Every sperm – and germ cell protocol – is sacred

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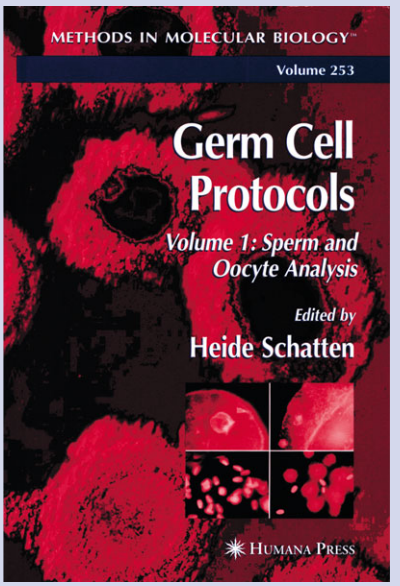
Germ cell development is essential for sexually reproducing organisms and, with due respect to Monty Python, fertilization is just the culmination of a vast repertoire of developmental accomplishments by these cells. Upon fertilization, germ cells directly contribute to both the genetic and epigenetic programs of the progeny, and have been designated as 'the guardians of the genome'. Because of their totipotency, germ cells are also referred to as the 'mother of all stem cells'. Accordingly, germ cell development is a vibrant and important area of research that crosses the boundaries of many disciplines: their inherent scientific value as specialized cell types; the unique development and fates of their stem cells; the social and ethical issues associated with reproductive biology, human health and assisted reproductive technologies; and their use in animal conservation approaches exemplify this importance. The challenges that lie ahead for us in each area of this field are enormous. However, the tackling of such challenges at the bench could become a little easier with the recent publication of *Germ Cell Protocols*.

The book is divided into two volumes. With 21 and 25 chapters, respectively, they cover a wide array of important germ cell topics among many animals, such as *C. elegans*, zebrafish, sea urchins, amphibians, ascidians, avians and numerous mammals, including humans. In total, the two-volume set thoroughly covers most research areas. Experts in the field will find these volumes useful when considering how to improve their own methodology by comparison with other cell/species types, and novices will benefit from the protocol nature of the book, which allows them to find rapidly many essential steps to conduct experiments for the first time.

The most attractive features of *Germ Cell Protocols* are the diversity of model systems and experimental strategies that are described by leading experts in each discipline to address the unique complexities of germ cell development. Current trends in the publication of primary research papers include the presentation of an abundance of experimental data combined with a lack of sufficient detail about the methodology for the reader to execute similar studies. In addition, the recent revolution in technological advances has made many complex procedures much easier to achieve, and technical problems that were previously impenetrable can now be overcome. Each chapter in the book is organized into Introduction, Materials, Methods and Notes. This simple and clever organization not only allows germ cell researchers to find the methods they need to employ more effectively, but also to identity the exact reagents and equipment they require, and to understand the intellectual basis of the methodology. As specific areas of research become more integrated with multiple disciplines, a precise guide to these new advances is an extremely valuable tool to have in the laboratory.

Volume 1 (Sperm and Oocyte Analysis) effectively describes how to manipulate mature germ cells, sperm and oocytes, from diverse organisms such as ascidians and pigs. The chapters in this volume largely deal with aspects of sperm and oocyte purification and manipulation, and instruct researchers on how to manipulate specific aspects of fertilization experimentally. For example, protocols on how to study sperm and oocyte maturation in vitro are described that use common techniques of fluorescence microscopy; several chapters describe how to purify biochemically membrane-associated kinase activities in the egg and sperm. Together, these methods allow researchers to study how germ cells become competent for fertilization in diverse animals and also explain how to visualize and assay the biochemical mechanisms associated with normal fertilization. But the understanding of how fertilization is achieved in vitro without a better understanding of how mature germ cells have arisen in the first place is unsatisfying to the developmental biologists among us.

Hence, Volume 2 (Molecular Embryo Analysis, Live Imaging, Transgenesis, and Cloning) contains detailed descriptions of how to ascertain and study early germ cell development that occurs during embryogenesis. Primordial germ cells (PGCs) are the precursors to gametes that arise during embryonic development. Studying PGCs in any organism is an enormous feat because they are often limited in



Germ Cell Protocols
Volume 1: Sperm and Oocyte Analysis
Volume 2: Molecular Embryo Analysis, Live Imaging, Transgenesis, and Cloning
Edited by Heide Schatten
Humana Press (2004) 336 pages (Volume 1)
428 pages (Volume 2)
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number and difficult to identify. The combined recent advances in imaging, transgenesis and stem cell technology have been used in many creative ways to visualize and manipulate the earliest aspects of germ cell development, PGC formation and migration. Chapter 1 of Volume 2, written by Hans Scholer and his associates, is a stellar example of the types of integrative methods that are being used to study germ-cell development. The use of various approaches, the detailed documentation of reagents and conditions, and descriptions of the wide-ranging effects of these approaches ensure that the reader will be able to take this work to the next level of understanding and application. In addition, many of the other chapters describe intricate nuclear cloning methods that are key to understanding how germ cells are programmed and reprogrammed for development, and how totipotency is achieved and manipulated. Overall, the protocols within Volume 2 guide researchers on how to use sophisticated tools in order to understand the molecular and cellular basis of the earliest events in germ cell development.

