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Kowalevsky, Comparative Evolutionary Embryology, and the Intellectual Lineage of Evo-Devo

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ABSTRACT Alexander Kowalevsky was one of the most significant 19th century biologists working at the intersection of evolution and embryology. The reinstatement of the Alexander Kowalevsky Medal by the St. Petersburg Society of Naturalists for outstanding contributions to understanding evolutionary relationships in the animal kingdom, evolutionary developmental biology, and comparative zoology is timely now that Evo-devo has emerged as a major research discipline in contemporary biology. Consideration of the intellectual lineage of comparative evolutionary embryology explicitly forces a reconsideration of some current conceptions of the modern emergence of Evo-devo, which has tended to exist in the shadow of experimental embryology throughout the 20th century, especially with respect to the recent success of developmental biology and developmental genetics. In particular we advocate a sharper distinction between the heritage of *problems* and the heritage of *tools* for contemporary Evo-devo. We provide brief overviews of the work of N. J. Berrill and D. T. Anderson to illustrate comparative evolutionary embryology in the 20th century, which provides an appropriate contextualization for a conceptual review of our research on the sea urchin genus *Heliocidaris* over the past two decades. We conclude that keeping research questions rather than experimental capabilities at the forefront of Evo-devo may be an antidote to any repeat of the stagnation experienced by the first group of evolutionary developmental biologists over one hundred years ago and acknowledges Kowalevsky's legacy in evolutionary embryology. *J. Exp. Zool. (Mol Dev. Evol.) 302B:19–34, 2004.* © 2004 Wiley-Liss, Inc.

INTRODUCTION

Alexander Kowalevsky (1840–1901) was one of the most significant 19th century biologists working at the intersection of evolution and embryology. His impact has not yet been documented in detail because historians of 19th century biology have tended to give pride of place to Ernst Haeckel, whose flamboyance and influence have had their own measure of importance. Inaccessibility also plays a role as many of Kowalevsky's papers are only available in German or Russian and have yet to be translated into English. (For complete documentation and biographical details, see Adams, '73; Mikhailov and Gilbert, 2002.) Kowalevsky is most often remembered for his discovery in 1866 of the chordate nature of the ascidian tadpole, which had two immediate effects: (a) ascidians were reclassified with chordates rather than molluscan invertebrates, and (b) many, especially Haeckel, found inspiration for recapitulatory ideas about adult stages of ancestors being traversed during the development of a descendant, thereby providing an ontogenetic window on the evolutionary past. Kowalevsky also worked on the embryology of a variety of invertebrates including amphioxus, brachiopods, and annelids, seeking generalizations of metazoan evolution and development through these investigations, aptly summarized in his Latin motto, '*In specialibus generalia quaerimus*' ('We seek the general in the specifics'). He also documented the existence of gastrulation processes across vertebrates and invertebrates alike (Brauckmann and Gilbert, 2004).

The reinstatement of the Alexander Kowalevsky Medal by the St. Petersburg Society of Naturalists for outstanding contributions to understanding evolutionary relationships among major groups in the animal kingdom, evolutionary developmental biology, and comparative zoology is timely now

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that Evo-devo has become a major research discipline in contemporary biology (Mikhailov and Gilbert, 2002). A full accounting of how Kowalevsky fits into a history of Evo-devo may still be incomplete but it can be partly understood by tracing the 20th century heirs to his intellectual lineage. Our aim is to explore some aspects of this lineage in comparative evolutionary embryology. To this end we briefly get our bearings in the 19th century before turning to a discussion of what constitutes a history for Evo-devo. The heritage of comparative evolutionary embryology explicitly informs the late 20th century emergence of research programs that mesh evolutionary and developmental themes. In particular we advocate a sharper distinction between the heritage of problems and the heritage of tools for contemporary Evo-devo. In order to illustrate the heritage of problems from comparative embryology we briefly outline the main contours of the research of N. J. Berrill and D. T. Anderson, respectively. Berrill's comparative studies of ascidian embryology, along with their application to the question of the origin of vertebrates, and Anderson's attempt to resolve annelid and arthropod phylogenetic relationships with careful descriptive embryology are noteworthy examples of research that resonates strongly with the questions found in contemporary Evo-devo. Looking back to the details of their investigative work and the conceptual themes exhibited by these biologists (e.g. heterochrony, homology, and evolutionary novelty) provides an appropriate contextualization for Raff's laboratory research on the sea urchin genus *Heliocidaris* during the past two decades.

Comparative evolutionary embryology and evo-devo history

The precise role of embryological investigations for the emergence of evolutionary thinking in biology remains an issue of debate. Since the 'demise' of the biogenetic law in the first half of the 20th century, there has been a tendency to downplay any importance that embryology might have had in the emergence of evolutionary studies despite demonstrable connections (Richards, '92). It is sufficient to note that many 19th century biologists involved in embryological investigations had evolutionary interests, which directs attention to research programs that crystallized after the publication of Darwin's *On the Origin of Species*. The evolutionary morphology of Ernst Haeckel and Carl Gegenbaur stands out in this regard. Haeckel and Gegenbaur, following on the earlier embryological research of individuals such as von Baer, produced an inspirational blending of evolutionary and developmental themes, most familiar of which is the biogenetic law, greatly expanding on Darwin's appreciation of the role of embryology in evolutionary classification (Gould, '77; Hoβfeld and Olsson, 2003; Laubichler and Maienschein, 2003). Haeckel had quickly grasped the importance of Kowalevsky's research on marine invertebrate embryology and brought into relief one of the key problem agendas of biologists in the 19th and 20th centuries; namely, classification and the origin of major taxonomic groups. The research program of Haeckel was fundamentally about discerning phylogenetic relationships among organisms using embryological patterns for data to establish relationships of homology, especially where paleontological help was unlikely to be forthcoming, such as in the origin of vertebrates, with all of its purported implications for *Homo sapiens* (Sander, 2002; Hoβfeld and Olsson, 2003). Haeckel's interest in the mechanistic details of the evolutionary process was less thorough, though it is often not carefully characterized (Gould, '77; Richardson and Keuck, 2002). The work of his students, collaborators, and fellow travelers continued to emphasize problems of phylogenetic classification, especially the nature of 'ancient' and 'transitional' ancestors, including metazoan precursors and the origin of paired limbs in vertebrates. Carl Gegenbaur worked alongside Haeckel for a time at the University of Jena (later moving to Heidelberg) and utilized comparisons of animal structure for elucidating phylogenetic relationships in order to discern the evolutionary laws of form (Nyhart, '95, 2003). In many ways his view of the relationship between ontogeny and phylogeny was more sophisticated than Haeckel's, recognizing the critical nature of distinguishing 'false' evidence of homology in development ('caenogenesis') due to processes such as larval adaptation from true evidence for homology found in developmental processes ('palingenesis') (Laubichler and Maienschein, 2003). In the American context, the students of William Keith Brooks at Johns Hopkins University, such as T.H. Morgan, carried out a very similar enterprise (Maienschein, '91). Toward the end of the century, attention began to focus on the precise detailing of cell lineages and their implications for homology, and thus phylogeny (Guralnick, 2002; Laubichler and Maienschein, 2003).

Whether it is Haeckel, Gegenbaur, Lillie, Brooks, or the early Bateson and Morgan, the primary rationale for studying the ontogeny of various organisms was to reconstruct the past. This does not preclude the fact that many researchers, including Kowalevsky, maintained a vigorous interest in developmental mechanisms such as gastrulation (Brauckmann and Gilbert, 2004). Various debates broke out over subproblems of interest such as the monophyly of arthropoda, the origin of vertebrates, the fin-limb transition, and the origin of birds or mammals (Nyhart, '95; Bowler, '96). We refer to this intellectual lineage as comparative evolutionary embryology and its most natural contrast in historical context is developmental mechanics (Entwicklungsmechanik), which took as one of its starting points an explicit rejection of the methodology inherent in the phylogenetic problem agenda at that time. Wilhelm Roux, Hans Driesch, and others attempted to forge a causal investigation of development that did not entangle itself in phylogenetic speculations (Oppenheimer, '67, 62–91, 117–172), which appeared to repeatedly end in stalemates (Nyhart, 2002). The rhetoric of the superiority of experimental embryology over the painstaking descriptive observations most commonly associated with comparative evolutionary embryology coincided with a general trend towards experimentalism in the early 20th century (Allen, '76). Thus the shift towards manipulative investigation of developmental mechanics was juxtaposed with a loss of interest in questions of phylogenetic relationships and evolutionary questions more generally. Nevertheless, an abiding interest in the intersection of evolution and development was sustained by those who continued to work in comparative evolutionary embryology, an effort that has yet to receive its full due in accounts of the historical backdrop for Evo-devo. Although overshadowed by the empirical success of experimental embryology and its descendent disciplines, key issues such as phylogenetic relationships and what we now call the origin of evolutionary innovations and the significance of developmental constraints were persistent problems for investigators working in comparative evolutionary embryology (Love and Raff, 2003).

