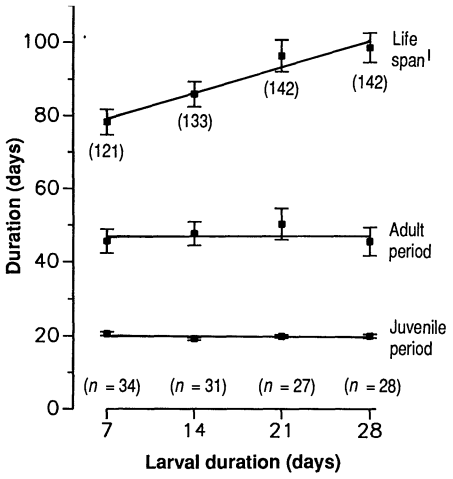

Fig. 1. Mean durations (± SE) of juvenile periods, adult periods, and life-spans for individuals that were held for increasing durations as larvae. Life-span is defined as the summation of larval-, juvenile-, and adult-period durations plus 5 days of embryonic development preceding hatching. Maximum achievable life-spans are given below the life-span data. Regressions were not statistically significant for juvenile or adult durations. Regression of life-span was significant ($F = 18.96$, $r^2 = 0.145$; slope = 1.03 ± 0.24, $P < 0.0001$).

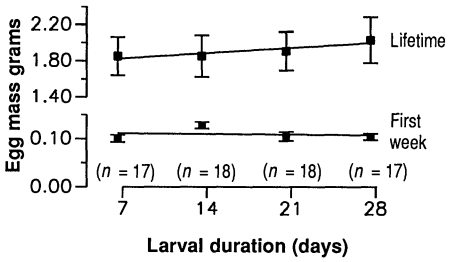

Fig. 2. Mean reproductive output (± SE) during the first week of reproduction and over the lifetime for individuals that were held for increasing durations as larvae. Regressions were not statistically significant for first-week or lifetime reproduction.

developmental temperatures, and larvae continued to feed actively through the larval period (Table 1).

Published accounts of the effects of an extended larval period on the post-larval life of other marine invertebrates have been limited to assessments of juvenile growth and survival. Extending larval duration did not affect either of these parameters in a planktotrophic prosobranch mollusc, but variably increased or decreased juvenile growth and survival in three planktotrophic echinoderm species (*14*). It should be noted that not all marine invertebrate larvae halt development upon attainment of metamorphic competence; larvae of some gastropods continue to grow and develop after the onset of competence (*15*). In *Drosophila* and *Caenorhabditis*, when development was slowed or halted, the larval period increased, the adult period remained unchanged, and life span increased, as observed in *P. sibogae* (*16*).

Our results and the work discussed above support evolutionary theories of life-span determination by demonstrating that a hiatus in development delays senescence by delaying the onset of the life-history stages in which senescence occurs. However, none of these data distinguishes between mutation accumulation and antagonistic pleiotropy as causes of aging (*4*). By either mechanism, a suspension of developmental schedule during larval life will delay the onset of the post-larval stages during which deleterious genetic effects are expressed.

While our experimental data for *Phestilla sibogae* indicate that the onset of adulthood is developmentally determined, they do not necessarily support the theory of "developmentally programmed aging" (*17*) in which senescence is expressed as a process similar to that which characterizes development in earlier life-history stages. Under stable conditions, the "programmed" nature of pre-adult development (other than the competent larval period) results in low variation in the duration of early life-history stages. Using coefficients of variation (CV) to compare relative variation in the duration of life-history stages (*18*), we found that values for the precompetent (embryonic and larval development necessary to attain metamorphic competence; $CV = 15$) and juvenile ($CV = 12$) periods were about equal. In contrast, variation in duration from first egg laying to the onset of senescence (defined as a sharp drop followed by a continual decline in total daily egg mass production; $CV = 40$) and adult period ($CV = 41$) were much greater, suggesting that the factors influencing the duration of adult life are different from those influencing the duration of pre-adult stages.

When life-span in *P. sibogae* was extended during the larval period, adult weight at first reproduction (Table 1) and reproductive output (Fig. 2) were not significantly affected. Similar results were observed in *Caenorhabditis elegans* (*16*). An increase in life-span through a suspension of developmental schedule during an early, nonreproductive life-history stage minimizes maintenance-cost increases. Consequently, allocation of energy to growth, maintenance, and reproduction during adult life (*19*) is not significantly affected by the larval events that influence life span.