Despite the strengths that this set has to offer, several shortcomings could be considered when updating future volumes. With all the genomic resources and genome data that are now available for several of the organisms covered in this book, it is unfortunate that a significant presentation of such resources is missing here. We could envision at least two chapters on the genomic resources and databases that are available to the community, and on how researchers can integrate bioinformatics-based approaches with the biochemical and cellular protocols currently discussed in these volumes. A table of antibody/reagent/cell line resources would be useful to assist readers with identifying and obtaining essential elements for their work. As the germ cell is a cell type that, in some species, is synonymous with rarity, a greater availability of community resources is important if the field is to make rapid and efficient progress.

Although we realize that these volumes are intended to be protocols, chapters do sometimes suffer from a lack of comparison to or integration with each other, or even a lack of cross-referencing. As a result, they often appear to stand alone and sometimes to lack the 'big picture' that is important for a first-time user of a protocol. The protocols are also generally just that – methodology. Although chapters end with a 'Notes' section, which contains helpful tidbits about each protocol discussed, little is presented by way of troubleshooting or explaining why one approach is used instead of another, or the consequences of using alternatives. It is often these types of discussions that make a protocol dynamic and that contribute to improvements by other investigators. Several chapters could also be enhanced with figures or diagrams to help the reader understand the protocol better, or to see sample outcomes from the documented steps. In the chapters that are well illustrated, the illustrations largely take the form of black and white photos, which do not portray the richness of the cell type when viewed under the microscope.

Overall, both volumes of *Germ Cell Protocols* have widespread appeal, from basic developmental biologists, to veterinarians and to human clinicians, and will become a highly valuable resource in our respective laboratories.

Developmental hematopoiesis from fly to human

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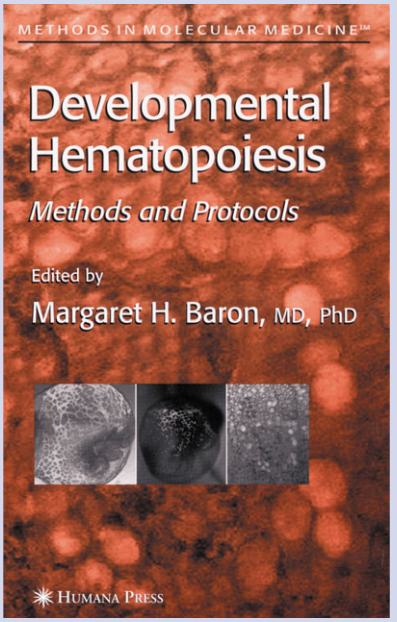
Developmental hematopoiesis is an exciting multi-disciplinary field at the crossroads of developmental biology, hematopoiesis, stem cell biology and genetics. This field began nearly a century ago with detailed observations on the formation of the first blood cells, the primitive erythrocytes, in the yolk sac of the developing chicken embryo, and it has incorporated many new scientific techniques and adopted alternative model organisms throughout its history. More recently, the intense focus on the clinical potential of stem cells has underscored the importance of understanding the developmental origins of the hematopoietic system and has encouraged research in this area. In this rapidly moving field, *Developmental Hematopoiesis: Methods and Protocols* is a timely book that provides a comprehensive overview of current methods and protocols available in developmental hematopoiesis. When I first saw this book advertised, I immediately ordered a copy for my lab. Yes, I do have a book fetish, but how could a group working in the field of developmental hematopoiesis do without a volume with such a promising title? I can now attest that this book is indeed worth its purchase price.

The editor, Margaret Baron, has put together a list of chapters covering an impressive breadth of topics contributed by recognized experts in developmental hematology. All the chapters start with a brief overview of their subject with references to the relevant literature or to other protocol books where appropriate; this is especially helpful to novices in the field. Most chapters contain in-depth coverage of the materials, protocols and potential pitfalls of the technique under discussion, while nuggets of wisdom are found in the 'Notes' section at the end of each chapter. The latter are extra bits of valuable information of the type that usually do not make it into journal articles, and their inclusion in this volume gives the reader a sense of being taught on a one-to-one basis. For example, it is useful to be cautioned against injecting zebrafish embryos

through the yolk as this is sticky and will clog the needle, and to be warned not to attempt to isolate mouse epiblast and visceral endoderm from more than four litters at once to avoid having explants sit around for too long before culture.

The book is divided into four sections. The first section provides an overview of the genetic approaches that are available in the mouse model for studying the molecular mechanisms of developmental hematopoiesis. The second part deals with mouse transplantation models and their analyses. Experimental assays in various model organisms ranging from *Drosophila* to human, rightfully take up the largest part of the book (327 out of the 480 pages). This allocation probably reflects the growing realization that the overall molecular mechanisms that drive hematopoiesis in ontogeny are well conserved between species, and that important insights can perhaps be more readily obtained from non-mammalian model systems. The last section provides an in-depth discussion of recent functional genomic approaches to the study of hematopoietic stem cell biology, with a special focus on the stem cell niche. Together, the chapters in these sections provide a good overview of the experimental approaches available in the different model organisms. Although there is some unavoidable overlap between chapters, this is not a problem because it more frequently results in the conveyance of additional information than in exact duplication.