Making a clear distinction between developmental mechanics and comparative evolutionary embryology illuminates a common view that embryology was excluded from the Modern Synthesis (Hamburger, '80, '88). Two different kinds of embryology were not integrated into the momentous discussion in the 1940s that produced what we call the synthetic theory of evolution. One way to reconstruct the potential significance of these two forms of developmental investigation for contemporary studies of evolution and development is to conceptually distinguish between scientific problems and the tools used to investigate those problems. Many of the contemporary tools for exploring the evolution of development have arisen by descent through the lineage of an experimental approach to embryology, most notably those from developmental genetics, such as molecular genetic manipulation (Duffy, 2002), laser ablation, tissue recombination, bead implantation, in vivo electroporation (Weaver and Hogan, 2001), and various gene expression observation methods such as in situ hybridization and immunolocalization (Rapley and Walker, '98; Carroll et al., 2001, ch. 2). It is because of the dominance of these genic focused techniques in contemporary developmental biology that this stream is seen as so crucial to the history of Evo-devo. But, if we shift our attention away from the tools of investigation to the agenda of problems within contemporary Evo-devo, experimental embryology is not the appropriate intellectual ancestor for most of the 20th century.

This is a crucial part of the heritage of Kowalevsky and it needs to be highlighted, because of the widespread focus on the molecular biological techniques that have allowed the discovery of homologous regulatory genes, homologous developmental pathways, and changing patterns of homeotic gene expression over the past two decades (McGinnis, '94; Holland, '99; Gilbert, 2000a, 2003; Arthur, 2002; Wilkins, 2002; McGinnis and Krumlauf, '92; Gilbert and Bolker, 2001; Gilbert and Burian, 2003). Figure 1 represents a common historical perspective that characterizes Evo-devo primarily in terms of developmental genetics as the bridge between genic based accounts of evolution and a molecularized embryology by only attending to the lineage of tools. Highlighting the differences between the contributions from the experimental embryology and comparative embryology (Fig. 2) emphasizes the unique marriage of new experimental tools and perennial problems, as well as heterogenous disciplinary perspectives, that characterizes many contemporary Evo-devo research

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This is not to say there are *only* two distinguishable threads of embryological research. One could also draw attention to the nascent genetical approaches to development, exemplified by key individuals such as C.H. Waddington (Gilbert, '91, 2000b) and Boris Ephrussi (Burian et al., '91).

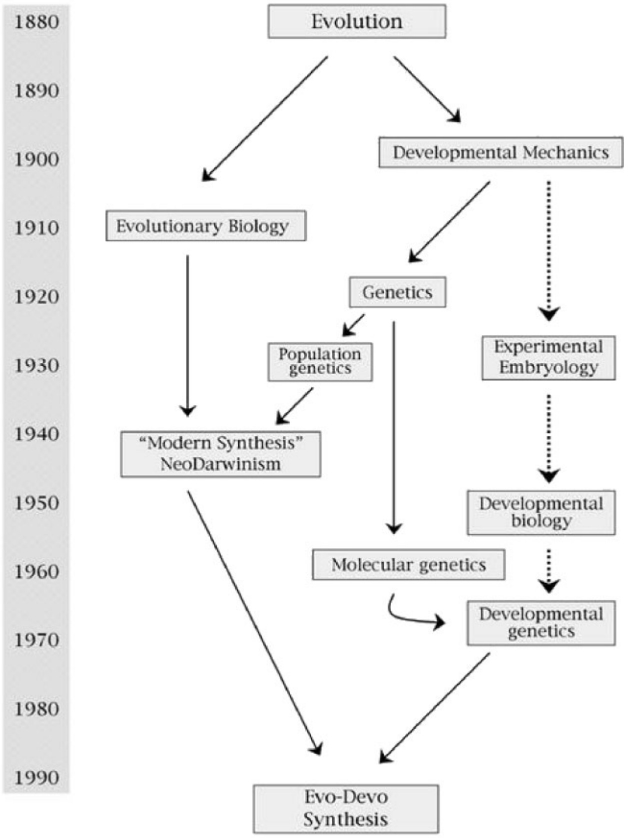

Fig. 1 A schematic representation of the intellectual lineage of experimental embryology and the heritage of tools used in modern Evo-Devo [dotted arrows] (Love and Raff, 2003). Reproduced by permission of Blackwell Publishing.

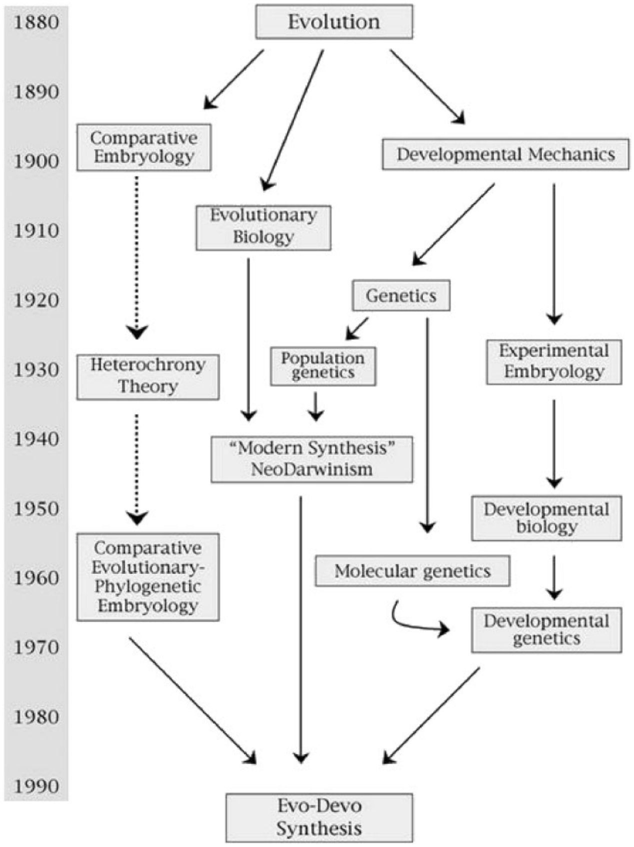

Fig. 2 A schematic representation of the intellectual lineage of experimental embryology and comparative embryology that emphasizes both the heritage of tools and heritage of problems [dotted arrows] found in modern Evo-Devo. (Love and Raff, 2003). Reproduced by permission of Blackwell Publishing.

programs (Love, 2003; Love and Raff, 2003). Attention to comparative evolutionary embryology themes in the research of Kowalevsky redirects our attention to what was going on in embryology after (and also during) the period of the Modern Synthesis that provided theoretical and experimental inspiration to Kowalevsky medal winners who were all trained prior to the emergence of any recognizable research program in modern developmental genetics. Instead of focusing on highly visible individuals such as Gavin de Beer (de Beer, '58; Hall, 2000), we attempt to highlight the research of two different individuals who illustrate the kinds of work that sustained comparative evolutionary embryology in the period immediately before the emergence of modern Evo-devo: N.J. Berrill and D.T. Anderson.

Berrill and the comparative embryology of ascidians

It is quite natural, for at least two reasons, to turn our attention to the work of Norman John ('Jack') Berrill (1903–1996). First, N.J. Berrill carried out a comparative program of investigation of ascidian embryos in the 1920s–1940s, the same group from which Kowalevsky made his profound discovery, in which there was a considerable component that resonates with current Evo-devo research. In tracing the history relevant to a discussion of the intersection between evolution and development in an introductory textbook, Berrill recounts that "Soon after the publication of Darwin's *Origin of Species* and its general acceptance by the scientific world of the day, the discovery was made by the Russian embryologist

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Although not represented in Figure 1 or 2, researchers from other disciplines such as morphology and paleontology had already begun stirring up theoretical interest surrounding the significance of development for evolutionary theory prior to the early 1980s (Love, 2003, 2004).

Kowalevsky that the sessile ascidians, previously classed with the filter-feeding bivalve mollusks produced larvae that were unmistakably of a chordate character, with a swimming tail complete with notochord and dorsal, tubular spinal cord, and muscle tissue along each side. The conclusion was that ascidians had descended from free-swimming chordate ancestors in the distant past and retained the tadpolelike chordate larvae as a relic thereof. The discovery launched a general investigation into the life cycles of animals in the belief that the life histories represented in a condensed form the evolutionary history of the species or class'' (Berrill, '66, 714). Berrill not only reminds us of the significance of Kowalevsky's ascidian embryology in this section entitled 'Evolution and Development' from his undergraduate textbook, but also that the relationship between evolution and development was not primarily about recapitulation but phylogenetic reconstruction. Before turning to descriptions of neoteny and relative growth he made a remark that appears prescient from our present vantage point of the excitement surrounding Evo-devo: ''A question arises concerning to what extent, if any, the course of development sheds light on the evolutionary past of the particular species. At one time it was thought to throw a great deal, but now not very much. The truth may lie between'' (Berrill, '66, 714).

The second reason for reviewing the work of Berrill follows from the high profile role of his work in de Beer's oft-cited and influential synthesis, *Embryos and Ancestors* (de Beer, '58). Berrill's work receives three prominent highlights. In his chapter 'Heterochrony', de Beer cites Berrill's studies on lower chordates that provided a developmental mechanism for getting a larger basic vertebrate embryo from the tiny ascidian. Additionally, in his review of theories of the origin of chordates, de Beer draws attention to Berrill's knowledge of larval adaptation and marine ecology in tracking this key evolutionary juncture and rejecting spurious speculations. Finally, in conjunction with the discussion of recapitulation, Berrill is cited for emphasizing that the repetition of ancestral features in the development of descendants does not imply a biogenetic law for phylogenetic reconstruction but rather signals that these repeated stages or features may be of special importance for understanding the evolution and development of the concerned lineages. These themes dominate Berrill's studies of tunicate development in the *Philosophical Transactions of the Royal Society of London* (Berrill, '30, '31, '35a, b, '36).

