

Differentiation trees, a junk DNA molecular clock, and the evolution of neoteny in salamanders

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Abstract

Obligate neotenic salamanders die if forced to metamorphose. We suggest that this can be explained by assuming: 1) their "excess" DNA is "junk" DNA; 2) the "adult" specifying portion of the DNA becomes junk DNA and is available for repeated duplication. This suggests a "new" junk DNA molecular clock. We obtain remarkable agreement in "predicting" the amount of DNA per nucleus in present day non-obligate neotene salamanders from this molecular clock. These observations are consistent with the idea that the development of these animals is describable in terms of differentiation trees whose branches (gene cascades) corresponding to adult somatic tissues accumulate deleterious mutations over evolutionary time. We show that the amount of DNA per nucleus increases linearly with the phylogenetic age of salamander families. The lack of constraints by natural selection, on unused adult branches, may account for the large amount of so-called "junk DNA" in obligate neotenic salamanders. The effects of this excess DNA, via increased cell size, suggest a positive feedback, ecophysiological explanation for such junk DNA: adaptation to cool water environments is enhanced by the lower metabolism associated with more DNA, larger cells and slower developmental time.

Introduction

One of the major, unsolved problems of evolutionary biology is the relationship between the development of the individual organism and its potential for contributing

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to further evolution (Bonner, 1965, 1982; Oster and Alberch, 1982; Horder, 1983; Raff and Kaufman, 1983; Arthur, 1988).

We have proposed a new theory of the development of tissues in eukaryotic organisms (Gordon, 1993) rooted in a biomechanical model for differentiation (Gordon and Brodland, 1987; Björklund and Gordon, 1993, 1994) based on ultrastructural observations (Burnside, 1971, 1973; Martin and Gordon, 1995) and time lapse observations of waves of differentiation (Brodland et al., 1994; Gordon, 1995; Gordon et al., 1994; cf. Ready, Hanson and Benzer, 1976) during primary

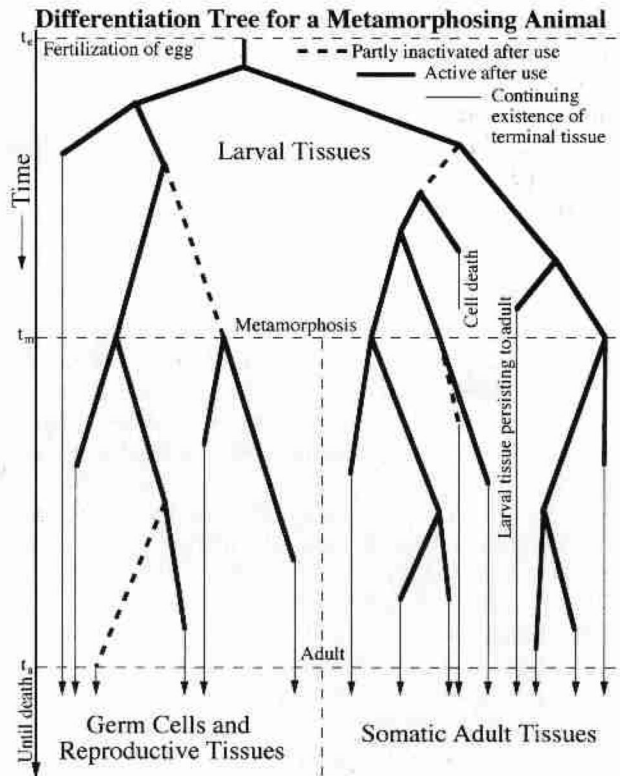


Fig. 1. Differentiation tree for a metamorphosing animal. Developmental time is indicated from top to bottom. The organism starts as a fertilized egg at time t_e . Each thick line represents a gene cascade, its products, and the morphogenetic movements and cell shape changes, etc., of all the cells in a single, contiguous tissue. Dashed thick lines represent gene cascades that are partly or completely shut down when the cells go through subsequent steps of differentiation. The nodes represent the synchronous erection of cell state splitters in each cell in a tissue. Each cell in the tissue resolves the instability of its cell state splitter by becoming one of two new cell types. At the time of metamorphosis, t_m , germ cells, reproductive tissues, and adult somatic tissues are further differentiated from some of the larval tissues. Differentiation to an adult is complete at time t_a . The straight lines descending from each terminal node indicate the continued existence of terminally differentiated cell types, unless "programmed" cell death (apoptosis) occurs.

neural induction. The basic idea is that differentiation may be viewed as a temporal alternation of intracellular physical instability resolving mechanisms and cell type specific gene cascades. Each tissue is split into two new tissues whose cells then unfold different sets of genes and gene products. The bifurcation of tissues may be represented by a binary tree, which we call the differentiation tree of an organism (Fig. 1). Therefore, a differentiation tree is a tissue lineage tree. The latter concept goes back to Von Baer (1828, see Patterson, 1983). Slack (1983) gives a tissue lineage tree for amphibians.

There are many testable ramifications of a theory of development based on differentiation trees (Gordon, 1995). We will demonstrate how differentiation trees can provide a reasonable framework for organizing the known facts about the evolution of neotenic salamanders. Since facultative and obligate neoteny appear to be responses to the local environment (reviewed below), this paper demonstrates the first of what may prove to be many links between differentiation trees, ecology and evolution. In particular, we find that the quantity of DNA per nucleus increases linearly with geological time in obligate neotenic salamanders, yielding a new "junk" DNA molecular clock.

Review of neoteny

The phenomenon of neoteny is defined as the attainment of reproductive maturity while retaining larval external morphology or larval reproduction via delayed somatic development (Pierce and Smith, 1979). Anderson and Worthington (1971) and Nussbaum (1976) support this definition by showing that larva of *Ambystoma ordinarium* and *Dicamptodon ensatus* reach sexual maturity at approximately the same size as transformed individuals. It is presumed that the relationship between size and age remains the same for both neotenic and metamorphosing salamanders.

Metamorphosis

Before attempting to discuss neoteny in salamanders we must first understand the normal condition of metamorphosis. Metamorphosis is a series of abrupt structural, physiological, biochemical and behavioural changes (Duellman and Trueb, 1986) involving:

- 1) regression of structures and functions that are significant only to the larva;
 - 2) transformation of larval structures into a form suitable to the adult;
 - 3) development of structures and functions *de novo* that are essential to the adult.
- Metamorphosis influences almost every tissue in the body. Table 1 shows the major morphological changes. It is interesting to note that reproductive tissues (germ line and reproductive organs) are not amongst those reported to change upon transformation (Table 7.2 in Duellman and Trueb, 1986), supporting our suggestion that they are formed by independent branches of the differentiation tree (Fig. 1). Direct confirmation of their lack of change on transformation would be worth seeking.