It is not surprising that the majority of the experimental protocols presented in this book relate to the mouse model, as this is the most widely used system in which to study developmental hematopoiesis. The strengths of the mouse model (apart from it being mammalian) include the availability of antibodies for the identification and purification of different classes of hematopoietic stem, progenitor and mature blood cells; the variety of in vitro and in vivo functional assays; the availability of genetics and genomics; and the existence of in vitro embryonic stem (ES) cell culture systems that recapitulate the earliest events in embryonic blood cell formation. All these aspects and more are addressed in the current volume. There is an overview of knockout, knock-in and transgenic strategies in mice, from which one can choose the best approach to study the hematopoietic role of a gene of interest. Another chapter explains the complex mouse breeding strategies required to identify quantitative trait loci involved in hematopoietic stem cell (HSC) biology. Several chapters comprehensively address the isolation and manipulation of (pre-) hematopoietic tissues, including the epiblast, yolk sac, the para-aortic splanchnopleura/aorta-gonad-mesonephros region, liver and thymus, from different developmental stages of mouse embryos for functional analysis. These tissues, or HSCs purified from them, can be assayed for their hematopoietic potential in vitro in, for example, hematopoietic induction assays (epiblast), colony-forming assays in culture, whole-explant cultures on stromal cell lines that support hematopoiesis, fetal thymus organ cultures and a derivative thereof that allows for the analysis of the lineage potential of single cells, and in vivo in transplantation assays to monitor HSC potential in hematopoietic and vascular lineages. The common techniques for adoptive transfer into adult recipients are not addressed in detail in this book, as these protocols are well established and described in detail in a previous volume of this series. This book does, however, offer instructive protocols for the lesser known, but equally important, neonatal and in utero transplantation strategies. For those preferring cell models over embryos, there are detailed protocols for the hematopoietic and vascular differentiation of mouse ES cells, and for the inducible expression of transgenes in ES cells. Methods are also presented for the in vitro expansion of primary multipotent and erythroid progenitors from mouse fetal liver or human cord blood for biochemical and other analyses that require larger cell numbers. Finally, two detailed chapters on imaging describe a whole-mouse-embryo culture system for studying the onset of hematopoiesis in the yolk sac and a procedure to follow the fate of individual hematopoietic cells in vitro, including information on the optimal filters to use for detecting GFP, CFP, YFP and RFP, singly and multiply.



Developmental Hematopoiesis: Methods and Protocols
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Humana Press (2004) 480 pages
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The human system is represented in two chapters: one on the analysis of hematopoietic sites and cells in the human embryo; and one on the generation of hematopoietic cells from human ES cells, which may have important implications for future therapies. For most investigators, the use of early human embryos will not be a possibility, but the detailed description is helpful in highlighting the differences between the human and the mouse model, and in appreciating the difficulties in human embryo research.

The non-mammalian model organisms covered in the book are the chicken, *Xenopus*, zebrafish and the fruit fly *Drosphila melanogaster*. The chicken embryo has a long-standing history in developmental hematopoiesis. Seminal experiments on the developmental origins of the hematopoietic system were performed in this species, as its accessibility and flat morphology make it well suited for the grafting experiments described in *Developmental Hematopoiesis*.

Xenopus, zebrafish and *Drosophila* all produce large numbers of offspring that develop externally. The unique feature that made *Xenopus* a valuable model organism for developmental hematopoiesis is the clear spatial separation of primitive

and definitive hematopoiesis, which takes place in the ventral blood islands and dorsal lateral plate mesoderm, respectively. This spatial separation allows for the detailed analysis of the developmental origin of these two distinct cell populations. Two alternative approaches to map the hematopoietic fate of pre-gastrulation embryonic regions – and how to interfere with this – are discussed in detail. In addition, similar to the chicken, the externally development of the embryos makes it possible to perform grafting experiments to examine the hematopoietic potential of various mesodermal tissues, and detailed protocols for this type of experiments are provided.

The power of genetics in zebrafish and *Drosophila* make these the model organisms of choice in, for example, high-throughput genetic screening. Among the strengths of the zebrafish model are the relatively short generation time, the transparency and accessibility of its embryos, and the presence of a 'complete' hematopoietic system with myeloid and lymphoid cell lineages. Two excellent chapters describe the basic techniques for the manipulation and analysis of hematopoiesis in the zebrafish embryo by in situ hybridization, (transient) transgenesis, morpholino-mediated knock down of genes of interest and imaging of live embryos.

Although the hematopoietic system in *Drosophila* is limited to myeloid-like cell lineages, studies into the embryonic development of these cells have revealed a remarkable conservation of the molecular players required at the onset of hematopoiesis between *Drosophila* and vertebrate species. The detailed chapter on how to analyze hemocyte development by in situ hybridization and immunohistochemistry will be helpful to those of us who wish to make use of fruit fly genetics to identify novel players in developmental hematopoiesis.

Overall, *Developmental Hematopoiesis: Methods and Protocols* more than meets its aim to be a resource for students, post-docs and more experienced investigators interested in the origins of hematopoiesis. Although personal training might still be required to perform the more-complex experiments successfully, such as dissections of early embryos, in utero transplantation or injection of zebrafish/*Xenopus* embryos, the wealth of detail and information in the individual chapters make this book a valuable guide to our understanding and use of the available experimental strategies in this growing field.

Following Ariadne's thread in the labyrinth of mouse phenotypes

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Because developmental biology and the use of the mouse in genetic studies have been my passion for the past two decades, my interest was immediately sparked when I laid eyes upon this book. The many years of using good old Mickey as a research tool has led to the widespread application of ever more sophisticated methods of creating genetic changes in the mouse, to, for example, follow cell behaviors, mark lineages and manipulate early developmental processes. Our final goal has always been to find phenotypes that can shed light on the intricate processes of normal development and – if something goes wrong – on disease processes. It was not surprising to see that both of the authors of this book are teaching at the Cold Spring Harbor course 'Molecular Embryology of the Mouse'. These yearly events, where mouse embryology and genome manipulation techniques are taught, are perhaps the most comprehensive learning opportunities for anyone interested in entering this exciting field. The next step in the process, however – following the tedious road of analyzing the phenotypes we have created – has thus far not been matched by any course or reference book. This new publication, *Mouse Phenotypes: A Handbook of Mutation Analysis*, is meant to fill this gap. The authors have chosen the motto of their book very well, quoting our much-admired 'mother' of mouse developmental genetics, Anne McLaren: 'A mouse without a phenotype is non-existent'.

