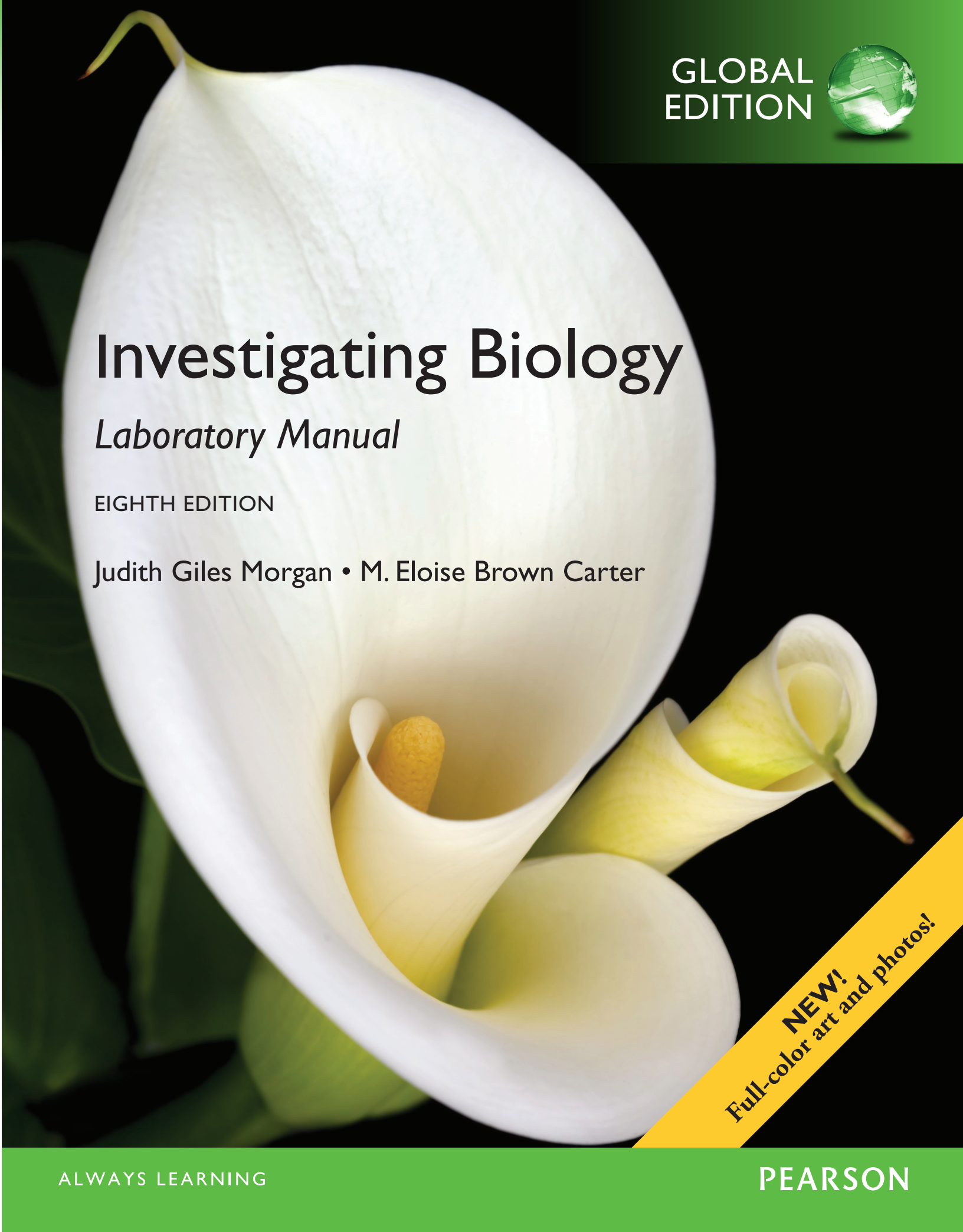

GLOBAL EDITION
Investigating Biology
Laboratory Manual
EIGHTH EDITION
Judith Giles Morgan • M. Eloise Brown Carter
NEW!
Full-color art and photos!
ALWAYS LEARNING
PEARSON

LABORATORY SAFETY:

GENERAL GUIDELINES

1. Notify your instructor immediately if you are pregnant, color blind, allergic to any insects or chemicals, taking immunosuppressive drugs, or have any other medical condition (such as diabetes, immunologic defect) that may require special precautionary measures in the laboratory.
2. Upon entering the laboratory, place all books, coats, purses, backpacks, etc. in designated areas, not on the bench tops.
3. Locate and, when appropriate, learn to use exits, fire extinguisher, fire blanket, chemical shower, eyewash, first aid kit, broken glass container, and cleanup materials for spills.
4. In case of fire, evacuate the room and assemble outside the building.
5. Do not eat, drink, smoke, or apply cosmetics in the laboratory.
6. Confine long hair, loose clothing, and dangling jewelry.
7. Wear shoes at all times in the laboratory.
8. Cover any cuts or scrapes with a sterile, waterproof bandage before attending lab.
9. Wear eye protection when working with chemicals.
10. Never pipet by mouth. Use mechanical pipeting devices.
11. Wash skin immediately and thoroughly if contaminated by chemicals or microorganisms.
12. Do not perform unauthorized experiments.
13. Do not use equipment without instruction.
14. Report all spills and accidents to your instructor immediately.
15. Never leave heat sources unattended.
16. When using hot plates, note that there is no visible sign that they are hot (such as a red glow). Always assume that hot plates are hot.
17. Use an appropriate apparatus when handling hot glassware.
18. Keep chemicals away from direct heat or sunlight.
19. Keep containers of alcohol, acetone, and other flammable liquids away from flames.
20. Do not allow any liquid to come into contact with electrical cords. Handle electrical connectors with dry hands. Do not attempt to disconnect electrical equipment that crackles, snaps, or smokes.
21. Upon completion of laboratory exercises, place wall materials in the disposal areas designated by your instructor.
22. Do not pick up broken glassware with your hands. Use a broom and dustpan and discard the glass in designated glass waste containers; never discard with paper waste.
23. Wear disposable gloves when working with blood, other bodily fluids, or mucous membranes. Change gloves after possible contamination and wash hands immediately after gloves are removed.
24. Place gloves, swabs, toothpicks, etc. that may have come in contact with body fluids in a disposable autoclave bag.
25. Leave the laboratory clean and organized for the next student.
26. Wash your hands with liquid or powdered soap prior to leaving the laboratory.

The biohazard symbol indicates procedures that may pose health concerns.

The caution symbol points out instruments, substances, and procedures that require special attention to safety. These symbols appear throughout this manual.

