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Intraspecific heterochrony and life history evolution: Decoupling somatic and sexual development in a facultatively paedomorphic salamander

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ABSTRACT Morphological features such as size and shape are the most common focus in studies of heterochronic change. Frequently, these easily observed and measured features are treated as a major target of selection, potentially ignoring traits more closely related to fitness. We question the primacy of morphological data in studies of heterochrony, and instead suggest that principal sources of fitness, such as life history characteristics, are not only the chief targets of selection, but changes in them may necessitate changes in other (subordinate) elements of the organism. We use an experimental approach to investigate the timing of metamorphosis and maturation in a facultatively paedomorphic salamander, *Ambystoma talpoideum*. We determine that individuals possessing the well-known paedomorphic phenotype are peramorphic with regard to maturation, through the process of predisplacement (an earlier onset of maturation). Combining the well studied ecology of dimorphic *A. talpoideum* populations with theories of heterochronic mechanisms and life history evolution, we conclude that age at maturation is the principal target of selection and that morphological changes are secondary effects. Increased attention to the intimate connection between life history evolution and heterochrony is the most promising route to a better understanding of both.

Heterochrony, evolutionary changes in patterns of development, has experienced a rebirth since Gould's magnum opus (1), and recently several synthetic reviews of the topic have been published (2–4). To understand how patterns of growth and development evolve, researchers have used a wide array of techniques and conceptual approaches, including quantitative genetics (5), paleontology (6), comparative developmental biology (7), and experimental ecology (8). However, McKinney and Gittleman (9) carefully note a major deficiency in the modern approach to heterochrony since Gould (1): there has been surprisingly little attention paid to the close relationship between life history evolution and heterochrony. Within each of these broad frameworks of evolutionary biology the timing of maturation is a critical event influencing both the morphology and fitness (i.e., lifetime reproductive success) of the individual (1, 2, 10) as well as the demographic structure of populations and species (11, 12). With this in mind, we turn to a classic example of heterochrony, paedomorphosis in ambystomatid salamanders, and experimentally investigate the evolution of derived developmental and life history patterns.

Members of the family Ambystomatidae (Amphibia: Caudata) are moderately sized, morphologically conservative salamanders widely distributed throughout North America. All species have aquatic eggs and larval stages. The larval

phase ends at metamorphosis when individuals lose features associated with aquatic life (e.g., external gills and extensive tail fins). Usually, metamorphosed juveniles attain maturity on land and return to aquatic habitats seasonally to reproduce. The duration of the larval period varies widely within the family: most species have a brief larval period (e.g., <3 months in *Ambystoma maculatum*; ref. 13) although some larvae overwinter and do not metamorphose until >12 months posthatching (e.g., some *A. tigrinum*, ref. 14). Other species have abandoned the complex life cycle and bypass metamorphosis altogether (e.g., the Mexican axolotl, *A. mexicanum*, ref. 15). These individuals mature while retaining the larval morphology, a derived state termed complete or larval paedomorphosis (meaning “child-shape” or “underdeveloped,” ref. 1, 4).

Paedomorphosis evolves by modifications of the timing and/or rate of key developmental events (reviewed in refs. 2, 4, 16). The most sophisticated models predict that larval paedomorphosis in salamanders may be achieved through three processes (Fig. 1) operating either singly or in consort: neoteny (also referred to as “deceleration,” ref. 4), the decelerated rate of somatic development; postdisplacement, the delayed onset of metamorphic change or somatic differentiation; or hypomorphosis, the precocious cessation (or negative offset) of somatic differentiation. Each of these processes has a corollary that shares a common morphological result termed peramorphosis (meaning overdeveloped): for example, the opposite of postdisplacement is predisplacement, the earlier onset of metamorphosis. Gould (1) has asserted that paedomorphosis in salamanders is the result of neoteny, although he was mindful to note that a comparison of ontogenies of groups with a well established ancestor-descendant relationship—the data necessary for determining heterochronic changes—are often conspicuously absent from the literature. It is important to note that all paedomorphic *Ambystoma* are derived from naturally metamorphosing ancestors (17); therefore we must focus on the developmental changes exhibited in derived paedomorphic forms relative to their closest metamorphosing ancestor (2). For the axolotl, this is most likely *A. tigrinum* (18). However, once cladogenesis (speciation) occurs, each lineage evolves independently, and subsequent changes in the developmental programs of both lineages are likely to confound inference of heterochronic patterns. Populations where both metamorphic and paedomorphic life cycles are manifested simultaneously are termed facultatively paedomorphic, and we believe it is in these populations where we can elucidate the selective pressure(s) and distinguish the heterochronic process(es) that result in paedomorphosis.