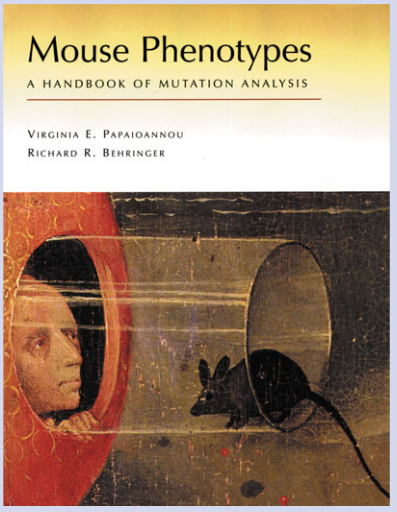
My journey through the chapters began with how to design a construct to generate mutations, and continued all the way to the completed analysis of complex and subtle phenotypes. However, this book is not meant to be read straight through from page 1 to page 235. Neither is it a collection of independent compartments. Instead, the chapters are linked with a roadmap, and I am directed to the exact path relevant to my particular situation simply by answering a few key questions at each critical step. Clear signposts guide me through the jungle of methods and swamps of possible pitfalls, making sure that I spend my time and resources on the most relevant experiments. To make this book complete, it is sprinkled full with helpful hints and tips on how to avoid costly mistakes. If you were expecting to find protocols for how to perform various assays, you will be disappointed. However, you will soon realize that adding protocols would have been a mistake: they could not possibly be fitted into one book. Instead, the authors have taken a wise approach by providing references to literature on versatile hands-on protocols. Having said this, you may expect the book to be heavy on theoretical reading – wrong again! The layout provides an excellent overview, and the language is very pleasant and easy to read. The text is to the point, without too much history and with just enough background to make it comprehensive.

For a newcomer to the field of mouse genetics, this book is a must. Those who

have once burned their fingers in the process of mouse mutation analysis will get the feeling of 'oh yeah, now I know what I did wrong last time' and will be encouraged to go ahead and try again. For those of us who 'have been there, seen that', it offers a wealth of new ideas for alternate approaches. The third edition of *Manipulating the Mouse Embryo* (Nagy et al., 2003) is a logical companion to this book, and it is not surprising to see that many references are made to protocols that can be found there. The intention to keep the text 'clean' by avoiding references is good, but occasionally I got lost when searching for specific data. For example, when looking for chromosomal segments in the genome of the 129 mouse strain that might be difficult to target, or for the mutant variant of *tk* that does not cause male sterility, I am guided to Appendix 1. Once in the appendix, I find myself lost as to where to retrieve the information I am looking for.

The first two chapters contain the all-important theory. The focus is on practical advice for making every project a success. First, it describes the various types of methods for generating mutants: chemical and X-ray induced mutagensis, gene-trap approaches, retroviral infections, transposons and classical transgenics. The second chapter gives a detailed analysis of the practical sides of gene targeting strategies, and lists the pros and cons of different methods. A step-by-step guide took me through the process, leaving out no important considerations. Positive and negative selection markers, how best to make a null allele, point mutations, conditional alleles and reporter 'knock-ins' are all discussed.

In the following two chapters, the time has come to actually make a mouse that carries a specific mutation. Because the most versatile (and therefore also often the most challenging) approach requires the use of embryonic stem (ES) cells, the generation of chimeras and germline transmission, these chapters heavily focus on these techniques. Here, I got answers to all those questions that often remain after I've read the literature, tried the assays and found that nothing seems to work. The authors describe the possible reasons as to why a gene-targeting experiment might not be working and suggest trouble-shooting techniques for tackling problems such as no correctly targeted clones, no chimeras, no offspring from chimeras or no germline transmission. Once a mutation has successfully been passed through the germline, the task of maintaining the mouse line begins, and this book offers detailed discussions of important topics, such as genetic background modifier effects, backcrossing schemes to obtain co-isogenic lines, how to delete selection cassettes in vivo and special techniques for dealing with infertility phenotypes. The authors provide many useful suggestions for how to test for genetic interactions and for redundancy between members of a gene family. I found only some minor omissions, such as the possible 'tricolor' phenotype of E14TG2 ES cell line chimeras (depending on the host embryo used to make them, the ES-derived fur is not always pale yellow), and I would have liked a more extensive discussion of embryo transfer as a means for re-deriving pathogen-loaded lines.



Mouse Phenotypes: A Handbook of Mutation Analysis

By Virginia E. Papaioannou and Richard R. Behringer

Cold Spring Harbor Laboratory Press (2005)
235 pages
ISBN 0-87969-640-0
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Chapters 5 and 6 are the ones most readers will probably be looking for, as they discuss the analysis of phenotypes that manifest at pre- and postnatal stages. Also here are lots of practical tips for avoiding common mistakes, and for performing tricky analyses with very limited resources by using innovative simple techniques. One such example is how to reliably count and image the blastomeres in late pre-implantation-stage embryos. The text is complete, covering important steps such as failure of the embryo to divide, hatch or implant (including in vitro assays for assessing the developmental potential of trophoblast and inner cell mass cells). The book then turns to looking at postnatal phenotypes. This section starts with a discussion of perinatal lethality and its possible causes, such as developmental delay, cranial nerve, cardiovascular, skeletal and diaphragm defects, and cleft palate. For each case, the authors offer advice on which organ system is likely to be affected, and on how to go about further and deeper analysis. With the same systematic precision, they take the reader on the quest of finding postnatal/pre-weaning phenotypes – both lethal and subtler ones. Finally, we arrive at the much dreaded 'no phenotype' finding. The authors point out quite correctly that there is no such thing – we just have not looked carefully enough! So, they give further advice on how to do a proper search by using, for example, neurological and behavioral tests, genetic background effects and environmental challenges.