INVESTIGATING BIOLOGY

Laboratory Manual

Eighth Edition
Global Edition

Judith Giles Morgan
Emory University

M. Eloise Brown Carter
Oxford College of Emory University

PEARSON

Boston Columbus Indianapolis New York San Francisco Hoboken
Amsterdam Cape Town Dubai London Madrid Milan Munich Paris Montreal Toronto
Delhi Mexico City São Paulo Sydney Hong Kong Seoul Singapore Taipei Tokyo

The authors and publisher believe that the lab experiments described in this publication, when conducted in conformity with the safety precautions described herein and according to the school's laboratory safety procedures, are reasonably safe for the students to whom the manual is directed. Nonetheless, many of the described experiments are accompanied by some degree of risk, including human error, the failure or misuse of laboratory or electrical equipment, mismeasurement, spills of chemicals, and exposure to sharp objects, heat, bodily fluids, blood, or other biologics. The authors and publisher disclaim any liability arising from such risks in connection with any of the experiments contained in this manual. If students have questions or problems with materials, procedures, or instructions on any experiment, they should always ask their instructor for help before proceeding.

Vice President/Editor-in-Chief: Beth Wilbur
Senior Acquisitions Editor: Josh Frost
Senior Editorial Manager: Ginnie Simione Jutson
Project Editor: Brady Golden
Managing Editor, Production: Michael Early
Production Project Manager: Jane Brundage
Production Management and Composition: S4Carlisle Publishing Services, Norine Strang
Head of Learning Asset Acquisition, Global Edition: Laura Dent
Manager, Media Production, Global Edition: M. Vikram Kumar
Senior Manufacturing Controller, Global Edition: Trudy Kimber
Acquisitions Editor, Global Edition: Shona Mullen
Assistant Project Editor, Global Edition: Priyanka Shivadas
Art: Precision Graphics
Cover Design Production: Lumina Datamatics Ltd.
Photo Researcher: Maureen Spuhler
Photo Editor: Donna Kalal
Image Rights and Permissions Manager: Michael Farmer Image Permissions Bill Smith Group, Stephanie Ramsay Manufacturing Buyer: Stacey Weinberger
Executive Marketing Manager: Lauren Harp
Text Printer: CPI Digital UK
Cover Printer: CPI Digital UK
Cover Photo Credit: Robyn Mackenzie /123RF

Pearson Education Limited
Edinburgh Gate
Harlow
Essex CM20 2JE
England

and Associated Companies throughout the world

Visit us on the World Wide Web at:
www.pearsonglobaleditions.com

Authorized adaptation from the United States edition, entitled Investigating Biology Laboratory Manual, 8/e, Global Edition, ISBN 978-1-292-06130-6, by Judith Giles Morgan and M. Eloise Brown Carter, published by Pearson Education © 2015.

ISBN 10:1-292-06130-8
ISBN 13: 978-1-292-06130-6

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

14 13 12 11 10 9 8 7 6 5 4 3 2 1

Typeset by S4Carlisle Publishing Services, Norine Strang in Warnock Pro/10.5 pt.

Printed and Bound in Great Britain by CPI Group (UK) Ltd Croydon, CRO 4YY

Preface

Science is a way of thinking much more than it is a body of knowledge.

Carl Sagan

Our knowledge and understanding of the biological world is based on the scientific enterprise of asking questions and formulating and testing hypotheses. Scientists gather data, then evaluate and interpret their results, communicating their findings through papers and presentations. An important aspect of learning biology is participating in the process of science and developing creative and critical reasoning skills. Our goal in writing this laboratory manual is to present a laboratory program that engages students in the scientific process and encourages scientific thinking. We want students to experience the excitement of discovery and the satisfaction of solving problems and connecting concepts. For us, investigating biology is more than just doing experiments; it is an approach to teaching and learning that promotes inquiry.

The laboratory exercises are designed to encourage students to ask questions, to pose hypotheses, and to make predictions before they initiate laboratory work. Students are asked to synthesize results from their observations and experiments, then draw conclusions based on evidence. Whenever possible, students apply their results to new problems and case studies. *Investigating Biology* provides students with many opportunities to design their own open-inquiry investigations as part of the laboratory. In addition, students can pursue independent investigations using the suggestions and extensions provided at the end of lab topics. Scientific writing and communication are emphasized throughout the laboratory manual and supported by an appendix that includes instructions for writing each section of a scientific paper, obtaining information from primary sources, and preparing oral and poster presentations. Instructors are given suggestions for organizing a laboratory writing program.

Investigating Biology uses a stepwise approach to developing scientific knowledge and skills, as students in early lab topics practice asking questions, developing hypotheses, and designing experiments. Early laboratory experiences build on knowledge (e.g., how cells and enzymes function, genetics, and then biodiversity as an expression of variation and evolution). At the same time, students are developing laboratory and thinking skills, such as pipetting, using instruments, analyzing results, organizing and managing teams, and developing systematic approaches to experimental design and problem solving. Lab topics are a mixture of directed inquiry and open inquiry, with more directed investigations in the initial lab topics and increasing opportunities for open inquiry in subsequent lab topics. In the open-inquiry investigations, students are presented with a topic and preliminary experiment, then encouraged to develop their own questions, design their investigations, and write their proposals before implementing their own investigations. They must then collect and interpret their data. We use a similar approach for scientific writing. Students write individual sections of a scientific paper for initial lab topics, receiving feedback and the opportunity to revise. Then, for open-inquiry investigations, they are prepared to use their writing expertise to write a complete scientific paper.

This incremental approach to knowledge, skills, and disciplined ways of thinking is a hallmark of our approach throughout the lab manual and is adaptable to programs with large laboratory sections or small groups of students. *Investigating Biology* provides a comprehensive introduction to the diverse topics and subdisciplines in the biological sciences, always with an emphasis on scientific investigation.

We are convinced that involving students in the process of science through investigating biological phenomena is the best way to teach. The organization of this laboratory manual with a mix of directed-inquiry and open-inquiry investigations complements this approach to teaching and learning.

New in the Eighth Edition

The eighth edition of *Investigating Biology* offers enhanced opportunities for students to participate in science. You will find two newly designed lab topics for protists and fungi, new and revised open-inquiry exercises, suggestions for extending lab topics with independent investigations, new case studies for practicing problem solving, a revised section on student media, two new appendices on instrumentation and techniques and for metric measurement, and a revised appendix to support scientific writing in the laboratory. Adopters of the eighth edition will notice an enhanced emphasis on recurring themes in biology, including structure and function, unity and diversity, transmission of genetic information, energy transformations, and the overarching theme of evolution. These themes are developed across hierarchical levels from cells to organisms to ecosystems.

The eighth edition includes major revisions that reflect new molecular evidence and our current understanding of phylogenetic relationships for plants, invertebrates, protists, and fungi. The new Lab Topic 17 Fungi provides extensive coverage of the major fungi groups, including lichens. Lab Topic 13 Protists has been revised and expanded with additional examples of all the major clades. Now both newly revised lab topics include suggestions and exercises for open-inquiry investigations. In addition, the sequence of the lab topics has been reorganized to reflect the closer relationship of the fungi and animal kingdoms.