Berrill ('30) tackles the ''simple'' ascidians from a ''functional viewpoint'' and attends to the ''typical'' development observed in this group. Despite this typological perspective that would appear to minimize variation, Berrill is careful to highlight variation throughout, such as in the size of ascidian eggs and nature of their perivitelline structures. One intriguing aspect of his developmental explanations is his preference for accounts in terms of mechanical causes. For example, in discussing the turgidity of notochord cells, he claims that, ''[i]t seems probable then that the correlated increase in volume of individuals cells, assuming they be contained within a relatively non-elastic cylinder, i.e., of mesoderm and ectoderm, is sufficient to account for the sliding movements and interdigitation described…''(Berrill, '30, 47).

Berrill ('31) sets out his account of the abbreviation of development in the family Molgulidae in contrast to simple ascidians. This family provides a robust case study for the roles of viviparity and yolk accumulation on the truncation of ontogenetic processes, phenomena now more commonly collected under the term 'direct development' (Hanken, 2003). Berrill discusses the four logical combinations of reproductive mode (oviparous versus viviparous) and type of development (urodele [indirect] versus anural [direct] development). He held that oviparous, indirect development was the ancestral character state and attempted to detail putative mechanistic and 'teleological' (selection) reasons for these transformations, which involved a subtle deployment of knowledge concerning the marine larval ecology of the different species. Berrill became convinced that direct development had arisen multiple times independently in different ascidian lineages.

In the third installment, Berrill ('35a) explored the theme of differential retardation and acceleration of development. He focused on the origin of viviparity, which he attributed to the relative shortening of the oviduct, while concentrating on the significance of cell size and yolk accumulation. Figure 3 gives a taste of how he visually represented these comparative embryological studies. Part 4 (Berrill, '35b) turns to the phenomenon of asexual reproduction via budding, canvassing the compound ascidians more directly while trying to draw out generalizations regarding the morphology, morphogenetic processes, and histological properties of budding. Finally, Berrill

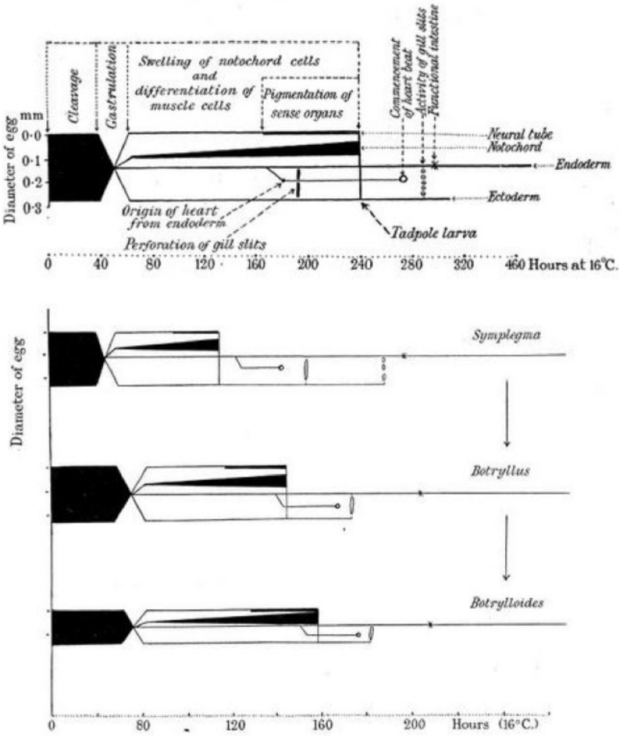

Fig. 3 An example of Berrill's comparative embryological analyses of developmental features focusing on egg size in three genera of ascidians. The top panel, based on the ontogeny of *Clavelina lepadiformis* at 16° C, is the key to understanding the features of the bottom panel graphs, which he believed constituted a genuine phyletic series through decrease in egg size and retardation of ontogenetic events (direction of arrows). (Berrill, '35, 303, 322) Note the numerous heterochronies for most of the features. Reprinted by permission of the Royal Society of London.

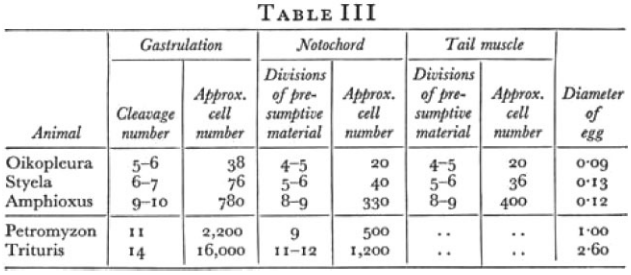

TABLE III

	Gastrulation		*Notochord*		*Tail muscle*		
Animal	*Cleavage number*	*Approx. cell number*	*Divisions of pre-sumptive material*	*Approx. cell number*	*Divisions of pre-sumptive material*	*Approx. cell number*	*Diameter of egg*
Oikopleura	5–6	38	4–5	20	4–5	20	0·09
Styela	6–7	76	5–6	40	5–6	36	0·13
Amphioxus	9–10	780	8–9	330	8–9	400	0·12
Petromyzon	11	2,200	9	500	..	..	1·00
Trituris	14	16,000	11–12	1,200	..	..	2·60

Fig. 4 Berrill's synthesis of developmental data that was deployed in his arguments concerning the origin of the vertebrates (Berrill '55) The key element in his thinking was the relationship of the increase in egg size to the number of cell division cycles at the time of gastrulation that resulted in a greater number of cells at the time when chordate features differentiate. Reprinted by permission of Oxford University Press.

('36) attempts to "trace the probable course of evolution within ascidians." These studies served as the basic platform for his later discussion of vertebrate origins (Berrill, '55), where he followed up on Garstang's hypothesis that the chordate lineage was derived from a neotenous larval ascidian. (Garstang, '28) Ironically, he held that Garstang's putative transitional scenario was still tainted with recapitulatory themes and Berrill attempted to articulate different mechanistic and selective explanations for the origin of chordates from ascidian tadpoles. For example, with respect to developmental mechanics, he attempted to explain the origin of vertebrates via a mechanism of size increase by slippage in the number of cell division cycles relative to gastrulation to get a larger basic vertebrate embryo from a tiny ascidian. (Fig. 4) These themes about the role of egg size, mode of development, and evolution were not confined to the tunicates. Berrill can also be seen applying these ideas to other marine invertebrates, such as hydromedusae (Berrill, '50).

Berrill devoted considerable attention to fields and gradients throughout his embryological discussions (Berrill, '61) and it comes as little surprise that his formative influences included C.M. Child, D'Arcy Thompson, and Joseph Needham (Scriver, '99). The influence of Thompson can be seen through the preference for mechanical explanations of ontogeny and in the invitation to contribute to a festschrift for him. (Berrill, '45) Later in life he would comment that, "[i]n contemporary biology the analytical-reductionist approach now holds sway, whereas the complementary holistic-spatial-intuitive approach (the classical one, perhaps) is to a great extent ignored" (Berrill, '84, 2).

Anderson and the ontogeny and phylogeny of annelids and arthropods

Another important thread in the comparative program leading to modern Evo-devo research was contributed by D.T. Anderson, who carried out a number of comparative embryological studies. Anderson, in his best-known work, marshaled the descriptive comparative embryology of various segmented invertebrates in an effort, "to utilize the morphological facts of embryonic development in these animals in the furtherance of speculations on their phylogenetic relationships" (Anderson, '73, Preface). The sources for this synoptic volume were culled from a variety of detailed articles on the basic embryology of different invertebrate species (Anderson, '59,'62, '66a,b,c, '69; cf. Anderson, 2001). Anderson's work was important in two ways. The first was in re-awakening the debate about the phylogenetic origins of the arthropods. His work supplied a body of comparative embryology and a solid hypothesis for the polyphyletic origin of arthropods. This hypothesis led to tests of arthropod monophyly versus polyphyly by the growing enterprise of molecular systematics. This phylogenetic stream has been of great significance to Evo-devo, arising out of an interesting juxtaposition of new theoretical underpinnings in systematics (cladistics) and, close on its heels, an abundant new data set from which to build hypotheses about the interrelationships of animal and plant species. The utilization of molecular evidence to overturn traditional morphological phylogenetic inferences (including Anderson's hypothesis of arthropod polyphyly) has recently been a prevalent theme. From an initial challenge to the assumed monophyly of metazoans (Field et al., '88) to a more recent 18S ribosomal DNA analysis that grouped arthropods, tardigrades, onychophorans, nematodes, nematomorphs, kinorhynchs, and priapulids into a new clade of molting animals (Ecdysozoa) (Aguinaldo et al., '97), molecular studies are quickly determining taxonomic orthodoxy (Adoutte et al., '99, 2000). Although there is some opposition to these results (Nielsen, 2001), the current momentum lies with the molecular approach. Although fascinating, we are not able to explore these issues further here.