Chapter 7 deals with dominant effects and offers advice on how to deal with the special challenges these situations may present in ES cells, chimeras and heterozygous offspring. Dominant reproductive problems are not forgotten, and the mechanisms behind both dominant and semi-dominant effects are discussed in detail, including haploinsufficiency, and mutations in X-linked, Y-linked and imprinted genes.

The last chapter re-visits both Chapters 2 and 5 in that it deals with early lethal phenotypes. The usefulness of conditional and inducible alleles now becomes fully clear, and the book offers advice on how to test for and use these methods. The authors then describe sophisticated methods of using different kinds of chimeras as a means of rescuing and analyzing otherwise 'impossible to reach'

phenotypes, such as the use of tetraploid embryos in combination with ES cells. However, I was surprised that a discussion of the advantages of using F1 hybrid cell lines for this purpose is missing from this chapter.

Two very well-thought through appendices bring the book to an end. In the first, I can find useful literature and web-based resources; the second provides a list of published phenotype analyses that give good examples of the work that lies ahead for the reader.

It is not difficult to imagine why no author has thus far attempted to take the very ambitious challenge of writing a book such as this. Richard Behringer and Virginia Papaioannou have accomplished this task in a truly impressive manner. If you are a student or researcher entering the field of mouse genetics, the money you invest in acquiring this book will be worth every penny. The book will provide you with a guided tour of how to make the most complete analysis of every possible phenotype you may stumble upon. While holding onto the authors' hands through this maze, you can rest assured that you will have exploited all available possibilities, while at the same time not having wasted energy on working towards dead ends. To put it simply, this book 'puts it all together'. It is truly a work we all have been waiting for!

Reference

Nagy, A., Gertsenstein, M., Vintersten, K. and Behringer, R. (2003). *Manipulating The Mouse Embryo: A Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

An ambitious experiment

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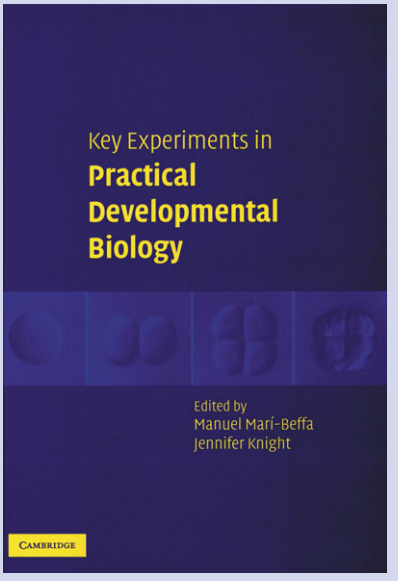
Key Experiments in Practical Developmental Biology details influential experiments in the field for undergraduate or postgraduate students to replicate in the laboratory class. The editors have set themselves an ambitious goal. Many of the experiments in this book are, as suggested by the editors, straightforward. For example, some of the crosses and phenotypic analyses described require care and good microscopes, but no tremendous manipulative skill. Students may be asked to set up a cross then determine, for example, the bristle numbers on a fly notum, or petals and sepals on an *Arabidopsis* flower. Of course, such experiments do require that one can obtain and maintain all the special strains that are cited. I had expected that the fly strains used in this book would be standard ones available from the Bloomington Stock Center; however, many are not, so presumably the authors of such chapters would need to provide them.

Beyond setting up the experiments, many of the techniques that are described here, although in standard use in a research lab (such as in situ hybridization to embryonic mRNAs), are still unreliable in a teaching lab. Perhaps the editors should have insisted that their authors use procedures that adapt well to undergraduate exercises. For example, the X-gal staining of *lac*Z reporter strains is a technique that usually works well, and it is exploited in this book in some instances. But even when *lac*Z reporter strains are available, such as those for studying early gene expression in blastoderm-stage fly embryos, the author of a particular chapter still suggests that in situ hybridization is used, without making clear the disadvantages of X-gal staining.

Beyond some of the genetic crosses and analyses, there are several well-designed and straightforward drug applications, such as the application of retinoids to amphibian limbs or to fish embryos, and some straightforward surgery or dissociation experiments. However, the chapters often turn to things that I cannot imagine trying with even a small class. Having taught both undergraduate lab courses and advanced classes in embryology at Cold Spring Harbor and the Marine Biological Laboratory at Woods Hole, I believe it would be rash to expect most of these experiments to be feasible in a university class setting. Even if a lab were well equipped with microscopes, manipulators and first-class facilities for a variety of animals, many of the manipulations are just plain difficult, and particularly so if there has been no skilled demonstration beforehand. By all means, examine the fates of dissociated sea urchin blastomeres, but if any student can do a micromere transplant in an undergraduate class, the instructor should insist that they switch immediately to honors research. Even if a lab class can obtain growth factors to put onto beads, the technique of bead implantation into chick embryos takes considerable skill and practice, and the description in this book is no substitute for a demonstration; in inexperienced hands, these experiments have a negligible success rate. Of course, one wants students to gain insights into the nature of



Key Experiments in Practical Developmental Biology
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understanding by doing experiments and seeing the reality and limitations of generating empirical data, but experiments need to be designed to give students a reasonable shot at success.