Lab Topic 1 Scientific Investigation has been completely revised with a return to an investigation of cardiovascular fitness. Students have additional options to design the experiment, as they choose among dependent variables and select the parameters for the experimental procedure. We have included new ways of measuring pulse/heart rate utilizing technology available on iPads and iPhones. The lab topic continues to emphasize the scientific process and establishing a fundamental way of working and thinking that will be featured in the continuing laboratory program.

In a previous edition we introduced a new laboratory, Lab Topic 16 Bioinformatics: Molecular Phylogeny of Plants. This lab topic connects organismal biology, molecular genetics, and evolution, using the techniques of computer science to analyze nucleotide sequences and develop phylogenetic trees. We have provided suggestions for teaching, additional analysis tools, and new questions that involve "tree thinking."

In the eighth edition, population genetics is covered in one lab topic with new problems and examples that connect ecology, evolution, and genetics.

Two new appendices have been added: Appendix B The Metric System and Appendix C Instrumentation and Techniques. Appendix C includes information and procedures for the use of pipettors, micropipettes, calipers, and both digital and electronic spectrophotometers.

We have continued to revise and propose ideas for the student-designed investigations in Lab Topic 5 Cellular Respiration, Lab Topic 11 Population Genetics, Lab Topic 13 Protists, Lab Topic 21 Plant Growth, Lab Topic 26 Animal Behavior, and Lab Topic 28 Ecology II. These open-inquiry laboratory experiences allow students to independently investigate questions, thus providing team research opportunities in the introductory laboratory program. For almost all other lab topics, we have further developed the open-inquiry section: "Investigative Extensions." For programs interested in providing independent or team research opportunities, students may pursue these investigations. Additional support for the investigations is provided in the *Preparation Guide for Investigating Biology Laboratory Manual* available to instructors. In Appendix A Scientific Writing and Communication, we updated a section on oral and poster presentations to introduce students to other formats for scientific communication.

The most dramatic innovation in the eighth edition is the use of full-color photographs and figures incorporated throughout the laboratory manual. Students will better visualize the procedures, structures, organisms, and life cycles within the context of their reading, thinking, and investigating. For example, students will find color photographs located in the text as they are observing the stages of mitosis or the diversity of plants and animals. Life cycles and stages of development are color coded to provide information that is essential to understanding processes and structures.

Other changes are more subtle but represent fine-tuning based on our experiences and those of instructors and students who used the first seven editions. We have revised and added to the questions at the end of each lab topic. Questions in "Reviewing Your Knowledge" allow students to test their knowledge and comprehension. Questions in "Applying Your Knowledge" allow students to apply their understanding to new problems that feature current research and societal issues. These questions reinforce the investigative approach of the laboratory manual in which students develop their skills in analyzing results, synthesizing, and communicating as they participate in the scientific process. We have integrated new scientific knowledge from molecular biology that has changed and invigorated areas of study in systematics and evolution. We have updated taxonomic classifications to be consistent with *Biology: A Global Approach,* 10th edition, Global Edition. However, we recognize that these will continue to change as new research reveals evolutionary relationships.

With increasing use of technology in the laboratory by instructors and students, we have provided all data tables in Excel format at *masteringbiology .com* under the Study Area. These tables can be modified and used in open-inquiry investigations, to teach data analysis, and as models for developing independent projects. All websites have been updated, and new Web sources and resources are included. We continue to support the use of media and technology with the section in each lab topic called "Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables," which coordinates with *Biology: A Global Approach* student media materials.

The *Preparation Guide* has been revised to coordinate with and support the laboratory manual.

Writing and scientific communication continue to be a strong component of the laboratory manual. In the eighth edition, we enhanced support for the writing program by reorganizing and revising Appendix A Scientific Writing and Communication with easily located information on each section of the scientific paper, notes for successful writing, suggestions for locating appropriate references, and a "Plan for Writing a Scientific Paper." We provide tips for preparing and presenting oral papers and posters and encourage instructors to include poster presentations to report findings from open-inquiry investigations. The writing program is supported by materials in the *Preparation Guide* as well as coordinating with Web resources and the latest editions of guides to scientific writing and presentations.

In all of these changes and modifications, our objective has been to provide laboratory experiences that are challenging to students and allow them to participate in authentic scientific investigations. We are keenly aware of the constraints on laboratory programs, including number of students and sections, preparation of laboratories and instructors, and the expense and mentoring that are required to teach inquiring young scientists. We hope we have provided choices and options that can meet the needs of a wide range of programs and instructors.

Laboratory Topics

The laboratory topics build on information and techniques in previous exercises. Various laboratory exercises incorporate a combination of directed and open-ended procedures. There are basically three types of lab topics included in the manual:

1. *Directed-Inquiry Investigations,* in which exercises have been constructed to involve students in the process of science. We have organized these lab topics to include introductory information from which students develop hypotheses and then predict the results of their experiments. They collect their data and summarize the data in tables and figures of their own construction. The students must then accept or reject their hypotheses, based on their results. Examples of these directed-inquiry investigations include Lab Topic 3 Diffusion and Osmosis, Lab Topic 4 Enzymes, and Lab Topic 6 Photosynthesis.
2. *Key Theme Investigations,* in which laboratory exercises have been designed and reorganized with a focus on key themes of biology, for example, the unity and diversity of life. In these thematic exercises, students summarize and synthesize their results and observations connecting to the key themes. They use their observations as evidence in support of these major concepts and apply their understanding to new problems. Examples of these laboratories (and their underlying themes) include Lab Topic 2 Microscopes and Cells (unity and diversity of life);

Lab Topics 14 and 15 Plant Diversity I and II (adaptation to the land environment); and Lab Topics 22 to 24 Vertebrate Anatomy I, II, and III (structure and function).

3. *Open-Inquiry Investigations,* in which students generate their own hypotheses and design their own experiments. These exercises begin with an introduction and a simple experiment that demonstrates procedures. Then students are given suggestions and encouraged to develop their own questions and methodologies for further investigation. Examples of these open-inquiry investigations include Lab Topic 5 Cellular Respiration and Fermentation, Lab Topic 11 Population Genetics, Lab Topic 13 Protists, Lab Topic 17 Fungi, Lab Topic 21 Plant Growth, Lab Topic 26 Animal Behavior, and Lab Topic 28 Ecology II.

Lab topics are designated as *Directed-Inquiry, Key Theme,* or *Open-Inquiry Investigations* in the table of contents.

Scientific Communication: Writing and Presenting

Scientists communicate their results in writing and in presentations to research groups and at meetings. Undergraduates need instruction in writing and an opportunity to practice these skills; however, instructors do not have the time to critique hundreds of student research reports for each exercise. Throughout this lab manual, teams of students work together on improving their skills. They are asked to organize and present their results to their peers during the discussion and summary sessions in the laboratory. Students are also required to write as part of each laboratory. They summarize and discuss their results and then apply information to new problems in the questions at the end of the laboratory.