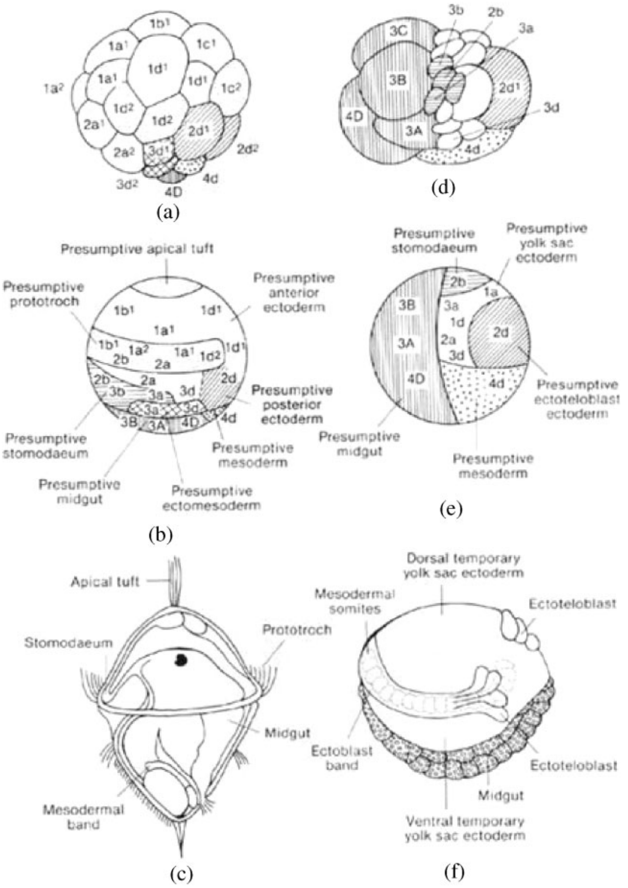

Fig. 5 An example of how Anderson's comparative embryology was adapted by Raff and Kaufman (2003). Cell lineage homologies allow an analysis of how developmental patterns and larval forms evolve. Reprinted by permission of DT Anderson and Indiana University Press.

More to the point of the evolving themes of an experimental discipline of evolutionary developmental biology, Anderson, like Berrill and others such as Gary Freeman (Goldstein and Freeman, '97), recognized the importance of comparative embryology and carried out these detailed studies, as well as synthesizing large quantities of existing data. Anderson did an exhaustive literature search on annelid and arthropod embryonic development and used this existing cell lineage and fate map data to prepare presumptive fate maps for a number of annelids and arthropods. These provided the raw material for comparisons among taxa, which Anderson made in minute detail. These fate maps also provided an enormous resource for comparisons of cell allocation in evolution of related groups that exhibit different developmental modes, e.g. annelid classes. These comparisons were adopted by Raff and Kaufman ('83; Fig. 5), to illustrate the potential power of this approach for cell lineage studies as an evolutionary and comparative tool. Cell lineage had been a major approach to embryology at the turn of the century, but only returned to a high level of prominence in developmental biology in the 1980s, notably with the publication of the *Caenorhabditis elegans* cell lineage (Sulston et al., '83). The full potential of cell lineage analyses for evolutionary purposes had largely lain fallow since the pioneering studies of F.R. Lillie on the clam *Unio* (Lillie, 1895; cf. Guralnick, 2002). Anderson's work provided an enormous database of fate map comparisons that inspired the further use of cell lineage work in Evo-devo. In addition, as we will note below, it was Anderson's continued interest in describing and comparing the embryology of invertebrates, especially the evolution of life history strategies in marine species, that provided the point of departure for our laboratory's work on mechanisms of developmental evolution in sea urchins.

Old problems, new tools: the evolutionary developmental biology of Heliocidaris

During the past two decades, Evo-devo has emerged as an active and recognized research discipline, which has its foundations both in the enduring problems of comparative evolutionary embryology and phylogenetics, as well as in the powerful experimental approaches of the new developmental genetics. The results are appearing from a number of contemporary laboratories, including (in hardly an exhaustive list) studies of ascidians (Jeffery et al., '99), amphioxus (Holland, 2002), hemichordates (Takacs et al., 2002; Lowe et al., 2003), a swarm of arthropods (Hughes and Kaufman, 2002), nematodes (Sommer, 2000), fish (Parichy and Johnson, 2001; Yamamoto et al., 2003), frogs (Callery et al., 2001), snakes (Cohn and Tickle, '99), starfish (Hart et al., '97), and sea urchins (Raff, '96; Wray, 2000). All of these systems offer particular advantages in addressing specific problems in the evolution of development, such as the role of Hox genes in the evolution of body form. As our laboratory has focused on the evolution of sea urchin larvae, we will use this example to exemplify the synthesis of comparative developmental biology and developmental genetics.

Heliocidaris is a genus of Australian sea urchins that has two species, *tuberculata* and *erythrogramma*, separated by about 4 myr (Zigler et al., 2003). We were attracted to these animals, or more precisely to their embryos, following a description by D. T. Anderson's laboratory of the development of *H. erythrogramma* (Williams and Anderson, '75). This species produces a large (430 micron diameter) egg that develops directly into a juvenile sea urchin in three to four days. *H. tuberculata* has a more typically sized small egg (90 microns) that develops via a feeding pluteus larva over about a six week period. This is the ancestral form of development for sea urchins and it is from this mode of development that the direct-developer, *H. erythrogramma*, has evolved. This pair of species has provided a dramatic and useful comparative system for studying the evolution of development and life history. This is not the place to review the extensive data that *Heliocidaris* has yielded; rather, we would like to discuss the overarching conceptual themes that have motivated and guided the work.

Two broad kinds of questions were asked in the studies of *Heliocidaris erythrogramma*. The first questions were developmental investigations meant to reveal the mechanistic workings of the embryo, and the second were evolutionary and comparative questions. These questions led to experimental approaches that combine traditional embryological methodology with the last two decades of developmental genetics. Among the first studies necessary to define the development of *H. erythrogramma* was the determination of its embryonic cell lineage (Wray and Raff, '90), which allows one to ascertain what cells of an embryo give rise to the different parts of the larva. A second step was to determine basic features of its embryology (Henry et al., '90). These are the kind of data that have been used to define the basics of embryonic development since the 1890s. Despite the antiquity of cell lineage studies, new technical innovations have made this one of the most informative types of developmental data. In addition, such data are also powerful tools in making evolutionary comparisons when a phylogenetic framework exists. The cell lineage pattern of *H. erythrogramma* is highly derived with respect to that of indirect-developing sea urchins that are basal to the direct developer (Raff, '99).

Other developmental studies of *H. erythrogramma* have addressed inductive interactions between both embryonic and developing adult tissues by microsurgical techniques (Minsuk and Raff, 2002; Henry et al., '90). These basic approaches go back to Spemann, and numerous other studies on induction in vertebrate embryos. Microsurgical approaches have been refined in many laboratories to include a wide variety of organisms, and they are now commonly combined with in situ hybridization to examine effects of tissue interactions on gene expression. We have also made use of cross species hybrids. (Fig. 6) These have a long history in studies of sea urchin development, particularly in attempts to understand maternal effects. Our studies were made with evolutionary questions in mind (Raff et al., '99; Raff et al., 2003). Most of our recent research on *H. erythrogramma* has fallen into a broadly developmental genetic framework, with an emphasis on understanding patterns of gene expression, isolating candidate genes, and manipulation of gene expression in embryos (Haag and Raff, '98; Kauffman and Raff, 2003; Ferkowicz et al., '98; Haag et al., '99; Wilson et al., unpublished observations). The major intellectual impetus to our

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We should note that Raff's formal training was in biochemistry and developmental biology, which likely biased the laboratory's research approaches. It is clear that Evo-devo studies can be profitably approached from other disciplinary foundations, such as microevolutionary biology (e.g., Nijhout and Emlen, '98; Monteiro et al., 2003).

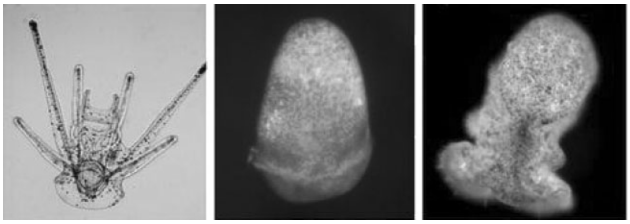

Fig. 6 Developing larvae of sea urchins of the genus *Heliocidaris*, in which radical changes in development have evolved. *H. tuberculata* (left), *H. erythrogramma* (center), and the crossspecies hybrid (right). Reprinted by permission of the Company of Biologists.

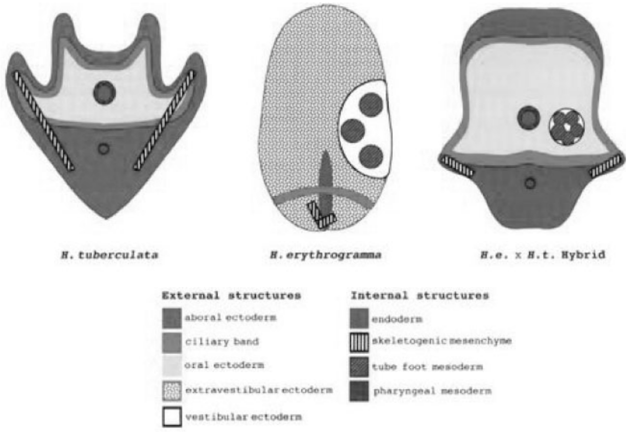

Fig. 7 Larval Territories in *H. tuberculata*, *H. erythrogramma*, and the cross-species hybrid (Nielsen et al., 2000). Note that *H. erythrogramma* lacks oral ectoderm (yellow) and that it is restored in the H.e. x H.t. hybrids. Reprinted by permission of Blackwell Publishing.

research on *H. erythrogramma* was, however, driven by the questions derived from evolutionary embryology, as well as from the utility of these two closely related species for comparative studies. These can be deconstructed into several categories of questions arising from comparative and evolutionary traditions.