We focus on the timing of metamorphosis and maturation as the key developmental landmarks in experimental populations of *Ambystoma talpoideum*, a facultatively paedomorphic salamander from the southeastern United States. Our reasons for concentrating on these events are straightforward. Larval paedomorphosis is a radical departure from the ancestral life cycle, one that is adaptive and thus shaped by natural selection (8, 19, 20). Selection is most likely to act on those characters

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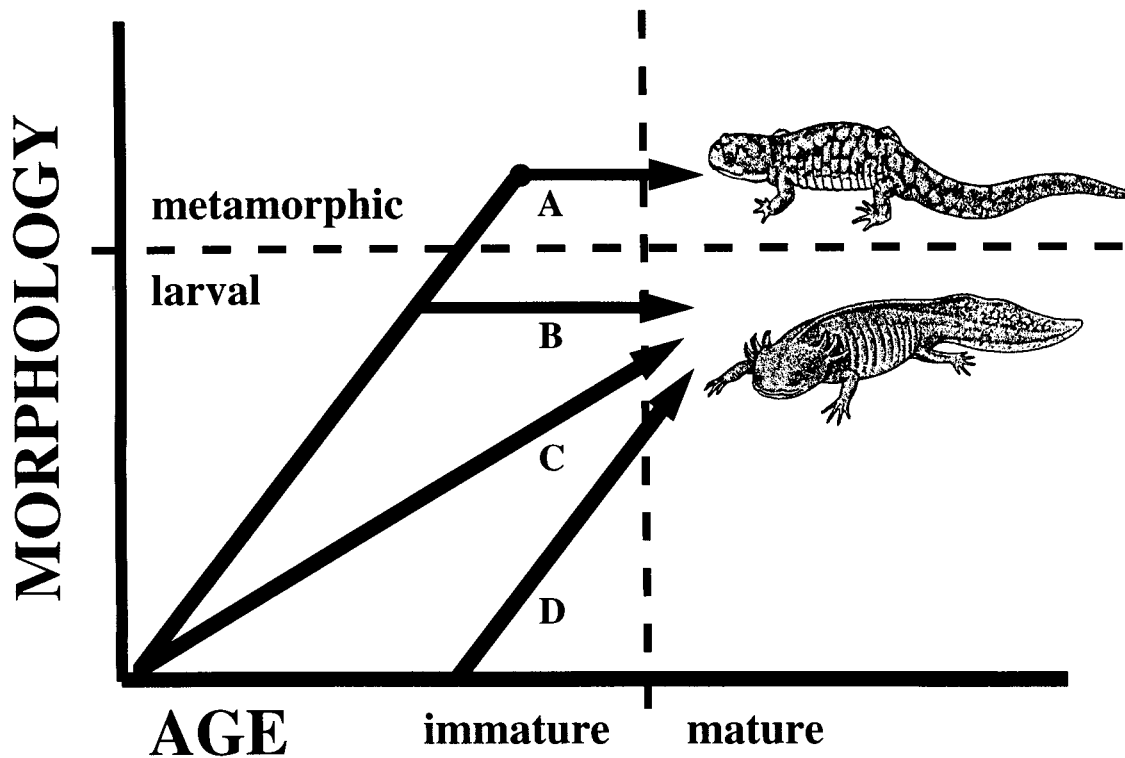


FIG. 1. Heterochronies that may result in paedomorphosis in salamanders. Lines (A–D) represent hypothetical developmental trajectories. The ancestral pattern is represented by metamorphic adults (A). Derived patterns resulting in paedomorphic adults include (B) hypomorphosis, where somatic development ceases before the metamorphic threshold (dashed horizontal line); (C) deceleration, where somatic development proceeds at a slower rate than A with the maturation threshold (dashed vertical line) being reached before the metamorphic threshold; (D) postdisplacement, where somatic development is initiated at a later ontogenetic age than A; or some combination of B–D (e.g., see ref. 4). Images modified from refs. 4 and 47.