We have also incorporated a scientific writing program into our lab manual in a stepwise fashion. Students must answer questions and summarize results within the context of the laboratory exercises. For directed-inquiry investigations, students are required to submit one section of a scientific paper. For example, they might submit the Results section for one experiment in Lab Topic 3, and the Discussion section for one experiment in Lab Topic 4. Once students have experience writing each section, they write at least one complete scientific paper for an open-inquiry investigation, for example, Lab Topics 13, 17, 21, and 26. Instructions for writing a scientific paper, developing an oral presentation, and creating a poster are included in Appendix A, which also contains suggestions for developing an organized writing program.

Instructors may also choose to organize a session for oral or poster presentations similar to scientific meetings. Appendix A includes advice and references for these other forms of scientific communication.

See Appendix A for additional information on scientific writing and presentations.

Integration of Other Sciences and Mathematics

Students often view biology as a separate and isolated body of knowledge. We have attempted to integrate biology, chemistry, some physics, and geology whenever possible. For example, in Lab Top 16 Bioinformatics: Molecular Phylogeny of Plants, students must use bioinformatics to analyze their data from molecular biology. Students use computer models to understand relationships and test hypotheses in Lab Topic 11 Population Genetics: The Hardy-Weinberg Principle and Lab Topic 28 Ecology II: Computer Simulations of a Pond Ecosystem. We also provide opportunities for students to quantify observations, analyze and summarize results in tables and figures, and, ultimately, to use these data to construct arguments in support of their hypotheses.

Special Features

Reviewing Your Knowledge: Students recall terminology and content by describing and explaining fundamental concepts. Students examine their results and then use evidence as they evaluate their understanding and knowledge. (Bloom's Taxonomy Level I: Knowledge and Comprehension)

Applying Your Knowledge: As instructors, we want our students to be challenged to think and to develop critical thinking skills. Throughout this manual, students are asked to work logically through problems, critique results, and modify hypotheses. To emphasize these skills further, we have developed a section in each laboratory topic called Applying Your Knowledge, in which students are asked to apply their knowledge to new problems and to make connections between topics. The questions encourage students to use their knowledge as they solve problems developed from current research as well as issues in science, medicine, and society. (Bloom's Taxonomy Level II: Application and Analysis; Synthesis and Evaluation)

Excel Tables: All data tables used for recording and analyzing results are available in Excel format for modification and use in the laboratory. These tables are designated in the table title and can be downloaded to laboratory computers for student use at www.masteringbiology.com in the Study Area.

Images and Data: Full-color images and figures are integrated throughout the lab manual. Lab Topic 16 Bioinformatics: Molecular Phylogeny of Plants is supported by images in the text as well as the masteringbiology.com website. Folders found under the Study Area contain images of the plants used in the investigation along with the edited and ready-to-use nucleotide sequences for each species. These can be downloaded to laptops or accessed on the website.

Investigative Extensions and Case Studies: Students and instructors have expressed interest in extending laboratory topics to open-inquiry investigations or simply to pursue additional questions that connect the topic to current research and issues. At the end of most lab topics, we have provided questions to prompt student-designed investigations. For a few, we provide case studies that build on the lab topic and require additional reading and research.

Student Media and Web Resources: Providing media and Web resources can enhance teaching and learning in the laboratory. For students, we have included references to videos that connect to their laboratory activities. These are designated in the text with a media icon. Also, at the end of each lab topic, we have included the section **Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables,** which directs students to the website for **activities** and **investigations** that can be used to prepare for the laboratory or review and practice after the laboratory. These media resources are available at www.masteringbiology.com under the Study Area.

Safety Considerations: Safety concerns are noted in the text by the use of icons for general safety and for biohazards. Note the **Laboratory Safety Guidelines** printed on the inside front cover.

Notes to Students: To assure student success, cautionary reminders and notes of special interest are also highlighted in the text.

Appendixes: Information needed in several laboratory topics is included in the appendixes: scientific writing and communication, instrumentation and techniques, the metric system, using chi-square analysis, and dissection terminology.

Instructional Support

The *Preparation Guide* provides valuable suggestions and essential information for the successful implementation of the laboratory topics.

Preparation Guide: A detailed *Preparation Guide for Investigating Biology* accompanies the laboratory manual. It contains materials lists, suggested vendors, instructions for preparing solutions and constructing materials, schedules for planning advance preparation, and suggestions for organizing materials in the lab. Also, the new edition includes sources for a new, hardier strain of mutant *Drosophila* flies for sepia/aldehyde oxidase developed for Lab Topic 9 Mendelian Genetics II: *Drosophila*. The *Preparation Guide* is essential for successfully preparing and teaching these investigative laboratories. It is available electronically at masteringbiology.com. Instructors can download preparation and ordering lists as needed and customize these for their program.

Acknowledgments

The development of our ideas, the realization of those ideas in laboratory investigations, and the preparation of this laboratory manual are the result of collaborations with many colleagues over the years. We are indebted to our teaching assistants, whose critical evaluations and insightful suggestions helped shape the exercises. Several colleagues made especially helpful or critical contributions to our efforts, including Evelyn Bailey, Steve Baker, Jim Brown, Joy Budensiek, Nitya Jacob, and Theodosia Wade. Many thanks to Kevin Cook, Bloomington Drosophila Stock Center of Indiana University, for developing the mutant strain of *D. melanogaster* used in Lab Topic 9 Mendelian Genetics II: *Drosophila*. We are grateful to Jacobus de Roode, Emory University, for sharing photographs and new research on the behavior of monarch butterflies, as well as suggestions for the new exercise in Lab Topic 1 Scientific Investigation. We are grateful for the support and guidance provided by the editorial and production group at Benjamin Cummings. Our thanks to Beth Wilbur, Josh Frost, Brady Golden, Lauren Harp, Mike Early, Jane Brundage, Maureen Spuhler, and Marilyn Perry. We are especially indebted to all the laboratory educators who have shared their ideas, hints for success, and philosophies of teaching with us, particularly our friends in the Association for Biology Laboratory Education (ABLE). We are grateful to our reviewers for sharing their words of encouragement and criticism, which were essential to the success of our work. We thank our students who, over the years, have provided the ultimate test for these investigations and our ideas. Finally, we extend special appreciation to our families for their good humor, patience, and encouragement.