Heterochrony

The classical ''universal'' basis for interpretations of the evolution of development through much of the 20th century was heterochrony (Gould, '77). The first analysis of *H. erythrogramma* showed that there are apparent global heterochronies between direct and indirect developers, and these heterochronies gave the first grounds for evolutionary interpretations (Raff, '87). These observations are analogous to those made by Berrill but in themselves do not yield penetrating insights into mechanisms of change. As a better genetic understanding of development has appeared, heterochrony as a primary means of defining evolutionary changes has given way to more mechanistically based explanations. Indeed, once more detailed embryological and anatomical understanding of *H. erythrogramma* embryos and larvae were obtained, evolutionary interpretations could be based on firmer grounds. Heterochronies did not disappear, but could be interpreted at different hierarchical levels on the bases of particular molecular changes or alterations in the timing of gene expression (Ferkowicz and Raff, 2001).

Homology, modules, and convergence

Homologies, or more accurately inferences of homologies, provide the basis for all evolutionary comparisons of morphological features as well as of gene sequences. They are no less important to understanding the evolution of embryos and larvae. *H. erythrogramma* larvae differ overall in appearance from the larvae of indirect developers but some homologous features are apparent because they are conserved in morphology. Others are not obvious to inspection. (Fig. 7) This has led us to seek homologies by means of two approaches. The first is the use of molecular markers, using genes that have tissue-specific patterns of expression in indirect developers (Raff and Sly, 2000). In a number of cases, tissues of *H. erythrogramma* express the same markers in presumptive homologous tissues as do indirect developers. In other cases, there have been changes. The patterns of gene expression noted in such studies confirm the idea of territories that arose from the cell lineage studies of indirect-developing sea urchins made by Cameron and colleagues ('87). *H. erythrogramma* has territories that correspond to those of indirect developers but has apparently lost one of them, the oral ectoderm.

We have taken a second approach using cross-species hybrids in which *H. erythrogramma* is fertilized by sperm of *H. tuberculata* (Raff et al., '99) The resulting hybrid embryos produce a pluteus-like morphology and they exhibit recognizable territories, including an oral ectoderm (Nielsen et al., 2000). These results confirmed those from molecular markers, and have led us to other interesting issues. Perhaps the most important is the recognition that the territories of sea urchin embryos correspond closely to developmental modules and the criteria used to define modules can be applied (Raff, '96). This places the study of gene expression territories as homologous features into the various theoretical frameworks available to account for the evolution of modules (Wagner, '96), and embryological studies can be made that are predicated on developmental concepts of modularity, e.g. fields (Gilbert et al., '96).

Gene expression results and morphology suggest that homologous features exist in the two larvae, and that part of the evolutionary transformation of development amounts to modifications of shared homologues. This has allowed us to define particular transformations at various levels. Thus, the pluteus aboral ectoderm can be considered a homologue to the extravestibular ectoderm of *H. erythrogramma*, although cell shapes are different, and expression of a key aboral ectoderm gene has been lost (Kissinger et al., '97; Nielsen et al., 2000). In the case of the oral ectoderm, a crucial territory in the pluteus that evidently has been lost in *H. erythrogramma*, a quite different kind of event has taken place. (An alternative hypothesis postulates that the extravestibular ectoderm of *H. erythrogramma* is a novel module fusing the oral and aboral ectoderm territories, Love and Raff, unpublished observations.) These different observations have provided the foundation for studying the role of homologous features in evolution and, in conjunction with different hypotheses, have played a key role in seeking candidate regulatory genes that might play crucial roles in the transformation of larval form. (Raff, '99) The expression and function of such genes has allowed us to identify and experimentally test potential large effect genes.

Evolution of maternal effects

The evolution of larval forms might well be thought of as a matter of the evolution of embryonic and larval features per se, with gains, losses, and modifications lying in genes expressed during different stages of ontogeny. To a large extent this is true, but early in our comparisons of *H. erythrogramma* embryology with that of indirect-developing sea urchins, we discovered that important features of axial specification had shifted to maternal specification (Henry et al., '90). Maternal effects refer to any consequences on developing embryos that arise from the action of genes acting in the mother or during oogenesis. The actions of maternal effect genes can provide nutrients in the egg, as well as maternal mRNAs and elements of egg structure that supply developmental information. All of these aspects of maternal effects have changed in the evolution of *H. erythrogramma*. Thus, oogenesis has been modified by a novel phase of egg provisioning that produces the large lipid-rich egg (Byrne et al., '99).

The composition of the maternally provisioned lipids has changed, as have mechanisms for synthesizing lipid components (Villinski et al., 2002, in press). Evolution of maternal information was noted in our early embryological experiments (Henry et al., '90), and studies of cross species hybrids also show pronounced evolutionary changes in egg organization (Raff et al., '99). The changes in maternal effects observed in *H. erythrogramma* are important in that they show that dramatic novel features have been gained in addition to the losses in larval features that are morphologically obvious. The new larval form is not merely a reduced entity; gains as well as losses underlie the evolution of the direct developing larva.

Large effect genes in rapid evolution, regulatory punctuated equilibrium, and convergence

Recent theoretical work in microevolution has suggested that genes of both large and small effect may play prominent roles in evolution (Orr, '98). Empirical observations of evolution in disparate organisms indicate that a small number of genes may have large roles in morphological change in short periods of evolutionary time (Orr, 2001; Peichel et al., 2001). This idea played a role in the early history of Evo-devo in the ideas of Goldschmidt with his views that genes producing macromutations, and thus large and sudden evolutionary effects, could drive rapid evolution. We now have a more balanced view that suggests that differences in macro-and micro-evolutionary phenomena can be bridged.

We have sought candidate regulatory genes for major steps in the evolution of *H. erythrogramma*. The oral ectoderm, which plays a substantial role in the regulation of pluteus development but is lost in *H. erythrogramma*, offers a promising source of such genes. We have isolated the genes encoding transcription factors that have major regulatory functions in the development of oral ectoderm and its interaction with other larval territories (Wilson et al., unpublished observations; Zhou et al., 2003). We have shown both from changes in expression patterns and effects in missexpression and knockout studies that some of them appear to be involved in the loss of pluteus features in *H. erythrogramma*. One gene in particular, *goosecoid*, has large effects in the restoration of pluteus-like features when misexpressed in *H. erythrogramma* (Wilson et al., unpublished observations). The existence of these genes and our ability to manipulate their action in living embryos offers some of the technical and conceptual advantages of developmental genetics to address classic Evo-devo questions in non-model organisms with complex life histories.

The swift evolution of *H. erythrogramma* and evidence for the action of genes with large phenotypic effects support a hypothesis of the rapid evolution of direct development with a few key genes playing major roles in the evolutionary transition. These observations implied that regulatory punctuated equilibrium occurs in sea urchin larval evolution. To test for punctuated equilibrium, we made hybrids of *H. erythrogramma* with both close (4 myr diverged) and distant (40 myr diverged) indirect-developing species. Both gave similar hybrids, consistent with slow evolution of pluteus developmental regulatory systems (Raff et al., 2003).

Convergence is one of the truly prevalent phenomena of macroevolution, but it is little studied at the level of developmental evolution. Our ability to make hybrids between distantly related sea urchin species has allowed us to ask about underlying regulatory convergence. Direct-developing larvae have arisen independently many times in various clades of sea urchins. These larvae are morphologically similar (Sly et al., 2003). To test for possible underlying regulatory convergences, we made hybrids between two independently evolved direct developers in families that are 70 myr diverged. The development of these hybrids was surprisingly harmonious, and produced no pluteus-like features, consistent with the hypothesis of convergence (Raff et al., 2003).

Speculative biology: larval origins

The creation of evolutionary scenarios has been a prominent part of evolutionary biology. This allows specific concrete findings to be integrated with other results, for evolutionary events to be placed in a temporal sequence, and for broader hypotheses to be generated. In the case of the evolution of larval features and life history, polarization can be provided by inferences of character evolution from phylogenetic analyses (Jeffery et al., 2003), and time limits from molecular clocks (Zigler et al., 2003). In the case of direct-developing sea urchins, the basal state of a small egg that proceeds via indirect development and a feeding pluteus larva is well established (Raff, '96). Several questions arise. What selective pressures triggered the change in development? How rapid was it? What was the order of developmental changes that took place? Are independently evolved direct developers convergent in their underlying developmental changes? At this point we do not know what selective pressures drove the evolution of direct development within the *H. erythrogramma* lineage, although there is a substantial literature on ecological correlates of developmental modes in marine animals (Olive, '85), as well as on selective pressures that affect the evolution of developmental features (West-Eberhard, 2003).