that are important to organismal fitness, such as age at metamorphosis (21) and age at maturation (22). Because morphological reorganization consumes considerable time and energy (17, 23), we hypothesize that, all other things being held equal, salamanders foregoing metamorphosis may have more energy available for growth and (or) sexual development. For example, we expect that those individuals bypassing metamorphosis should either attain maturation earlier than metamorphic salamanders, or attain maturation at the same time as metamorphs but at a larger body size. In the first case, energy and available resources can be channeled to the rapid development of gonads and gametes rather than extensive morphological rearrangement (24, 25). Thus we expect if paedomorphs and metamorphs mature at the same body size, paedomorphs should mature at a younger age. Alternatively, if the two adult morphs mature at the same point in time, we might predict paedomorphic individuals to have increased body size relative to metamorphic individuals, with the energy and resources required for metamorphosis having been committed to overall growth in paedomorphs. Other complex trade-offs between life history pathways and components of fitness (e.g., morph-specific fecundity, mating success) are also likely to be important in determining the evolution and maintenance of facultative paedomorphosis. Herein we present the results of an experiment investigating the timing of metamorphosis and differences in the timing of maturation between metamorphic and paedomorphic *A. talpoideum*, and discuss the selective pressures that likely account for the heterochronic patterns observed.

METHODS

Experimental Design. We raised populations of larval *A. talpoideum* in experimental artificial ponds in a two-factor

randomized design. These experimental ponds have become a stalwart of amphibian ecological and evolutionary studies, and offer a practical mixture of ecological realism and experimental rigor (26, 27). We reared larvae at two densities (18 larvae per pond = low density; 36 larvae per pond = high density) within the range of natural densities (28), and collected whole populations at four times (September, October, November, and December). Each treatment combination was replicated four times, for a total of 32 experimental populations.

Procedures. Experimental populations were derived from the progeny of metamorphic adult *A. talpoideum* intercepted during breeding migrations at Rainbow Bay, a temporary pond in Barnwell County, South Carolina. Because Rainbow Bay dries annually (29, 30), all members of the breeding population are necessarily metamorphic, indicating this as the life history pattern ancestral to the population. The members of this population, nonetheless retain the ability to become paedomorphic when released from the pressure exerted by pond drying, both under natural (31) and experiment (29) situations. Under constant water conditions, expression of the adult morphs is near parity (29).

Thirty-two experimental ponds (polyethylene cattle tanks, 1.83 m diameter, $\approx 1,300$ l volume) were randomly assigned treatment combinations and positions in an array at the Reactor Research Park (University of Missouri, Columbia), and were filled with $\approx 1,000$ l tap water on 4–5 March 1996. Each pond was equipped with an internal adjustable standpipe to standardize and limit water depth. We added 1 kg of homogenized leaf litter to each pond on 12 March 1996 and added equal-sized aliquots of a concentrated zooplankton solution to each pond several times before the addition of larvae (8, 29). Ponds were covered during the day to prevent colonization of predacious insect larvae (e.g., odonates) and uncovered at night to allow for natural colonization of other

insects (e.g., chironomids) that serve as a food source. On 12 March 1996, 10 males and 18 females were introduced to a 1,500-l experimental pond, containing a leaf litter mixture to serve as a site for oviposition, and allowed to mate at random. Adults were removed from the breeding pond once oviposition was observed (14–20 March 1996). Eggs were allowed to develop and hatch in the breeding pond. Hatchling *A. talpoideum* larvae were randomly assigned to experimental ponds according to density treatments on 3 May 1996 (day 1).

We monitored the ponds daily and searched for metamorphs every 2–3 nights beginning 1 June 1996 until the end of the experiment on 2 December 1996. Metamorphs were collected via dipnet, measured for snout-vent length (the distance from the tip of the snout to the posterior margin of the cloacal aperture) in mm, and given a unique mark (toe-clip). We released metamorphs into polyethylene tanks filled with ≈ 10 cm topsoil and ≈ 10 cm leaf litter and a 30 cm \times 30 cm coverboard for refuge (referred to hereafter as “pens”). The natural soil and leaf litter substrate contained an *ad libitum* food supply; arthropods, annelids, and other invertebrates were observed during pen construction and dismantling. One pen was available for each collection month, and density in the pens did not exceed natural densities of juveniles (32).

