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Overview

A laboratory manual for developmental biology offering basic, easy to use, laboratory investigations (18 experiments) spanning various models including echinoderm (Sea Urchin), amphibian (Frog), chick embryo, and fern gametophyte.

Preface

As with the earlier editions, the goal of this edition of *Patterns and Experiments* is to facilitate and encourage developmental biology and embryology laboratory experiences that bring students together with fascinating and dynamic developing systems. Professional biologists and nonbiologists both often relate that the study of some aspect of development of a living organism has been a memorable highlight in their educational experience. How fascinating it is to watch those tiny clusters of cells as one makes that first marathon set of observations of a batch of developing sea urchin embryos. How exciting it is to return to the lab to find a vigorously beating heart in an in vitro cultured chick embryo where there had been no visible heart and a much simpler form only twenty-four hours earlier.

My own view of biology and my career plans changed when I had that experience. I want to say to students who will use this manual that I envy you the excitement that comes with those first opportunities to experiment with living, developing organisms. I hope that a few of you might be inspired to go on to careers researching developmental processes and sharing the fascination of development with your own students. This is a truly exciting time in developmental biology because we are now able to investigate directly many of the genetic mechanisms underlying various developmental processes. However, as you begin your study of developmental biology, whether you pursue that study only in this course or study development for many years to come, I would like to offer one bit of advice from the perspective of many years in developmental biology. As intently as you may study certain individual developmental processes, please try not to lose sight of the whole developing organism and the still broader picture of the role of development in the perpetuation of species. Much of the fascination and beauty of development is to be found at those levels.

This third edition of *Patterns and Experiments* includes a number of additions and new features. Several of the additions are to the considerably expanded section on echinoderm development. There are much more detailed directions for caring for sea urchin and sand dollar embryos and larvae (Laboratory 1 and Appendix A). Several colleagues have reported that their students have been frustrated with their inability to observe development beyond the earliest stages, and I think that these directions will make it much easier for students to observe additional parts of development. The simpler and more effective procedure for blastomere separation that has been incorporated into Laboratory 2 should make it easier for students to conduct "twinning" experiments like those that have such a rich history in developmental biology's past. Laboratory 2 also includes a fascinating new experiment on the somewhat surprising, but very adaptive, capacity of echinoderm embryos and larvae to regenerate lost cilia. Also, reorganization of the echinoderm portion of the manual led to creation of a new part (Laboratory 3) that includes investigation of differentiation of an enzyme system. This investigation provides students a chance to study specific localized genetic activation in differentiation. Also, "Suggestions for Further Investigation of Echinoderm Development" was reorganized and substantially rewritten.

There is an important addition to the chick embryo section as well. In Laboratory 11, an earlier brief suggestion about investigating heart duplication has been expanded to a full experiment on heart rudiment separation and heart tube duplication that includes informative new illustrations.

Numerous other updates and additions, including several added illustrations, have been made throughout the manual. Well over one hundred new references have been incorporated into the "Suggestions for Further Investigation" that appear at the ends of the portions of the manual. Each set of references has been updated, and the majority of the new references are to works that have been added to the very dynamic literature of developmental biology since publication of the second edition of *Patterns and Experiments* in 1995.

I've also added citations to a number of the useful websites, many of which have come into being since 1995 as well. I've tried for a modest mix of specialized websites as well as general ones that provide links to many more of the valuable resources now available on the World Wide Web and which are likely to incorporate additional links to important sites that surely will be developed in the coming years.

Developmental biology is not a discipline isolated from other aspects of biology. This is particularly evident, for example, in regard to the worldwide ecological problem of declining populations of numerous amphibian species recognized during the 1980s and 1990s. Appendix G contains some suggestions concerning responsible use of amphibians in teaching that are relevant to this problem. That appendix also contains suggestions of strategies for teaching developmental biology without sacrificing adult vertebrate animals, which is an option that a number of biologists, including me, prefer to choose.

I thank the colleagues and students who have used the earlier editions of this manual and have taken the time to share some of their experiences in developmental biology. They have made insightful comments about the manual and have offered helpful suggestions and criticisms. A number of those suggestions led to additions to the second edition, and others have influenced the development of this third edition. I also warmly thank the many colleagues from colleges and universities across the United States and Canada who have participated over the years in my summer workshops on the Developmental Biology Teaching Laboratory at the University of Maine's Darling Marine Center. Those developmental biologists have brought their own individual perspectives and expertise to the workshop sessions, and we've shared some remarkable learning experiences in that beautiful setting. I owe them and my Darling Center colleagues a great deal.

Finally, I wish once again to offer my thanks to Peter Volpe who was my colleague and mentor in preparation of the first edition of this manual. Several of the amphibian development labs, especially Laboratories 4, 5, 6, and parts of Laboratory 8 have been only slightly updated and have remained largely as Peter conceived them, as has Appendix B. Some years ago, Peter's main interests moved into the areas of human development, medical genetics, and biomedical ethics, and he turned his full energy and attention to those pursuits. Thus, his direct involvement with this manual ended with the first edition, but his influence remains evident in a number of places. The manual's current title stands as a recognition of his original contributions.

Leland G. Johnson

L A B O R A T O R Y

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**Fertilization and Early Development of
Sea Urchins and Sand Dollars**

Echinoid echinoderms (sea urchins and sand dollars, which are also known as irregular urchins) have been the subjects of many investigations of fertilization and early development, and much of our understanding of developmental processes in animals has come from this research. Sea urchin and sand dollar gametes are readily obtained just before, and during, the breeding season and their developing embryos can be cultured in seawater or salt solutions that approximate the osmotic and ionic properties of seawater. Eggs and embryos of many species are quite translucent, so it is possible to observe a number of cell activities during early development, using a light microscope.

In this laboratory, you will have opportunity to observe development from fertilization through assembly of the pluteus larva, which is the swimming, feeding larval form that is characteristic of many of the echinoid echinoderms.

Techniques

Please read and understand the techniques for obtaining gametes for fertilization and for the observation of embryos before you begin this laboratory.

Obtaining Gametes

As in other echinoderms, the sexes are separate in sea urchins and sand dollars. In nature, gametes are discharged into the water, and the sperm swim freely until they reach an egg.

Since the sexes are difficult or impossible to distinguish by external features, sex of an individual animal must be determined by observing the gametes that it sheds. Injection of a small amount of potassium chloride into the coelom will induce an urchin to shed its gametes. The sex of the animal can then be determined by observing the color of gametes that are extruded from gonopores of the aboral (dorsal) surface of the animal within a few minutes after injection. The eggs of most sea urchins and sand dollars range in color from translucent yellow to pale orange, but eggs of some species are darker and may have a reddish cast. Sperm, when shed in mass, appear white or very light gray.