As discussed above, we have been able to obtain answers to other crucial questions and we here can suggest a rough scenario for the evolution of the ontogeny of *H. erythrogramma*. Essentially, the initial step probably involved the evolution of a large enough egg to allow facultative feeding. After that point, feeding structures might have been rapidly lost (Wray, '96). Such a step could have involved changes in expression of large effect genes important to development of feeding larval features. Our work has begun to trace such a regulatory scenario. Thus, we have found that oogenesis in *H. erythrogramma* involves a novel post-vitellogenic stage that results in large yolk poor, lipid rich eggs (Byrne et al., '99). We have also documented changes in expression and functions in *H. erythrogramma* of regulatory genes involved in the development of major pluteus features (Raff et al., '99; Wilson et al., unpublished observations; Zhou et al., 2003). These changes are expected to be important to the rapid loss of unneeded feeding features. Last, an acceleration of embryonic primary axial determination and rates of development of adult structures should have followed, in as yet poorly understood ways. We have documented a number of heterochronies in *H. erythrogramma* that show that these events have occurred, and we are now finding some of the regulatory genes involved (Ferkowicz et al., '98; Kauffman and Raff, 2003, and, unpublished observations).

There is a century-long speculative literature on larval origins and complex life histories. Although larval origins has not per se been a direct target of our work, *H. erythrogramma* has yielded a number of insights on how larvae may have originated (Sly et al., 2003). In essence, the observed rapidity of dramatic changes in larval structure, combined with a high frequency of gene co-option, suggests that feeding larval features might have been inserted into the ontogenies of basal direct-developing Cambrian bilaterians once these animals grew large enough to make sizable numbers of planktotrophic larvae to exploit novel ecological niches available to such larvae. Secondary direct developers like *H. erythrogramma* have arisen repeatedly in several lineages of animals with long conserved planktotrophic larvae in response to shifting ecological demands. Early development evolves often, rapidly, and with great exuberance.

Concluding remarks

The problem agenda derived from the intellectual lineage of comparative evolutionary embryology that we have emphasized has been enriched and facilitated by the ideas and experimental tools that have been developed in the past twenty years to investigate problems in developmental genetics. Streicher and Müller express a sentiment we share when they end a paper describing three dimensional reconstruction of embryos and gene expression patterns as follows: ''The comparison of 3D and 4D expression patterns of homologous genes in several species can become a powerful *tool* to approach the *problem* of phylogenetic transformation of organismal form, thereby addressing the fifth dimension of life, evolution. (Streicher and Müller, 2001; our emphasis) Keeping the distinction between tools and problems is even more critical when increasingly popular high-throughput genomic approaches involve scores of researchers, many of whom may have little familiarity with the rationale behind a particular laboratory strategy. The loss of emphasis on phylogenetic questions in the tradition leading up to developmental genetics and demand for organisms that can be easily manipulated experimentally is arguably one reason why Evo-devo suffers from the drawbacks of the model organism bias in contemporary developmental biology. (Hall, '99, 123–126) To illustrate with one salient case, the ascidian *Ciona intestinalis* has recently had its genome sequenced and is readily proving itself a new standard system for studying the evolution of development. (Dehal et al., 2002) But would *Ciona* have been Berrill's recommended choice of organism? Yes, if he was going to compare ascidians to other taxa, since he took *Ciona* to be representative of the ancestral form. But Berrill was worried that comparative investigations using widely separated taxa allowed for the obscuring of important information because of evolutionary processes. (Berrill, '35a, 256). Instead he likely would have fixed on two closely related species whose development was different in some critical respect, as in his focus on the Molgulidae. (Berrill, '31; cf. Jeffery and Swalla, '92).

Regardless of these differences, each research program is shaped by the investigative problems, such as the origin of chordates and vertebrates, and the reciprocal interaction between developmental and evolutionary processes. They also reflect fundamental differences in how comparative biology is executed: compare closely related species to understand particular developmental differences, or, assess the ontogenetic similarities and differences across wide taxonomic distances between 'representative' members of specific taxa. Both strategies are legitimate but the latter is highly preferred by many Evo-devo researchers. The heritage from comparative evolutionary embryology encourages a more balanced approach and in our estimation best characterizes the strategy we have adopted with *Heliocidaris*.

A different consequence of our analysis here and elsewhere (Love and Raff, 2003) suggests more historical attention should be paid to the diversity of embryological research in the middle years of the 20th century, especially for understanding what it meant for embryology to be excluded from the Modern Synthesis and also how these different strands of developmental research transformed themselves in the face of molecularization. One recent study has insightfully addressed the latter issue in a Belgian context with respect to Albert Dalcq (Theiffry, 2001). Individuals like de Beer, Berrill, and Anderson characterized a school of highly expert anatomists and comparative embryologists who carried on vigorous programs rightly construed as Evo-devo during the middle half of the 20th century. Their work was emphatically not in the mainstream of experimental embryology or molecular genetics, which jointly fostered the approaches in developmental genetics that have flourished in the past twenty years. More attention to the disciplinary specifics within embryology and their various relations to evolutionary research over the course of the 20th century is critical to avoid pitfalls that have plagued researchers trying to juxtapose evolutionary and developmental studies. Without this perspective it may only be a matter of time before another Bateson arises, discontented with the inability of contemporary methods to resolve biological questions of interest, complaining that, ''Out of the same facts … men [and women] of equal ability and repute have brought the most opposite conclusions.…From the same facts opposite conclusions are drawn. Facts of the same kind will take us no further. The issue turns not on the facts but on the assumptions. Surely we can do better than this'' (Bateson, 1894 ['92], vi). For the moment, it seems we are.

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LITERATURE CITED

Adams MB. 1973. Kowalevsky, Aleksander Onufrievich. In: Gillispie CC, editor. Dictionary of Scientific Biography, Volume VII. New York: Charles Scribner's Sons. p 474–477.

Adoutte A, Balavoine G, Lartillot N, de Rosa R. 1999. Animal Evolution: the end of intermediate taxa? Trends in Genetics 15:104–108.

Adoutte A, Balavoine G, Lartillot N, Lespinet O, Prud'homme B, de Rosa R. 2000. The new animal phylogeny: Reliability and implications. Proc Natl Acad Sci U S A 97:4453–4456.

Aguinaldo AMA, Turbeville JM, Linford LS, Rivera MC, Garey J, Raff RA, Lake JA. 1997. Evidence for a clade of nematodes, arthropods and other moulting animals. Nature 387:489–493.

Allen GE. 1976. Life Science in the Twentieth Century. New York: Wiley.

Anderson DT. 1959. The embryology of the polychaete Scoloplos armiger. Q J Microsc Sci 100:89–166.

Anderson DT. 1962. The embryology of Dacus tryoni (Frogg.) (Diptera, Trypetidae (=Tephritidae)), the Queensland fly. Journal of Embryology and Experimental Morphology 12:65–75.

Anderson DT. 1966a. The comparative embryology of the Diptera. Annu Rev Entomol 11:23–46.

Anderson DT. 1966b. The comparative embryology of the Oligochaeta, Hirudinea and Onychophora. Linneanciety (NSW) 91:10–43.

Anderson DT. 1966c. The comparative embryology of the Polychaeta. Acta Zool 47:1–42.

Anderson DT. 1969. On the embryology of the cirripede crustaceans Tetraclita rosea (Krauss), Tetraclita purpurascens (Wood), Chthamalus antennatus Darwin and Chamaesipho columna (Spengler), and some considerations of crustacean phylogenetic relationships. Philos Trans R Soc Lond B Biol S 256:183–235.

Anderson DT. 1973. Embryology and Phylogeny in Annelids and Arthropods. Oxford: Pergamon Press.

Anderson DT, editor. 2001. Invertebrate Zoology. 2nd ed. New York: Oxford University Press.

Arthur W. 2002. The emerging conceptual framework of evolutionary developmental biology. Nature 415: 757–764.

Bateson W. 1894 [1992]. Materials for the Study of Variation treated with especial regard to Discontinuity in the Origin of Species. Baltimore, MD: The Johns Hopkins University Press.

Berrill NJ. 1930. Studies in Tunicate Development. Part I.–General Physiology of Development of Simple Ascidians. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 218:37–78.

Berrill NJ. 1931. Studies in Tunicate Development. Part II.–Abbreviation of Development in the Molgulidae. Philos Trans R Soc Lond B Biol Sci 219:281–346.

Berrill NJ. 1935a. Studies in Tunicate Development. Part III.–Differential Retardation and Acceleration. Philos Trans R Soc Lond B Biol Sci 225:255–326.

Berrill NJ. 1935b. Studies in Tunicate Development. Part IV.–Asexual Reproduction. Philos Trans R Soc Lond B Biol Sci 225:327–379.

Berrill NJ. 1936. Studies in Tunicate Development. Part V.–The Evolution and Classification of Ascidians. Philos Trans R Soc Lond B Biol Sci 226:43–70.

Berrill NJ. 1945. Size and organization in the development of ascidians. In: Clark WEL, Medawar PB, editors. Essays on growth and form, presented to D'Arcy Thompson. Oxford: Clarendon Press. p 231–263.

Berrill NJ. 1950. Development and Medusa-Bud Formation in Hydromedusae. Q Rev Biol 25:292–316.

Berrill NJ. 1955. The Origin of Vertebrates. Oxford: Clarendon Press.