We harvested the contents of four replicate low density and four replicate high density ponds on 8 September (day 129), 6 October (day 157), 3 November (day 185), and 2 December (day 224). This period, from 4–8 months posthatching, covered the time during which nearly all metamorphosis takes place in natural populations (22, 33). Past this point, most immature larvae have been found to remain as immature larvae until the following spring when they metamorphose with the first of the new cohort. During each harvest, the appropriate ponds were drained and all leaf litter was thoroughly searched for nonmetamorphosed individuals. Similarly, the corresponding terrestrial pens were emptied each month and all metamorphic individuals were recovered. All individuals were sacrificed in a dilute ethanol solution and preserved in 7% formalin. Each individual was measured for snout-vent length and dissected to determine reproductive status. Mature or maturing males were identified by the presence of enlarged testes and/or pigmented vasa deferentia. Mature or maturing females were identified by the presence of enlarged yolky ovarian follicles and/or enlarged convoluted oviducts. We regarded all individuals exhibiting both the larval morphology (external gills and large tail fins) and sexual maturation as paedomorphs, as these individuals have “committed” to that life history pathway (29, 33); individuals that metamorphose < 1 yr posthatching do not emerge from natural or experimental ponds sexually mature (unpublished data from 8, 22, 29, 33). All other nonmetamorphic individuals were regarded as immature larvae.

Analysis. We performed a two-way ANOVA to detect differences in survival among the eight treatment combinations, using the arcsine-square root of the proportion of individuals surviving to the time of collection. We recorded size (snout-vent length in mm) and morph (metamorph, paedomorph, or immature larvae) of each individual, and calculated the proportion of each population (i.e., experimental pond) exhibiting each adult morph (metamorph or paedomorph). Preliminary analyses indicated that the proportion of paedomorphs and metamorphs in low density ponds did not differ significantly over time, thus we used a two-way ANOVA to determine whether the (arcsine-square root transformed) proportion of paedomorphs differed with regard to density and month of collection. The proportion comprising the immature larvae was uniquely determined by the proportion of paedomorphs in all high density ponds and was therefore excluded from analyses of population composition.

We performed equivalence tests (tests-of-no-effect, see ref. 34 for review) to determine whether the mean sizes of pae-

domorphs and metamorphs differed in each of the three treatment combinations where both morphs were present. In these tests, the null hypothesis is that there is a significant difference in body size of the two morphs, and if the null hypothesis is rejected the morphs are considered indistinguishable with regard to body size. In all comparisons, the null hypothesis was clearly rejected (t values = 11.23–52.31; all P values < 0.001). We thus regarded these adult morphs as representing a single class in a three-way ANOVA testing for differences in body size, with morph (adult morph or immature larvae), month of collection, and density as main effects. Body size was log-transformed for all statistical analyses.

RESULTS

Survival and Size. There were no significant differences in survival among the treatment combinations (density effect: $F_{1,24} = 2.01$, $P = 0.1686$; month of collection effect: $F_{3,24} = 1.87$, $P = 0.1613$; interaction effect: $F_{3,24} = 1.96$, $P = 0.1473$) as survival was high among all treatments (92–100%). Mean body size varied significantly with respect to both density ($F_{1,45} = 312.37$; $P < 0.0001$) and month of collection ($F_{3,45} = 26.14$; $P < 0.0001$), but not between immature larvae and adult morphs ($F_{1,45} = 0.24$; $P = 0.6247$), and there were no significant interactions among main effects. At each of the four collections, the low density survivors were significantly larger than their high density counterparts. Within each density survivors collected in September were significantly smaller than those in the remaining collections, and survivors collected December were significantly larger than those collected in October. These results demonstrate that initial larval density influenced the body size of individuals, but that growth was similar among the morphs at both densities.