Although the book frequently aims too high, it is still a useful read for students. The methods are spelled out somewhat more clearly than in the primary literature, and the reader achieves more sense of an experiment than from a developmental biology textbook. In the book, each experiment is introduced, with the materials outlined along with preparation work for staff. Then the experiment is described step-by-step, and finally the expected results are discussed, as are the likely causes of failure. Alternative exercises and teaching concepts are given at the conclusion of each chapter. Thus, even the technically challenging experiments provide useful material for discussion, even though an experienced instructor would be needed to turn them into feasible laboratory exercises.

One hesitates to turn a lab class into spectator sport, but the book would be wonderful if accompanied by a DVD of demonstrations. Perhaps then the students could be content with watching how experiments are carried out, and only perform some of the more straightforward exercises. There are also some obvious gaps in the book's content. Lineage tracing in embryos is about as straightforward, yet rich in interpretation and discussion, as other more-detailed manipulations, but it is not discussed in useful detail. And as for grafting, I would like to have seen some experiments that use the experimental embryologist's mainstay: the amphibian embryo.

Is there a solution to designing high-level experiments for undergraduates? If the facilities are there, then the best solution exploits the expertise of the local instructors. They can carry out the demonstrations and design an experiment that works at least half of the time in inexperienced hands.

A grand new view from the embryo

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The field of 'evo-devo', the study of evolutionary developmental biology, is one of the most exciting in modern biology. Its appeal lies in the promise of delivering answers to the age-old question of where did we come from? The answers come from not one but two perspectives, which are intimately related but at two entirely different time scales. How did we, and the fascinating myriad of organisms we share this planet with, develop from embryos? And how did we reach our dramatically different morphological forms and functions via evolution? That evolutionary forces must act on developing embryos to produce differing body plans seems obvious, and yet the modern synthesis of evolutionary and developmental biology is a relatively recent phenomenon sparked by the discovery of genetic conservation across taxa. The evo-devo field is currently moving forward in leaps and bounds, and although there are limitless questions left to answer, some general principles have already emerged. The time is thus ripe for an accessible text on evo-devo that will be appealing and informative to a wide readership.

This challenge has been taken up by Sean Carroll, an evolutionary developmental biologist from the University of Wisconsin, whose own work has played a central role in putting evo-devo studies on the map. Carroll's new book is titled *Endless Forms Most Beautiful*, a quote from the final paragraph of *The Origin of Species* (Darwin, 1859) and it nicely sums up Carroll's attitude to his subject. The book is easy to read with a well-balanced combination of personal anecdote and hard science, is not too long, has beautiful color plates and is very reasonably priced. It is enjoyable because of the palpable enthusiasm and excitement that Carroll brings to his subject. His appreciation for the beauty of the natural world in all its infinite variety is contagious, and his desire to explain to us how this variety arose keeps the pages turning rapidly. Although we did find some aspects of *Endless Forms* frustrating, because of an overly narrow focus that led to missed opportunities, we nevertheless felt that, on the whole, it is a successful book with much to commend it.

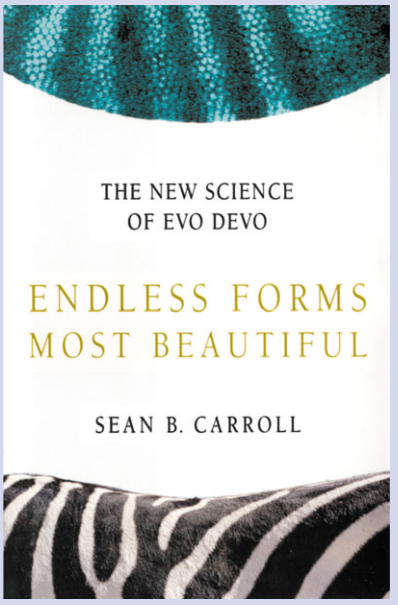
Endless Forms is essentially a popular science book with a unique emphasis on developmental biology and the genetics of evolutionary change. It has been some time since any books that fall into this general niche were published: Kenneth McNamara's *Shapes of Time* (McNamara, 1997) and Carl Zimmer's *At the Water's Edge* (Zimmer, 1998) being the most obvious contenders. Carroll's book gives the reader an exciting picture of the resurgence of comparative developmental biology following the discovery of the unexpected conservation of Hox genes across taxa in the 1980s. The potential of developmental biology for solving evolutionary conundrums is enthusiastically conveyed, and Carroll gives a concise, informative history of the modern merging of these two disciplines. However, he defines evo-devo narrowly as 'the comparison of developmental genes between species', thus giving his description of this new synthesis of evolution and development a decidedly genetic focus. As a consequence much of this book deals with the respective roles of gene products ('tool kit genes') versus cis-regulatory regions ('switches') in generating diversity.

The book is organized around four main questions: (1) what are the rules for generating form; (2) how is the information encoded; (3) how does diversity evolve; and (4) what explains large-scale evolutionary change? In the first half of the book, questions 1 and 2 are

addressed through a fast-paced tour of developmental and molecular biology. It is difficult for us to gauge exactly how easy these chapters would be to follow for a complete novice, but they are certainly written in an accessible way, and Carroll tries hard to use lay-terms and analogies, rather than to introduce too much specialized terminology. His embryos have longitude and latitude rather than animal-vegetal and dorsoventral axes, and regulatory elements are termed 'switches' that act as 'GPS integrators'. For the most part these devices work well, although just occasionally they seem more likely to obfuscate than clarify: a description of limb axes that includes 'top (back)' and 'back (pinkie)' in a single sentence had us tying our fingers in knots. Remarkably, in just a couple of short chapters the book manages to effectively summarize all the major themes that a standard undergraduate developmental biology course is likely to touch on. This is followed by 'The Dark Matter of the Genome', a chapter using the pleasing cosmological analogy of non-coding DNA as dark matter of the universe to lead into a fairly detailed discussion of transcriptional regulation. Developmental biologists should not expect any revelations from these chapters, but they do provide some excellent fodder for teaching development to students who are not majoring in biology. This first part of the book on the making of animals succeeds well, and sets up the reader for the evolutionary biology to follow.