Berrill NJ. 1961. Growth, Development, and Pattern. San Francisco: W.H. Freeman and Company.

Berrill NJ. 1966. Biology in Action: A Beginning College Textbook. New York: Dodd, Mead, & Company, Inc.

Berrill NJ. 1984. The Pearls of Wisdom: An Exposition. Perspect Biol Med 28:1–16.

Bowler PJ. 1996. Life's Splendid Drama: Evolutionary Biology and the Reconstruction of Life's Ancestry, 18601940. Chicago: University of Chicago Press.

Brauckmann S, Gilbert SF. 2004. Sucking in the gut: A brief history of early studies on gastrulation. In: Stern CD, editor. Gastrulation. Cold Spring Harbor: Cold Spring Harbor Press.

Burian RM, Gayon J, Zallen DT. 1991. Boris Ephrussi and the Synthesis of Genetics and Embryology. In: Gilbert SF, editor. Developmental Biology, A Comprehensive Synthesis: Volume 7, A Conceptual History of Modern Embryology. New York: Plenum Press. p 207–227.

Byrne M, Villinski JT, Cisternas P, Popodi EM, Raff RA. 1999. Maternal factors and the evolution of developmental mode: Evolution of oogenesis in Heliocidaris erythrogramma. Dev Genes Evol 209:275–283.

Callery EM, Fang H, Elinson RP. 2001. Frogs without polliwogs: evolution of anuran direct development. BioEssays 23:233–241.

Cameron RA, Hough-Evans BR, Britten RJ, Davidson EH. 1987. Lineage and fate of each blastomere of the eightcell sea urchin embryo. Genes Dev 1:75–85.

Carroll SB, Grenier JK, Weatherbee SD. 2001. From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design. Malden, MA: Blackwell Science, Inc.

Cohn MJ, Tickle C. 1999. Developmental basis of limblessness and axial patterning in snakes. Nature 399:474–479.

de Beer GR. 1958. Embryos and Ancestors. London: Oxford University Press.

Dehal P, Satou Y, Campbell RK, Chapman J, Degnan B, De Tomaso A, Davidson B, Di Gregorio A, Gelpke M, Goodstein DM, Harafuji N, Hastings KEM, Ho I, Hotta K, Huang W, Kawashima T, Lemaire P, Martinez D, Meinertzhagen IA, Necula S, Nonaka M, Putnam N, Rash S, Saiga H, Satake M, Terry A, Yamada L, Wang H-G, Awazu S, Azumi K, Boore J, Branno M, Chin-bow S, DeSantis R, Doyle S, Francino P, Keys DN, Haga S, Hayashi H, Hino K, Imai KS, Inaba K, Kano S, Kobayashi K, Kobayashi M, Lee B-I, Makabe KW, Manohar C, Matassi G, Medina M, Mochizuki Y, Mount S, Morishita T, Miura S, Nakayama A, Nishizaka S, Nomoto H, Ohta F, Oishi K, Rigoutsos I, Sano M, Sasaki A, Sasakura Y, Shoguchi E, Shin-i T, Spagnuolo A, Stainier D, Suzuki MM, Tassy O, Takatori N, Tokuoka M, Yagi K, Yoshizaki F, Wada S, Zhang C, Hyatt PD, Larimer F, Detter C, Doggett N, Glavina T, Hawkins T, Richardson P, Lucas S, Kohara Y, Levine M, Satoh N, Rokhsar DS. 2002. The Draft Genome of Ciona Intestinalis: Insights into Chordate and Vertebrate Origins. Science 298:2157–2167.

Duffy JB. 2002. GAL4 System in Drosophila: A Fly Geneticist's Swiss Army Knife. Genesis 34:1–15.

Ferkowicz MJ, Raff RA. 2001. Wnt gene expression in sea urchin development: Heterochronies associated with the evolution of developmental mode. Evol Dev 3:24–33.

Ferkowicz MJ, Stander MC, Raff RA. 1998. Phylogenetic Relationships and Developmental Expression of Three Sea Urchin Wnt Genes. Mol Biol Evol 15:809–819.

Field KG, Olsen GJ, Lane DJ, Giovannoni SJ, Ghiselin MT, Raff EC, Pace NR, Raff RA. 1988. Molecular Phylogeny of the Animal Kingdom. Science 239:748–753.

Garstang W. 1928. The Morphology of the Tunicata, and its bearings on the Phylogeny of the Chordata. Q J Microsc Sci 72:51–187.

Gilbert SF. 1991. Induction and the Origin of Developmental Genetics. In: Gilbert SF, editor. Developmental Biology, A Comprehensive Synthesis: Volume 7, A Conceptual History of Modern Embryology. New York: Plenum Press. p 181–206.

Gilbert SF. 2000a. Developmental Biology. Sunderland, MA: Sinauer Associates, Inc.

Gilbert SF. 2000b. Diachronic Biology meets Evo-Devo: C.H. Waddington's approach to Evolutionary Developmental Biology. Am Zool 40:729–737.

Gilbert SF. 2003. Opening Darwin's black box: teaching evolution through developmental genetics. Nat Rev Genet 4:735–741.

Gilbert SF, Bolker JA. 2001. Homologies of Process and Modular Elements of Embryonic Construction. In: Wagner GP, editor. The Character Concept in Evolutionary Biology. San Diego: Academic Press. p 437–456.

Gilbert SF, Burian RM. 2003. Development, Evolution, and Evolutionary Developmental Biology. In: Hall BK, Olson WM, editors. Keywords and Concepts in Evolutionary Developmental Biology. Cambridge, MA: Harvard University Press. p 61–68.

Gilbert SF, Opitz JM, Raff RA. 1996. Resynthesizing evolutionary and developmental biology. Dev Biol 173: 357–372.

Goldstein B, Freeman G. 1997. Axis specification in animal development. BioEssays 19:105–116.

Gould SJ. 1977. Ontogeny and Phylogeny. Cambridge, MA: Belknap/Harvard University Press.

Guralnick R. 2002. A Recapitulation of the Rise and Fall of the Cell Lineage Research Program: The Evolutionary-Developmental Relationship of Cleavage to Homology, Body Plans, and Life History. J Hist Biol 35:537–567.

Haag ES, Raff RA. 1998. Isolation and characterization of three mRNAs enriched in embryos of the directdeveloping sea urchin *Heliocidarus erythrogramma*: evolution of larval ectoderm. Dev Genes Evol 208:188–204.

Haag ES, Sly BJ, Andrews ME, Raff RA. 1999. Apextrin, a Novel Extracellular Protein Associated with Larval Ectoderm Evolution in Heliocidaris erythrogramma. Dev Biol 211:77–87.

Hall BK. 1999. Evolutionary Developmental Biology. Dordrecht: Kluwer Academic Publishers.

Hall BK. 2000. Balfour, Garstang, and de Beer: The First Century of Evolutionary Embryology. Am Zool 40:718–728.

Hamburger V. 1980. Embryology and the Modern Synthesis in Evolutionary Theory. In: Mayr E, Provine WB, editors. The Evolutionary Synthesis: Perspectives on the Unification of Biology. Cambridge, Mass.: Harvard University Press. p 97–112.

Hamburger V. 1988. The Heritage of Experimental Embryology: Hans Spemann and the Organizer. New York: Oxford University Press.

Hanken J. 2003. Direct Development. In: Hall BK, Olson WM, editors. Keywords and Concepts in Evolutionary Developmental Biology. Cambridge, MA: Harvard University Press. p 97–102.

Hart MW, Byrne M, Smith MJ. 1997. Molecular phylogenetic analysis of life-history evolution in asterinid starfish. Evolution 51:1848–1861.

Henry JJ, Wray GA, Raff RA. 1990. The dorsoventral axis is specified prior to first cleavage in the direct developing sea urchin Heliocidaris erythrogramma. Development 110: 875–884.

Holland LZ. 2002. Heads or tails? Amphioxus and the evolution of anterior-posterior patterning in deuterostomes. Dev Biol 241:209–228.

Holland PWH. 1999. The future of evolutionary developmental biology. Nature 402:C41–C44.

Hoβfeld U, Olsson L. 2003. The road from Haeckel. The Jena Tradition in evolutionary morphology and the origins of ''Evo-Devo.'' Biology and Philosophy 18:285–307.

Hughes CL, Kaufman TC. 2002. Hox genes and the evolution of the arthropod body plan. Evol Dev 4:459–499.

Jeffery CH, Emlet RB, Littlewood DT. 2003. Phylogeny and evolution of developmental mode in temnopleurid echinoids. Mol Phylogenet Evol 28:99–118.

Jeffery WR, Swalla BJ. 1992. Evolution of Alternate Modes of Development in Ascidians. BioEssays 14:219–226.

Jeffery WR, Swalla BJ, Ewing N, Kusakabe T. 1999. Evolution of the ascidian anural larva: evidence from embryos and molecules. Mol Biol Evol 16:646–654.

Kauffman JS, Raff RA. 2003. Patterning Mechanisms in the Evolution of Derived Developmental Life Histories: the Role of Wnt Signaling in Axis Formation of the Direct-developing Sea Urchin Heliocidaris erythrogramma. Dev Genes Evol 213:612–624.