Metamorphosis and Maturation. At low density, paedomorphs were present in the collections (September) before the appearance of any metamorphs (October) (Fig. 2). Furthermore, none of the metamorphs from any of the collections

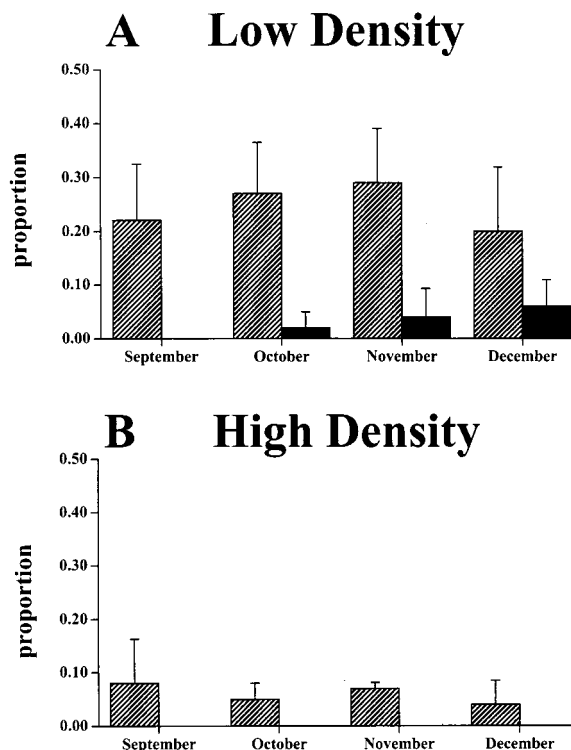


FIG. 2. Mean proportion of each adult morph (▨, paedomorphs; ■, metamorphs) present in each collection under (A) low density and (B) high density. Bar = 1 SEM.

showed any signs of sexual maturation. The expression of paedomorphosis varied significantly with regard to density ($F_{1,28} = 35.04$; $P < 0.0001$) but not with month of collection ($F_{3,28} = 0.32$; $P = 0.7301$), and the interaction between these two factors was not significant ($F_{3,28} = 1.24$; $P = 0.3212$). The proportion of paedomorphs in low density ponds was greater than that of low density ponds at each of the collections, and did not appear to increase or decrease appreciably throughout the experiment (Fig. 2). No metamorphs emerged from any of the high density ponds by the termination of the experiment.

DISCUSSION

We collected paedomorphic individuals from our experimental populations before collecting any metamorphic individuals, in accordance with our predictions. Furthermore, no metamorphs emerged from any of the high density ponds, although paedomorphs were present in each of the four collections, albeit at relatively low frequency. Mean body size within a given density over time and between densities at any point in time differed significantly in a predictable manner consistent with growth rates in natural populations (21, 33). There was no evidence that growth rate influenced the probability of becoming paedomorphic or metamorphic as metamorphs, paedomorphs, and immature larvae were of similar size within any given treatment combination (see also 33). In general, the sizes of metamorphs and paedomorphs are comparable to those collected in other experiments (e.g., refs. 28 and 33) and in natural populations (e.g., refs. 21 and 22).

The most conspicuous difference in the developmental patterns of the metamorphic (ancestral) and paedomorphic (derived) life histories pathways of *A. talpoideum* in our experimental populations is the presence of mature paedomorphs before any detectable metamorphic activity. Although the pattern of somatic development may be paedomorphic, the pattern of sexual development is clearly peramorphic via the process of predisplacement, the earlier onset or initiation of the development of a particular trait (2, 4), in this case maturation. It appears that at least two separate processes involving the development of somatic and reproductive organs operate to produce the paedomorphic phenotype.

In organisms with complex life cycles, metamorphosis is a necessary threshold that must be crossed to reach the next stage (35). For most amphibians, the transition from the larval to the postmetamorphic form must occur before the initiation of maturation (17): few caudate and fewer anuran amphibians are sexually mature at the completion of metamorphosis. In this context metamorphosis is largely a means to an end. When new metamorphosis and maturation (i.e., the "means" to the "end") are decoupled a developmental and life history patterns, such as larval paedomorphosis, may evolve (1). To understand the selective pressures resulting in decoupling these developmental landmarks, the reproductive ecology of the organisms must be considered.