Discussions of the evolution of complex life cycles in benthic marine invertebrates have not fully considered the role of larval-period duration (*20*). In the face of tidal, current, and boundary-layer processes that dominate larval dispersal (*21*), a chance encounter with an appropriate settlement site may take more time than is required to attain metamorphic competence, especially in species with rare or widely distributed settlement sites [for example, species such as opisthobranch molluscs with highly specific settlement requirements (*22*)]. A developmental hiatus during competent planktotrophic larval life eliminates costs due to premature aging and lost reproductive capacity, and so permits a flexibility in larval duration that facilitates larval settlement in optimal habitats.

Despite this developmental suspension of aging, extending larval life does have costs. A long larval period reduces the genetic representation of a lineage in a population by increasing larval mortality and by delaying reproduction, which increases lineage generation time (*23*). Oceanic processes spread these effects equally across all pelagic larvae of a species such that no single lineage obtains a consistent reproductive advantage. Species with lecithotrophic (that is, nonfeeding) larvae incur the added costs of reduced energy reserves and smaller size at settlement with increasing larval duration. These effects may alter post-larval growth (*24*) and reproduction. The consistent ability to settle at or soon after the attainment of metamorphic competence may permit a genetic lineage to overcome the costs of extended larval life, and may be prerequisite to an evolutionary shift from pelagic to nonpelagic, or from feeding to nonfeeding larval development. However, complex interactions of development, life-history and ecology will ultimately determine the extent to which a lineage diverges from its ancestral developmental mode (*20*).

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for 3 hours, and reweighed. The latter weight was subtracted from the former and divided by the number of individuals in the sample to give the ash-free dry weight per larva or juvenile. Ash-free dry weights for adult individuals and for daily egg-mass production from paired adults were similarly obtained.
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Rational Design of Peptide-Based HIV Proteinase Inhibitors

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A series of peptide derivatives based on the transition-state mimetic concept has been designed that inhibit the proteinase from the human immunodeficiency virus (HIV). The more active compounds inhibit both HIV-1 and HIV-2 proteinases in the nanomolar range with little effect at 10 micromolar against the structurally related human aspartic proteinases. Proteolytic cleavage of the HIV-1 *gag* polyprotein (p55) to the viral structural protein p24 was inhibited in chronically infected CEM cells. Antiviral activity was observed in the nanomolar range (with one compound active below 10 nanomolar) in three different cell systems, as assessed by p24 antigen and syncytium formation. Cytotoxicity was not detected at 10 and 5 micromolar in C8166 and JM cells, respectively, indicating a high therapeutic index for this new class of HIV proteinase inhibitors.

DURING THE REPLICATION CYCLE of HIV, *gag* and *gag-pol* gene products are translated as polyproteins. These are subsequently processed by a virally encoded proteinase to yield structural proteins of the virus core (p17, p24, p9, and p7), together with essential viral enzymes including the proteinase itself (*1*). On the basis of its primary amino acid sequence (*1*), its inhibition by pepstatin (*2*), and its crystal structure (*3*), HIV-1 proteinase has been classified as an aspartic proteinase that functions as a homodimer (*4*). This enzyme was first suggested as a potential target for AIDS therapy by Kramer *et al.* (*5*) when it was shown that a frameshift mutation in the proteinase region of the *pol* gene prevented processing of the *gag* polyproptein precursor. In this report we describe the rational design of a series of potent, selective inhibitors of HIV proteinase that show powerful antiviral activity against HIV-1 in vitro combined with low cytotoxicity to the host cell lines.

Although HIV proteinase can cleave a number of specific peptide bonds (*6*), it is unusual in being able to cleave the Phe-Pro and Tyr-Pro sequences found in *gag* and *gag-pol* gene products. Since the amide bonds of Pro residues are not susceptible to cleavage by mammalian endopeptidases, we reasoned that this could provide a basis for the rational design of HIV proteinase inhibitors selective for the viral enzyme.

Our strategy was based on the transition-state mimetic concept, an approach that has been used successfully in the design of potent inhibitors of other aspartic proteinases. Transition-state mimetics include hydroxyethylene isosteres (*7*), phosphinic acid (*8*), reduced amide (*9*), statine types (*10*), and hydroxyethylamine mimetics (*11*). Since the reduced amide **I** and the hydroxyethylamine **II** structures (Fig. 1) most readily accommodate the imino acid moiety characteristic of Phe-Pro and Tyr-Pro in retroviral substrates, we chose to study these structural types. During our studies we, and others (*12, 13*), have discovered that compounds containing the reduced amide function are relatively poor inhibitors. In contrast, we now report that compounds incorporating the hydroxyethylamine moiety are very potent and highly selective inhibitors of HIV proteinase.

Compounds based on the *pol* fragment Leu^{165}-Ile^{169}, containing the transition state

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