Kissinger JC, Hahn JH, Raff RA. 1997. Rapid evolution in a conserved gene family. Evolution of the actin gene family in the sea urchin genus Heliocidaris and related genera. Mol Biol Evol 14:654–665.

Laubichler MD, Maienschein J. 2003. Ontogeny, Anatomy, and the Problem of Homology: Carl Gegenbaur and the American Tradition of Cell Lineage Studies. Theory in Biosciences 122:194–203.

Lillie FR. 1895. The embryology of the Unionidae. J Morphol 10:1–100.

Love AC. 2003. Evolutionary Morphology, Innovation, and the Synthesis of Evolutionary and Developmental Biology. Biology and Philosophy 18:309–345.

Love AC. 2004. Morphological and Paleontological Perspectives for a History of Evo-Devo. In: Maienschein J, Laubichler M, editors. From Embryology to Evo-Devo: MIT Press.

Love AC, Raff RA. 2003. Knowing Your Ancestors: Themes in the History of Evo-Devo. Evol Dev 5:327–330.

Lowe CJ, Wu M, Salic A, Evans L, Lander E, Stange-Thomann N, Gruber CE, Gerhart J, Kirschner M. 2003. Anteroposterior Patterning in Hemichordates and the Origins of the Chordate Nervous System. Cell 113:853–865.

Maienschein J. 1991. Transforming Traditions in American Biology, 1880–1915. Baltimore, MD: The Johns Hopkins University Press.

McGinnis W. 1994. A Century of Homeosis, A Decade of Homeoboxes. Genetics 137:607–611.

McGinnis W, Krumlauf R. 1992. Homeobox Genes and Axial Patterning. Cell 68:283–302.

Mikhailov AT, Gilbert SF. 2002. From development to evolution: the re-establishment of the ‘‘Alexander Kowalevsky Medal.’’ Int J Dev Biol 46:693–698.

Minsuk SB, Raff RA. 2002. Pattern Formation in a Pentameral Animal: Induction of Early Adult Rudiment Development in Sea Urchins. Dev Biol 247:335–350.

Monteiro A, Prijs J, Bax M, Hakkaart T, Brakefield PM. 2003. Mutants highlight the modular control of butterfly eyespot patterns. Evol Dev 5:180–187.

Nielsen C. 2001. Animal Evolution: Interrelationships of the Living Phyla. New York: Oxford University Press.

Nielsen MG, Wilson KA, Raff EC, Raff RA. 2000. Novel gene expression patterns in hybrid embryos between species with different modes of development. Evol Dev 2:133–144.

Nijhout HF, Emlen DJ. 1998. Competition among body parts in the development and evolution of insect morphology. Proc Natl Acad Sci U S A 95:3685–3689.

Nyhart LK. 1995. Biology Takes Form: Animal Morphology and the German Universities, 1800–1900. Chicago: University of Chicago Press.

Nyhart LK. 2002. Learning from History: Morphology's Challenges in Germany ca. 1900. J Morphol 252:2–14.

Nyhart LK. 2003. The Importance of the ‘‘Gegenbaur School’’ for German Morphology. Theory in Biosciences 122: 162–173.

Olive PJW. 1985. Covariability of reproductive traits in marine invertebrates: implications for the phylogeny of the lower invertebrates. In: Conway Morris S, George JD, Gibson R, Platt HM, editors. The Origins and Relationships of Lower Invertebrates. Oxford: Oxford University Press. p 42–59.

Oppenheimer JM. 1967. Essays in the History of Embryology and Biology. Cambridge, MA: The M.I.T. Press.

Orr HA. 1998. The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. Evolution 52:935–949.

Orr HA. 2001. The genetics of species differences. Trends Ecol Evol 16:343–350.

Parichy DM, Johnson SL. 2001. Zebrafish hybrids suggest genetic mechanisms for pigment pattern diversification in Danio. Dev Genes Evol 211:319–328.

Peichel CL, Nereng KS, Ohgi KA, Cole BL, Colosimo PF, Buerkle CA, Schluter D, Kingsley DM. 2001. The genetic architecture of divergence between threespine stickleback species. Nature 414:901–905.

Raff RA. 1987. Constraint, flexibility, and phylogenetic history in the evolution of direct development in sea urchins. Dev Biol 119:6–19.

Raff RA. 1996. The Shape of Life: Genes, Development and the Evolution of Animal Form. Chicago: University of Chicago Press.

Raff RA. 1999. Larval homologies and radical evolutionary changes in early development. In: Bock GR, Cardew G, editors. Homology. Chicester: John Wiley & Sons. p 110–124.

Raff RA, Kaufman TC. 1983. Embryos, Genes, and Evolution: The Developmental-Genetic Basis of Evolutionary Change. New York: Macmillan Publishing Co., Inc.

Raff RA, Sly BJ. 2000. Modularity and dissociation in the evolution of gene expression territories in development. Evol Dev 2:102–113.

Raff EC, Popodi EM, Sly BJ, Turner FR, Villinski JT, Raff RA. 1999. A novel ontogenetic pathway in hybrid embryos between species with different modes of development. Development 126:1937–1945.

Raff EC, Popodi EM, Sly BJ, Turner FR, Morris VB, Raff RA. 2003. Regulatory punctuated equilibrium and convergence in the evolution of developmental pathways in direct-developing sea urchins. Evol Dev 5:478–493.

Rapley R, Walker JM, editors. 1998. Molecular Biomethods Handbook. Totowa, NJ: Humana Press.

Richards RJ. 1992. The Meaning of Evolution: The Morphological Construction and Ideological Reconstruction of Darwin's Theory. Chicago: University of Chicago Press.

Richardson MK, Keuck G. 2002. Haeckel's ABC of evolution and development. Biol Rev Camb Philos Soc 77:495–528.

Sander K. 2002. Ernst Haeckel's ontogenetic recapitulation: irritation and incentive from 1866 to our time. Ann Anat 184:523–533.

Scriver CR. 1999. Normal John Berrill: 28 April 1903-15 October 1996. Biog Mem Fellows R Soc 45:19–34.

Sly BJ, Snoke MS, Raff RA. 2003. Who came first-larvae or adults? Origins of bilaterian metazoan larvae. Int J Dev Biol 47:623–632.

Sommer RJ. 2000. Evolution of nematode development. Curr Opin Genet Dev 10:443–448.

Streicher J, Müller GB. 2001. 3D modelling of gene expression patterns. Trends Biotechnol 19:145–148.

Sulston JE, Schierenberg E, White JG, Thomson JN. 1983. The embryonic cell lineage of the nematode Caenorhabditis elegans. Dev Biol 100:64–119.

Takacs CM, Moy VN, Peterson KJ. 2002. Testing putative hemichordate homologues of the chordate dorsal nervous system and endostyle: expression of NK2.1 (TTF–1) in the acorn worm Ptychodera flava (Hemichordata, Ptychoderidae). Evol Dev 4:405–417.

Theiffry D. 2001. Rationalizing Early Embryogenesis in the 1930s: Albert Dalcq on Gradients and Fields. J Hist Biol 34:149–181.

Villinski JT, Villinski JC, Raff RA. 2002. Convergence in maternal provisioning strategy during developmental evolution of sea urchins. Evolution 56:1764–1775.

Villinski JC, Hayes JM, Villinski JT, Raff RA. 2003. Carbon-isotopic shifts associated with heterotrophy and biosynthetic pathways in direct-and indirect-developing sea urchins. Marine Ecology Progress Series. In press.

Wagner GP. 1996. Homologues, natural kinds and the evolution of modularity. Am Zool 36:36–43.

Weaver M, Hogan B. 2001. Powerful ideas driven by simple tools: lessons from experimental embryology. Nat Cell Biol 3:E165–E167.

West-Eberhard MJ. 2003. Developmental Plasticity and Evolution. New York: Oxford University Press.

Wilkins AS. 2002. The Evolution of Developmental Pathways. Sunderland, MA: Sinauer Associates, Inc.

Williams DHC, Anderson DT. 1975. The Reproductive System, Embryonic Development, Larval Development and Metamorphosis of the Sea Urchin Heliocidaris erythrogramma (Val.) (Echinoidea: Echinometridae). Aust J Zool 23:371–403.

Wray GA. 1996. Parallel Evolution of Nonfeeding Larvae in Echinoids. Syst Biol 45:308–322.

Wray GA. 2000. The evolution of sea urchin development. In: Olsson L, Jacobson C-O, editors. Regulatory Processes in Development. London: Portland Press. p 49–60.

Wray GA, Raff RA. 1990. Novel origins of lineage founder cells in the direct-developing sea urchin Heliocidaris erythrogramma. Dev Biol 141:41–54.

Yamamoto Y, Espinasa L, Stock DW, Jeffery WR. 2003. Development and evolution of craniofacial patterning is mediated by eye-dependent and -independent processes in the cavefish Astyanax. Evol Dev 5:435–446.

Zhou N, Wilson KA, Kauffman JS, Raff RA. 2003. Evolutionary changes in the the function of the Otp genes in the evolution of direct-development in the sea urchin H. erythrogramma. J Exp Zool (Mol Dev Evol) 300B: 58–71.

Zigler KS, Raff EC, Popodi EM, Raff RA, Lessios HA. 2003. Adaptive Evolution of Bindin in the Genus Heliocidarus is Correlated with the Shift to Direct Development. Evolution 57:2293–2302.