Pearson would like to thank the following people for their work on the Global Edition:

Contributor
Louise Beard, *University of Essex*

Reviewers
Audrey O'Grady, *University of Limerick*

Helen Page, *Teesside University*

Caroline Orr, *Teesside University*

Reviewers

Amanda Boose, *Gonzaga University*
Thomas Butler, *SUNY Rockland*
Sharon Fugate, *Madisonville Community College*
Troy Giambernardi, *Grand Rapids Community College*
Douglas Kane, *Defiance College*
Ann Kircher, *College of Alameda*
Stephanie Maruhnich, *Florida State College at Jacksonville*
Margaret Olney, *Saint Martin's University*
Lynn Petrullo, *College of New Rochelle*
Catherine Purzycki, *University of the Sciences*
Eugenia Ribiero-Hurley, *Fordham University*
Ana Ribiero, *College of Mount Saint Vincent*
John Rousseau, *Whatcom Community College*
Jenise Snyder, *Ursuline College*
Nina Theis, *Elms University*
Shauna Weyrauch, *Central Ohio Technical College*

Reviewers for Previous Editions

Mitch Albers, *Minneapolis Community College*
Carol Alia, *Sarah Lawrence College*
Connie L. Allen, *Edison Community College*
Phyllis Baudoin, *Xavier University of Louisiana*
Mariette Baxendale, *University of Missouri, St Louis*
Carol Bernson, *University of Illinois*
Charles Biggers, *Memphis State University*
Sandra Bobick, *Community College of Allegheny County*
Jerry Bricker, *Cameron University*
Jeffrey T. Burkhart, *University of LaVerne*
Linda Burroughs, *Rider University*
Neil Campbell, *University of California, Riverside*
Nickie Cauthen, *Clayton College and State University*
Deborah Clark, *Oregon State University*
Sunita Cooke, *Montgomery College*
Charles Creutz, *University of Toledo*
Diana R. Cundell, *Philadelphia University*
Jean DeSaix, *University of North Carolina, Chapel Hill*
Rebecca Diladdo, *Suffolk University*
John Doherty, *Villanova University*
Susan Dunford, *University of Cincinnati*
April Ann Fong, *Portland Community College, Sylvania Campus*
Caitlin Gabor, *Southwest Texas State University*
William Garnett, *Raymond Walters College*
Patricia S. Glas, *The Citadel*
Michelle F. Guadette, *Tufts University*
Regina Guiliani, *Saint Peter's College*
Tracy Halward, *Colorado State University*
Teresa Hanlon, *Washington and Lee University*
John Hare, *Linfield College*
Laurie Henderson, *Sam Houston State University*
Daniel Hoffman, *Bucknell University*
Alan Jaworski, *University of Georgia*
Virginia A. Johnson, *Towson State University*
Tamas Kapros, *University of Missouri, Kansas City*
Nikolai Kirov, *New York University*
George Krasilovsky, *Rockland Community College*
Laura G. Leff, *Kent State University*
Brian T. Livingston, *University of Missouri, Kansas City*
Raymond Lynn, *Utah State University*
Presley Martin, *Drexel University*
Barbara J. Maynard, *Colorado State University*
Dawn McGill, *Virginia Western Community College*
Jacqueline McLaughlin, *Pennsylvania State University, Lehigh Campus*
Michael McVay, *Green River Community College*
Charles W. Mims, *The University of Georgia*
Alison Mostrom, *Philadelphia College of Pharmacy and Science*
Greg Paulson, *Washington State University*
Shelley A. Phelan, *Fairfield University*
Marcia Pierce, *Eastern Kentucky University*
Jane Rasco, *University of Alabama*
Carolyn Rasor, *Southern Methodist University*
John Rousseau, *Whatcom Community College*
Michael R. Schaefer, *University of Missouri, Kansas City*
Daniel C. Scheirer, *Northeastern University*
Bin Shuai, *Wichita State University*
Sandra Slivka, *San Diego Miramar College*
Nanci Smith, *University of Texas, San Antonio*
Gerald Summers, *University of Missouri*
Sheryl Swartz Soukup, *Illinois Wesleylan University*
Michael Toliver, *Eureka College*
Geraldine W. Twitty, *Howard University*
Roberta Williams, *University of Nevada, Las Vegas*
Dan Wivagg, *Baylor University*
Carol Yeager, *The University of Alabama*

To Mary, Bill, Rob, Laura, Victoria, Kate, and Will, with love

J.G.M.

To Stefanie and Cyndi, with love

M.E.B.C.

Judith Giles Morgan M. Eloise Brown Carter

About the Authors

Judith Giles Morgan received her M.A. degree from the University of Virginia and her Ph.D. from the University of Texas, Austin. She is Professor Emeritus of Biology at Emory University. She developed a general biology laboratory curriculum for majors to incorporate an investigative approach and a TA training program for multisection investigative laboratories. She served as an officer and board member of the Association for Biology Laboratory Education. Dr. Morgan has worked to improve science education in the community through a number of enrichment programs for high school students.

M. Eloise Brown Carter earned her M.S. and Ph.D. from Emory University and is Professor of Biology at Oxford College of Emory University. With expertise in plant ecology, she has incorporated independent and team research into lecture and laboratory courses in introductory and advanced biology. Dr. Carter served as President of the Association of Southeastern Biologists. She has received several teaching awards, including Oxford's Phi Theta Kappa and Fleming Awards, and Emory's Williams Award. Dr. Carter also teaches in the Oxford Institute for Environmental Education, a program for precollege teachers to improve science education through development of schoolyard investigations.

Contents

Preface 3

1 Scientific Investigation 29

Laboratory Objectives 29

Introduction 29

Exercise 1.1 Questions and Hypotheses 30

Exercise 1.2 Designing Experiments to Test Hypotheses 32

Exercise 1.3 Designing an Experiment 38

Exercise 1.4 Presenting and Analyzing Results 42

Exercise 1.5 Interpreting and Communicating Results 48

Reviewing Your Knowledge 49

Applying Your Knowledge 52

Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables 56

References 56

Websites 57

2 Microscopes and Cells 61

Laboratory Objectives 61

Introduction 61

Exercise 2.1 The Compound Light Microscope 62

Exercise 2.2 Basic Microscope Techniques 65

Exercise 2.3 The Stereoscopic Microscope 69

Exercise 2.4 The Transmission Electron Microscope 72

Exercise 2.5 The Organization of Cells 74

Reviewing Your Knowledge 81

Applying Your Knowledge 82

Investigative Extensions 83

Student Media: BioFlix, Activities, Investigations, and Videos 84

References 84

Websites 84

3 Diffusion and Osmosis 87

Laboratory Objectives 87

Introduction 87

Exercise 3.1 Diffusion of Molecules 89

Exercise 3.2 Osmotic Activity in Cells 93

Exercise 3.3 Investigating Osmolarity of Plant Cells 99

Reviewing Your Knowledge 108

Applying Your Knowledge 109

Investigative Extensions 110

Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables 110

References 111

Websites 111

4 Enzymes 115

Laboratory Objectives 115

Introduction 115

Exercise 4.1 Experimental Method and the Action of Catechol Oxidase 116

Exercise 4.2 Inhibiting the Action of Catechol Oxidase 119

Exercise 4.3 Influence of Concentration, pH, and Temperature on the Activity of Amylase 122