The second half of the book tackles the questions of diversity and evolutionary change from the new evo-devo perspective. We found this section of the book to be less consistent in quality. A chapter on human evolution owes much to a previous article of Carroll's, and is rather disappointing in its lack of a true evo-devo perspective. We think a discussion of heterochrony here could have helped to broaden the book as a whole. By contrast, the discussion of melanism works much better, and the chapter on butterfly spots is one of the most fascinating pieces of popular science writing that we've read; indeed, it can stand alone as a primer on mimicry.

The chapter focusing on the modification of appendages, an area where Carroll's own work has had a major impact, is nicely done and right up to date, including exciting ongoing work in the Kingsley laboratory on sticklebacks. However, one of our primary criticisms of this part of the book is that it tends to give us the evo-devo world according to Carroll. That is a world controlled almost exclusively by changes in cis-regulatory elements. Studies in this area, many from Carroll's own lab, have been both successful and enlightening; but they do not provide a full story. There is no reason to suppose that important evolutionary changes at the genetic level will not be uncovered wherever they are looked for. As our understanding of gene regulation and protein function becomes more sophisticated, we will find evolution at every level of gene activity. In addition, whereas Carroll downplays changes in coding sequences, because pleiotropy makes it difficult for proteins to change while remaining functional, he de-emphasizes the role of gene duplication and other mechanisms in releasing genes from these constraints to allow new functions to evolve.



Endless Forms Most Beautiful: The New Science of Evo Devo and the Making of the Animal Kingdom
By Sean B. Carroll
W. W. Norton (2005) 288 pages.
ISBN 0-393-06016-0
$25.95 (hardback)

Carroll's focus on 'tinkering' with existing morphology via changes in cis-regulation, and on the somewhat obscure Williston's law of specialization and reduction in serial homologs, does not lead him to ignore larger scale changes and the acquisition of novelties. However, we finished the book feeling that these areas remained under-explored. For example, there is little to complain about in Carroll's description of the Cambrian explosion, but it neglects to discuss how the different phyla, and their already complex array of 'tool kit genes', arose in the first place. Sadly, despite Carroll's consistent attempts to use evo-devo to dispel intelligent design (ID) ideas, this omission can even be misinterpreted as fuel for the ID 'theory': a review of the book at Amazon.com from an ID proponent goes so far as to pose the question of 'Who put the tools in the tool kit?'

In parts of this second section of the book, we felt that the reader is left to guess at how evolution works. Carroll pronounces that evolution always proceeds by incremental gradual change, and does not present the ongoing debate of the relative importance of gradualism versus punctuated equilibrium. It is difficult for the reader to reconcile the origin of novelties with the idea that change is always incremental. For evolution of human characters such as brain size, we are told that there was likely to be 'selection for variants of many genes, responsible for small increments of differences…over sustained intervals of many thousands of generations'. Yet evolution of human brain size during the Pleistocene Epoch is described by Carroll as occurring through two rapid phases of expansion punctuated by a period of stability. The reader is left to try to connect the dots between the pattern seen in the fossil record and evolution proceeding in small increments. The concept of the hopeful monster is introduced, then quickly discarded as a specter vanquished by evo-devo. Here, we think, Carroll does Richard Goldschmidt a disservice. Although much of Goldschmidt's *The Material Basis of Evolution* has proved to be wrong, his discussion of macroevolution and its relationship to changes in developmental timing, embodied in the concept of the hopeful monster, has some enduring value. Unfortunately, Carroll doesn't mention Goldschmidt, and anachronistically discusses hopeful monsters in terms of Bateson's work on

discontinuous variation (Bateson, 1894). Although Carroll shows us how evo-devo has identified genes that allow or cause large morphological changes, he then tells us there is no need for nature to make jumps to produce diversity. What does explain large-scale evolutionary change? Ultimately, this question is not fully addressed.

Despite our criticisms, we feel that this book fills a much-needed gap. If you are a developmental biologist tightly focused on a single model organism and wondering what evo-devo is all about, then we highly recommend the non-developmental chapters of *Endless Forms*. Carroll suggests that the book is appropriate not only for students and educators, but also for those interested in complexity and for the large number of natural history buffs. The book should appeal not only to these readers, but also to a wider audience, and its conversational tone, together with its clever use of photographs and diagrams, will augment their enjoyment, as well as their understanding.