For *A. talpoideum*, rainfall and temperature determine the initiation of breeding migrations, when mature metamorphic adults leave their subterranean refugia and move to aquatic breeding sites (37). Variation in these abiotic parameters accounts for a large proportion of the variation in the timing of migrations (37, 38); paedomorphic adults, however, are present at the breeding site as soon as they attain maturation. Although breeding migrations have been shown to be heavily dependent on ecological factors, breeding *per se* has not. Breeding begins in late September and lasts as late as March (21, 22). When climatic conditions do not permit migration to occur until late in the breeding season, paedomorphic adults may complete reproduction before the arrival of metamorphic adults. For example, Ginger's Bay (within 10 km of Rainbow Bay) usually dries annually but, due to an exceptionally wet year, held water continuously from the winter of 1990 through

the summer of 1992 (39). Released from the ecological pressure of pond drying in the summer and fall of 1991, many larvae became paedomorphic in this population where the metamorphic pathway is the norm. In the winter of 1991 courtship, sperm transfer, insemination, and oviposition were completed by paedomorphic adults and hatching of some of their progeny occurred all prior to the arrival of metamorphic adults. All breeding paedomorphs in the population were <1 yr posthatching at the time reproductive activity was documented (39). Although metamorphs may reproduce initially at ≈ 1 yr posthatching, reproduction is often delayed an additional year (or more), with the size and age at metamorphosis acting as a primary influence on reproductive attributes (21). At Ellenton Bay (a permanent pond about 15 km from Rainbow Bay), Krenz and Sever (40) found that 50% of paedomorphic females had been inseminated and begun oviposition about 1.5 months before the climax of migration by metamorphs. Although the ages of both morphs were unknown in this study, it again demonstrates that migratory activity of metamorphs is constrained by local weather conditions, and thus may result in delayed reproduction relative to paedomorphs. Differences in maturation among the morphs of facultatively paedomorphic salamander species have largely been neglected, leading several authors to assume that maturation is synchronous (1, 4, 22, 42) and that breeding activity is concomitant with the arrival of terrestrial metamorphic adults.

We suggest age at maturation as the principal target of natural selection in the evolution of facultative paedomorphosis, with the retention of larval morphology following as secondary effect. Age at maturation is a major factor in an organism's life history, both in theory and in practice (11, 12, 42). The primary advantages of earlier maturation are increased survival to first reproduction and shortened generation times (11). Additionally, and perhaps more importantly, when competition and other density-dependent factors influence growth and survival as they do in *A. talpoideum* (28) and other amphibians (36, 43, 44), precocious maturation and early reproduction of paedomorphs provides growth and survival advantages to their offspring in extremely low density conditions. These early growth and/or survival benefits increase the overall quality of the offspring, which later translate into increased adult performance or fitness (21). The advantages of early growth are, of course, not exclusive to salamander or other amphibian larvae; e.g., the fitness benefits of early emergence in most plant communities are well documented (45).

The benefits of early maturation are sacrificed, however, if the age at first reproduction is the same as those that mature later; in fact, early maturation then may carry with it significant costs (ref. 11; e.g., reduced body size, reduced parental investment). Thus, if an individual matures early and then undergoes metamorphosis, the benefits of increased survival and higher quality offspring are lost, because the age at first reproduction then becomes dependent on the abiotic conditions that permit the breeding migrations to occur. Rather, to reap the benefits of early maturation the individual must bypass metamorphosis to retain the larval morphological characters that facilitate survival in the aquatic habitat. This completed, the age at first reproduction is constrained less by abiotic factors than by availability of mates and other considerations (e.g., mate choice). Therefore, selection favors the retention of the larval morphology in adult salamanders indirectly to allow for the potential fitness benefits of an early age at maturation to be realized through an early age at first reproduction (Fig. 3). Because most *A. talpoideum* retain the ability to metamorphose after initially reproducing as a paedomorphs (even after several years), we regard the paedomorphic morphology to result primarily from the indefinite postdisplacement of metamorphosis (Fig. 3). When it occurs, the transition from pae-