Reviewing Your Knowledge 133

Applying Your Knowledge 133

Investigative Extensions 135

Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables 136

References 137

Websites 137

5 Cellular Respiration and Fermentation 141

Laboratory Objectives 141

Introduction 141

Exercise 5.1 Alcoholic Fermentation 144

Exercise 5.2 Cellular Respiration 151

Exercise 5.3 Designing and Performing Your Open-Inquiry Investigation 158

Reviewing Your Knowledge 160

Applying Your Knowledge 161

Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables 162

References 162

Websites 162

6 Photosynthesis 165

Laboratory Objectives 165

Introduction 165

Exercise 6.1 The Wavelengths of Light for Photosynthesis 166

Exercise 6.2 Pigments in Photosynthesis 169

Exercise 6.3 Separation and Identification of Plant Pigments by Paper Chromatography 172

Exercise 6.4 Determining the Absorption Spectrum for Leaf Pigments 175

Reviewing Your Knowledge 180

Applying Your Knowledge 180

Investigative Extensions 182

Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables 182

References 182

Websites 182

7 Mitosis and Meiosis 185

Laboratory Objectives 185

Introduction 185

Exercise 7.1 Modeling the Cell Cycle and Mitosis in an Animal Cell 186

Exercise 7.2 Observing Mitosis and Cytokinesis in Plant Cells 191

Exercise 7.3 Observing Chromosomes, Mitosis, and Cytokinesis in Animal Cells 194
Exercise 7.4 Modeling Meiosis 197
Exercise 7.5 Meiosis in *Sordaria fimicola:* A Study of Crossing Over 203

Reviewing Your Knowledge 207
Applying Your Knowledge 209
Investigative Extensions 210
Student Media: BioFlix, Activities, Investigations, and Videos 212
References 212
Websites 213

8 Mendelian Genetics I: Fast Plants 217
Laboratory Objectives 217
Introduction 217

Exercise 8.1 Inheritance of Anthocyanin Gene 225
Exercise 8.2 Inheritance of Plant Color: Green, Yellow-Green, and Purple (Anthocyanin Present) 229
Exercise 8.3 Inheritance of Variegated and Nonvariegated Leaf Color 234

Reviewing Your Knowledge 238
Applying Your Knowledge 239
Investigative Extensions 240
Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables 242
References 242
Websites 242

9 Mendelian Genetics II: *Drosophila* 245
Laboratory Objectives 245
Introduction 245

Exercise 9.1 Establishing the Enzyme Reaction Controls 246
Exercise 9.2 Determining the Pattern of Inheritance of the Aldox Gene 251
Exercise 9.3 Determining Parental Genotypes Using Evidence from Progeny 255
Exercise 9.4 Mapping Genes 258

Reviewing Your Knowledge 263

Applying Your Knowledge 263

Investigative Extensions 264

Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables 265

References 265

Websites 265

10 Molecular Biology 271

Laboratory Objectives 271

Introduction 271

Exercise 10.1 Mapping DNA Using Restriction Enzymes and Electrophoresis 274

Exercise 10.2 Practice Problem for Mapping DNA 288

Reviewing Your Knowledge 292

Applying Your Knowledge 294

Investigative Extensions 295

Student Media: BioFlix, Activities, Investigations, and Videos 295

References 295

Websites 296

11 Population Genetics: The Hardy-Weinberg Principle 299

Laboratory Objectives 299

Introduction 299

Exercise 11.1 Testing Hardy-Weinberg Equilibrium Using a Bead Model 303

Exercise 11.2 Simulation of Evolutionary Change Using the Bead Model 306

Exercise 11.3 Open-Inquiry Computer Simulation of Evolution 321

Reviewing Your Knowledge 322

Applying Your Knowledge 323

Investigative Extensions 325

Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables 325

References 326

Websites 326

12 Bacteriology 329
Laboratory Objectives 329
Introduction 329
Exercise 12.1 Investigating Characteristics of Bacteria 331
Exercise 12.2 Ecological Succession of Bacteria in Milk 339
Exercise 12.3 Bacteria in the Environment 342
Exercise 12.4 Controlling the Growth of Bacteria 349
Reviewing Your Knowledge 354
Applying Your Knowledge 355
Investigative Extensions 356
Student Media: BioFlix, Activities, Investigations, and Videos 358
References 358
Websites 358

13 Protists 363
Laboratory Objectives 363
Introduction 363
Exercise 13.1 Supergroup Excavata 366
Exercise 13.2 Supergroup "SAR" 368
Exercise 13.3 Supergroup Unikonta 377
Exercise 13.4 Supergroup Archaeplastida 381
Exercise 13.5 Designing and Performing an Open-Inquiry Investigation 387
Reviewing Your Knowledge 390
Applying Your Knowledge 392
Student Media: BioFlix, Activities, Investigations, and Videos 393
References 393
Websites 393

14 Plant Diversity I: Bryophytes (Nonvascular Plants) and Seedless Vascular Plants 397
Laboratory Objectives 397
Introduction 397
Exercise 14.1 Bryophytes (Nonvascular Plants) 401
Exercise 14.2 Seedless Vascular Plants 407

Reviewing Your Knowledge 417

Applying Your Knowledge 418

Investigative Extensions 421

Student Media: BioFlix, Activities, Investigations, and Videos 421

References 421

Websites 422

15 Plant Diversity II: Seed Plants 425

Laboratory Objectives 425

Exercise 15.1 Gymnosperms 428

Exercise 15.2 Angiosperms 435

Reviewing Your Knowledge 447

Applying Your Knowledge 448

Investigative Extensions 450

Student Media: BioFlix, Activities, Investigations, and Videos 451

References 451

Websites 451

16 Bioinformatics: Molecular Phylogeny of Plants 455

Laboratory Objectives 455

Introduction 455

Exercise 16.1 Establishing Molecular Phylogenetic Relationships Among Rubisco Large Subunit Genes 458

Exercise 16.2 Analyzing Phylogenetic Trees and Reporting Results 466

Reviewing Your Knowledge 467

Applying Your Knowledge 468

Investigative Extensions 469

Student Media: BioFlix, Activities, Investigations, and Videos 470

References 470

Websites 470

17 The Kingdom Fungi 473
Laboratory Objectives 473
Introduction 473
Exercise 17.1 A Survey of Major Fungal Groups 475
Exercise 17.2 Designing and Performing an Open-Inquiry Investigation 487
Reviewing Your Knowledge 490
Applying Your Knowledge 491
Student Media: BioFlix, Activities, Investigations, and Videos 491
References 491
Websites 492