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Encyclopedia Drosophilidiana

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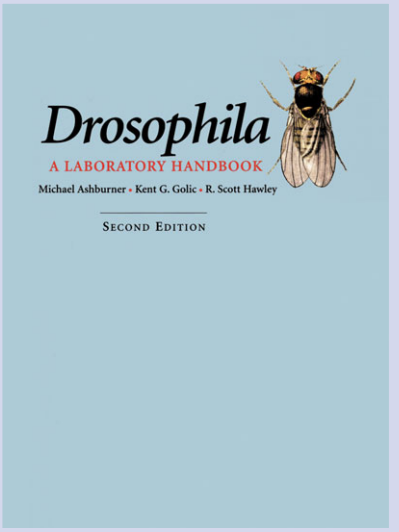
There are three foundational dates in the history of encyclopedism: 500 CE for the completion of the Babylonian *Talmud*, 1751 for the publication of the *Encyclopédie* (Diderot and D'Alembert, 1751) and 1989 for the initial publication of Drosophila*: A Laboratory Handbook* (Ashburner, 1989). For the first, it is unlikely that any reviews were solicited or written. For the second, one of its originators, Denis Diderot, reviewed it himself (favorably). For the third, now released in its second edition, the task of reviewing 1409 pages of the most comprehensive, meticulously organized, thoroughly referenced and exhaustively detailed compendium of Drosophilology ever to be undertaken is challenging, to say the least.

All three encyclopedic landmarks have had major impacts on science. Talmudic disputation and hair-splitting became the paradigm for cellular immunology in its heyday. Diderot and D'Alembert's *Encyclopédie* provided the first comprehensive compilation of the length and breadth of human knowledge, including a survey of natural philosophy (i.e. science). Ashburner's understated title, Drosophila*: A Laboratory Handbook*, belies the enormity and thoroughness of his original treatment of the subject, which has now become even more enormous and even more thorough (if such a thing is possible) in its new, triple-author incarnation as Ashburner, Golic and Hawley. It would suffice to say that any laboratory serious about working with the little fruit fly needs at least one copy of this book.

All three authors have fly credentials as sterling as one could want. Although not a progeny of the standard fly lineage (documented in Keith Maggert's *Fly Researcher's Pedigrees*; see http://flybase.bio.indiana.edu/allied-data/lk/pedigree.html), Ashburner has taught himself seemingly everything there is to know about the organism and its genetics, and has interacted with virtually everyone in the fly world. Golic and Hawley are progeny of the Larry Sandler lineage, steeped in the traditions of meiosis, hard-core chromosome mechanics, fly lore and scholarship. Moreover, they all have intimate knowledge as either the originators or practitioners of the new hybrid genetic/molecular techniques that endow the fly with the degree of versatility and experimental prowess that makes it stand above all other systems. But the breadth and depth of the subjects treated goes far beyond the combined research expertise of all three authors.

As one would expect, all genetics-related subjects are explored and documented – for example, chromosome cytology, chromosome aberrations, mutants and mutagenesis, mitotic recombination, mosaic techniques, aneuploidy, sex determination, and dosage compensation – as well as their contemporary counterparts in the molecular characteristics of the fly genome and the newer combined molecular-genetic techniques (such as, for example, homologous recombination). Beyond these subjects, reproductive biology, the principles and techniques of its husbandry, and diseases and parasites are



***Drosophila*: A Laboratory Handbook, 2nd Edition**
By Michael Ashburner, Kent G. Golic and R. Scott Hawley
Cold Spring Habor Laboratory Press (2005)
1409 pages
ISBN 0-87969-706-7
£115.00/$185.00 (hardback)

also covered. Several esoteric (but quintessentially fly) genetic subjects are also given an unusually full treatment here, including autosynaptic chromosomes, transvection and position effect. Taxonomy remains one of the most extensive portions of the volume, albeit with a new-found urgency borne of the comparative genome sequencing carried out in the past few years and still ongoing.

The new edition is not merely a re-issue of the original with a few additions (in contrast to a much smaller fly genetic primer published, and recently re-issued, by the same press). Many subjects have been expanded, some dramatically (for example, there are now separate chapters on male and female meiosis), others rearranged and consolidated. Major chapters have been deleted, for example, when they could no longer hope to encompass the material (such as the original chapter on development), or when they dealt with fundamentally non-genetic techniques that are well treated elsewhere (e.g. tissue culture). In their place, new chapters have appeared (such as the one on the genetic analysis of DNA repair).

Why would anyone, even in a fly lab, need such a hodge-podge of topics collected in one place? Because even in the age of PubMed and FlyBase, one cannot find everything one needs online. This is especially true of the older literature. Not that the references are not to be found in FlyBase, but, to be placed properly in context, they have to have been read and incorporated into a body of knowledge and understanding. Databases do not have this ability (not even 'advanced search' engines). In other words, this current, updated tome is a testament to the value (as well as the honor) that is associated with traditional scholarship, a practice that has all but disappeared from our world. Consistent with this tradition, this book mercifully avoids any discussion of *Drosophila* 'models' for human disease. It does, however, meet its present-day responsibilities by giving a detailed account of the available databases and other electronic reference resources available online.

When is the book useful? If you do not know anything about a subject, you can find an overview and guidance to the appropriate references. If you know a little bit about a subject and need to find more, you can be similarly edified and then directed to the relevant literature. If you need to know how long the pupal stage will last at 23°C, you can look it up. If you want a highly detailed laboratory manual or a textbook, you must look elsewhere, as that is not the role of an encyclopedia. This is not to say that there is no detail, but the detail is of an encyclopedic sort (e.g. vector maps, names of important stocks, and scads of vital statistics on the fly that are otherwise scattered throughout the literature).

Does this volume have any failings? Perhaps, depending on your perspective. (Reviewers, whether of books or grants, are always asked to point out deficiencies as well as strengths.) It is not exactly beach reading – but then neither is the *Encyclopedia Brittanica*. It is not exactly a page turner – but then neither is the *Talmud*. One can always nit-pick, but that would obscure the immensely impressive effort that has gone into this oeuvre. Those who lament the loss of scientific traditions and the knowledge that came with them can take comfort in the lengths to which this book goes to preserve them and to carry them into the future. We are all indebted to its authors.

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