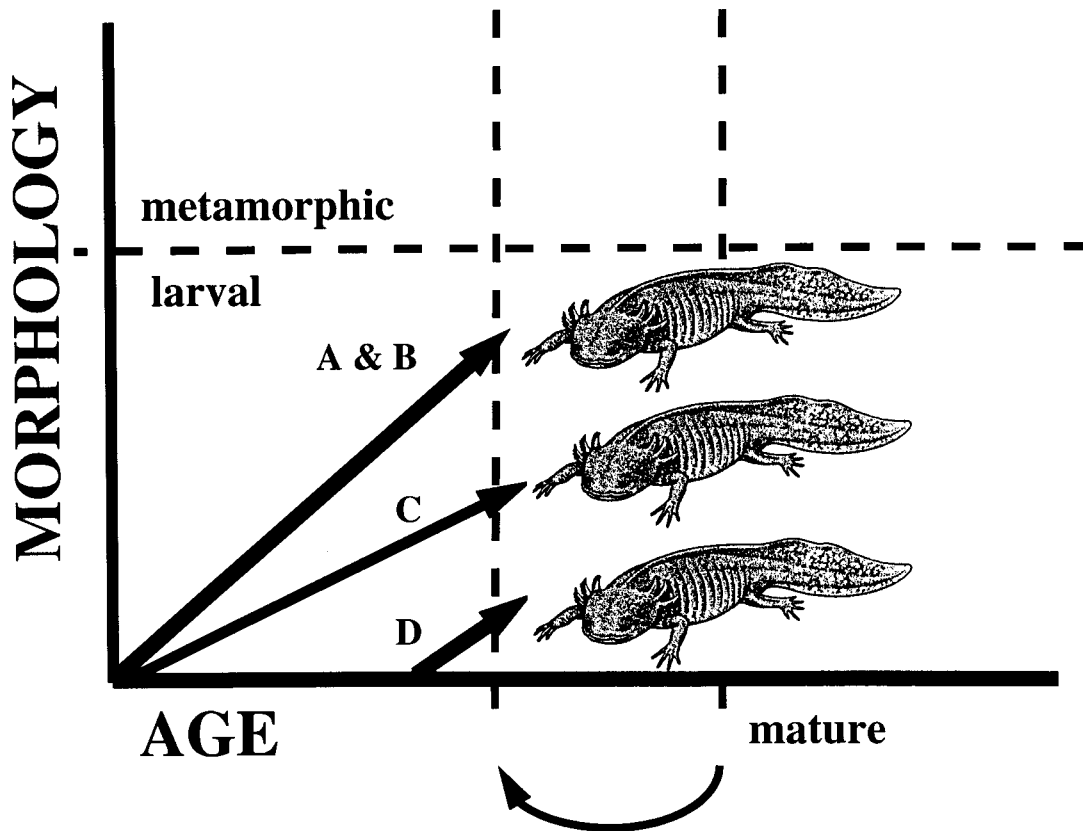


FIG. 3. Reproductive and somatic heterochronies that result in paedomorphosis in *Ambystoma talpoideum*. A significant decrease in the age at maturation, indicated by shifting the maturation threshold (dashed vertical line) to the left, requires the retention of the larval morphology, regardless of the developmental trajectory (A–D as in Fig. 1). When larvae become paedomorphic through predisplacement of maturation, metamorphosis becomes indefinitely postdisplaced (see text). Images modified from refs. 4 and 47.

domorphic to metamorphic adult does not happen until the spring, after the breeding season when other metamorphs (i.e., young-of-the-year) also leave the pond. This allows paedomorphs to react adaptively to drastic changes in the usually benign and often favorable aquatic habitat (19, 22).

Ambystomatid salamanders possess a considerable amount of developmental plasticity, and the retention of larval features in reproductive adults may arise through multiple pathways, selected by entirely different suites of ecological pressures (19). The value in our study is that we have compared the ontogenetic trajectories of organisms whose evolutionary history is known, and interpreted them in the context of ecological conditions and fitness benefits that have been vigorously studied. A comparative study of this sort is critical to explicating the evolutionary processes accounting for the apparent (morphological) results (1) and has rarely been accomplished (2). Instead of describing only changes in morphology (e.g., ref. 46), though informative, we have evoked an explanation congruent with heterochronic and life history theories as well as the ecology of the organisms under study. This holistic approach leads us to identify age at maturation, a principal component of individual- and population-level fitness, as the most likely target of selection, with retention of the larval morphology as a secondary effect. The result is a paedomorphic appearance, although the selective forces have been acting on life history characters in a manner consistent with peramorphic development. The error in diagnosing evolutionary changes in patterns of development based solely on morphology is that the primary target of selection, the very character driving the evolution of ontogeny, may be ignored.

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