18 Animal Diversity I: Porifera, Cnidaria, Platyhelminthes, Mollusca, and Annelida 495
Laboratory Objectives 495
Introduction 495
Exercise 18.1 Phylum Porifera—Sponges (*Scypha*) 500
Exercise 18.2 Phylum Cnidaria—Hydras *(Hydra)* 502
Exercise 18.3 Phylum Platyhelminthes—Planarians *(Dugesia)* 505
Exercise 18.4 Phylum Mollusca—Clams 508
Exercise 18.5 Phylum Annelida—Clamworms *(Nereis)* and Earthworms *(Lumbricus terrestris)* 511
Reviewing Your Knowledge 516
Applying Your Knowledge 517
Investigative Extensions 517
Student Media: BioFlix, Activities, Investigations, and Videos 518
References 518
Websites 518

19 Animal Diversity II: Nematoda, Arthropoda, Echinodermata, and Chordata 521
Exercise 19.1 Phylum Nematoda—Roundworms (*Ascaris*) 521
Exercise 19.2 Phylum Arthropoda 525
Exercise 19.3 Phylum Echinodermata—Sea Star 532
Exercise 19.4 Phylum Chordata 535

Reviewing Your Knowledge—Lab Topics 18 and 19 542
Applying Your Knowledge 542
Investigative Extensions 547
Student Media: BioFlix, Activities, Investigations, and Videos 548
References 548
Websites 549

20 Plant Anatomy 555
Laboratory Objectives 555
Introduction 555
Exercise 20.1 Plant Morphology 559
Exercise 20.2 Plant Primary Growth and Development 561
Exercise 20.3 Cell Structure of Primary Tissues 564
Exercise 20.4 Structure of Tissues Produced by Secondary Growth 576
Exercise 20.5 Grocery Store Botany: Modifications of Plant Organs 579
Reviewing Your Knowledge 580
Applying Your Knowledge 582
Investigative Extensions 584
Student Media: BioFlix, Activities, Investigations, and Videos 584
References 584
Websites 585

21 Plant Growth 587
Laboratory Objectives 587
Introduction 587
Exercise 21.1 Factors Influencing Seed Germination 592
Exercise 21.2 Plant Growth Regulators: Auxin 595
Exercise 21.3 Plant Growth Regulators: Gibberellins 599
Exercise 21.4 Designing and Performing an Open-Inquiry Investigation 603
Reviewing Your Knowledge 605
Applying Your Knowledge 605

Student Media: BioFlix, Activities, Investigations, Videos, and Data Table 607

References 607

Websites 608

22 Vertebrate Anatomy I: The Skin and Digestive System 611

Laboratory Objectives 612

Introduction 612

Exercise 22.1 Histology of the Skin 616

Exercise 22.2 Introduction to the Fetal Pig 619

Exercise 22.3 The Digestive System in the Fetal Pig 623

Reviewing Your Knowledge 630

Applying Your Knowledge 631

Case Study 633

Student Media: BioFlix, Activities, Investigations, and Videos 634

References 635

Websites 635

23 Vertebrate Anatomy II: The Circulatory and Respiratory Systems 637

Laboratory Objectives 637

Introduction 637

Exercise 23.1 Glands and Respiratory Structures of the Neck and Thoracic Cavity 638

Exercise 23.2 The Heart and the Pulmonary Blood Circuit 639

Exercise 23.3 The Heart and the Systemic Circuit in the Thorax 643

Exercise 23.4 The Systemic Circuit in the Abdominal Cavity 645

Exercise 23.5 Blood 650

Exercise 23.6 Fetal Pig Circulation 651

Exercise 23.7 Details of the Respiratory System 653

Reviewing Your Knowledge 655

Applying Your Knowledge 656

Case Studies 658

Student Media: BioFlix, Activities, Investigations, and Videos 658

References 658

Websites 659

24 Vertebrate Anatomy III: The Excretory, Reproductive, and Nervous Systems 661

Laboratory Objectives 661

Introduction 661

Exercise 24.1 The Excretory System 662

Exercise 24.2 The Reproductive System 665

Exercise 24.3 Nervous Tissue, the Reflex Arc, and the Vertebrate Eye 673

Reviewing Your Knowledge 679

Applying Your Knowledge 680

Case Studies 680

Student Media: BioFlix, Activities, Investigations, and Videos 681

References 681

Websites 681

25 Animal Development 683

Laboratory Objectives 683

Introduction 683

Exercise 25.1 Development in Echinoderms: Sea Urchin and Sea Star 688

Exercise 25.2 Development in an Amphibian 695

Exercise 25.3 Development in the Zebrafish 697

Exercise 25.4 Development in a Bird: The Chicken 702

Reviewing Your Knowledge 710

Applying Your Knowledge 711

Investigative Extensions 713

Student Media: BioFlix, Activities, Investigations, and Videos 714

References 714

Websites 715

26 Animal Behavior 717
Laboratory Objectives 717
Introduction 717
Exercise 26.1 Taxis in Brine Shrimp 719
Exercise 26.2 Kinesis in Pill Bugs 722
Exercise 26.3 Agonistic Display in Male Siamese Fighting Fish 725
Exercise 26.4 Reproductive Behavior in Fruit Flies 729
Exercise 26.5 Designing and Performing Your Open-Inquiry Investigation 733
Reviewing Your Knowledge 735
Applying Your Knowledge 735
Student Media: BioFlix, Activities, Investigations, and Videos 737
References 737
Websites 737

27 Ecology I: Terrestrial Ecology 741
Laboratory Objectives 741
Introduction 741
Exercise 27.1 Biotic Components 744
Exercise 27.2 Abiotic Components 757
Exercise 27.3 Data Analysis 761
Reviewing Your Knowledge 770
Applying Your Knowledge 771
Optional Exercise 27.1 773
Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables 773
References 774
Websites 774

28 Ecology II: Computer Simulations of a Pond Ecosystem 781

Laboratory Objectives 781

Introduction 781

Exercise 28.1 Computer Model of the Pond Ecosystem 783

Exercise 28.2 Investigating the Effects of Disturbance on the Pond Ecosystem 792

Reviewing Your Knowledge 801

Applying Your Knowledge 802

Investigative Extensions 804

Student Media: BioFlix, Activities, Investigations, and Videos 804

References 804

Websites 805

Appendix A Scientific Writing and Communication 809

Appendix B The Metric System 823

Appendix C Instrumentation and Techniques 825

Appendix D Chi-Square Test 833

Appendix E Terminology and Techniques for Dissection 837

Credits

Photographs

1.1a: Jaap de Roode. 1.1b: Jaap de Roode. 1.9: Barry Mansell/naturepl.com. 2.1a: Judith Morgan. 2.1b: Judith Morgan. 2.2: Eloise Carter. 2.3: Judith Morgan. 2.5a: Biophoto Associates/Science Source. 2.5b: Biophoto Associates/Science Source. 2.6: Melba/age footstock. 2.7: Richard Howey. 2.8: Yuuji Tsukii. 2.9: Dr. Ralf Wagner. 2.10: Manfred Kage/Science Source. 2.11: Perennou Nuridsany/Science Source. 2.12: Bruce Iverson/SPL/Science Source. 2.UN1: Dr. Yuuji Tsukii. 3.3: Photo Insolite Realite/Science Source. 3.7a: Eloise Carter. 3.7b: Eloise Carter. 4.UN1: Steve Shott/Dorling Kindersley, Ltd. 5.7: L. Brent Selinger. 6.3: Judith Morgan. 6.6: L. Brent Selinger. 7.03: Biophoto/Science Source. 7.9a: Don W. Fawcett/Science Source. 7.9c: B. A. Palevitz, Courtesy of E. H. Newcomb, University of Wisconsin. 7.11: Pearson Education. 7.12: Pearson Education. 7.13: Pearson Education. 7.14: Pearson Education. 7.15: Ed Reschke/Getty Images. 7.16: Pearson Education. 7.17a: Ed Reschke/Peter Arnold/Getty Images. 7.17b: Ed Reschke/Peter Arnold/Getty Images. 7.17c: Ed Reschke/Peter Arnold/Getty Images. 7.17d: M. Abbey/Science Source. 7.17e: Ed Reschke/Peter Arnold/Getty Images. 7.18: Judith Morgan. 7.21g (left): Michael Franklin/Michael Franklin Micro Imaging. 7.21g (right): Dr. George L. Barron, University of Guelph. 7.23: Michael Clayton. 7.UN4: J.L. Carson, Custom Medical Stock Photo. 8.3: JS Photo/Alamy. 9.4a: Judith Morgan. 9.4b: Judith Morgan. 9.5a: Gunter Reuter. 9.5b: Gunter Reuter. 11.5a–d: The genetic basis of adaptive melanism in pocket mice. M. W. Nachman, H. E. Hoekstra, and S. L. D'Agostino. Proc Natl Acad Sci USA. 2003 Apr 29;100(9):5268–73, fig. 1B. 11.6a: Eye of Science/Science Source. 11.6b: Eye of Science/Science Source. 12.2a: Christine Case. 12.2b: Centers for Disease Control and Prevention (CDC). 12.5: Barry L. Batzing. 12.10: L. Brent Selinger. 12.11: L. Brent Selinger. 13.1: John Mansfield. 13.2: Michael Abbey/Science Source. 13.3a: Frank Fox/Science Source. 13.3b: Eric Grave/Science Source. 13.4a: Colin Bates/Coastal Imageworks (coastalimageworks.com). 13.4b: David Hall/Science Source. 13.4c: Eloise Carter. 13.5: Fred Rhoades. 13.6b: Dr. David Patterson/Science Source. 13.7a: Commonwealth Scientific and Industrial Research Organization of Australia. 13.7b: DP Wilson/Frank Lane Picture Agency Limited. 13.8: Biophoto Associates/Science Source. 13.9: Claude Carre/Science Source. 13.10: Image Quest 3-D/Photoshot Holdings, Ltd. 13.11: Melba/age footstock. 13.12: Stephen Sharnoff. 13.13: Bruno in Columbus. 13.14a: Ed Reschke/Peter Arnold/Getty Images. 13.14b: D. P. Wilson/Eric & David Hoskins/Science Source. 13.14c: Wayne Armstrong. 13.15: Wolfgang Bettinghofer/David Patterson micro*scope. 13.15b: blickwinkel/NaturimBild/Alamy. 13.15c: Laurie Campbell/Photoshot/NHPA Limited. 13.16: Center for Aquatic and Invasive Plants. 14.4: Tony Wharton/Frank Lane Picture Agency Limited. 14.6: K.G. Vock/Okapia/Science Source. 14.7a: Pat Lynch/Science Source. 14.7b: Jody Banks. 14.8: Steven Lynch. 14.9: Gordon Dickson. 14.10: Shari L. Morris/age footstock. 14.11a: Hemera/Thinkstock. 14.11b: Judith Morgan. 14.12a: Thinkstock. 14.12b: Fred Spiegel. 14.14: Open University, Department of Earth Sciences. 15.1a (inset): Judith Morgan. 15.1a (main): Eloise Carter. 15.1b: Ken Brate/Science Source. 15.1c: Eloise Carter. 15.1d: Biophoto Associates/Science Source. 15.1e1: Keith Gunnar/age footstock. 15.1e2: Al Schneider. 15.3a: F. Rauschenbach/F1online/age footstock. 15.3b: Stephen Dalton/naturepl.com. 15.3c: Anthony Mercieca/Science Source. 15.3d: Merlin P. Tuttle/Science Source. 15.6: Ed Reschke. 15.7: Eloise Carter. 16.UN1: Nigel Cattlin/Science Source. 16.UN2: Eloise Carter. 16.UN3: Eloise Carter. 16.UN4: Eloise Carter. 16.UN5: Judith Morgan. 16.UN6: Eloise Carter. 16.UN8: Judith Morgan. 16.UN9: Judith Morgan. 16.UN10: Judith Morgan. 16.UN11: Eloise Carter. 17.2: Darlyne A. Murawski/National Geographic Creative. 17.3a: comar/Fotolia. 17.3b: seelevel.com. 17.4a: D. Mahlke/Blickwinkel/age footstock. 17.4b: Jubal Harshaw/Shutterstock. 17.4c: Biology Pics/Science Source. 17.5a: Judith Morgan. 17.5b: Photo by Joe H. Taft. 17.5c: Judith Morgan. 17.5d: Nancy Rose. 17.5 (inset): Judith Morgan. 17.6a: Judith Morgan. 17.6b: Judith Morgan. 17.6c: Judith Morgan. 18.3: Wilfried Bay-Nouailhat. 18.4: Paddy Ryan. 18.6: Edward L. Snow/Photoshot Holdings, Ltd. 18.9: Kjell Sandved/Science Source. 18.1: Robert Dunne/Science Source. 19.2a: Kjell Sandved/Photoshot. 19.4a: Luis Castaneda/Getty Images. 19.6: Jeff Rotman/naturepl.com. 19.8: Heather Angel/Natural Visions/Alamy. 20.2a: Judith Morgan. 20.2b: blickwinkel/Alamy. 20.2c: Judith Morgan. 20.2d: Jim Wehtje/Getty Images. 20.2e: Judith Morgan. 20.4b: Elisabeth S. Pierson. 20.6a: Ed Reschke/Peter Arnold/Getty Images. 20.6b: Ed Reschke/Peter Arnold/Getty Images. 20.7b: Ed Reschke/Peter Arnold/Getty Images. 20.7c: Natalie Bronstein. 20.9b: Professor Ray F. Evert. 20.9c: Michael Clayton. 20.11b: Michael Clayton. 21.7: Burkhard Schulz. 22.1a: Marian Rice. 22.1b: Marian Rice. 22.1c: Marian Rice. 22.1d: Marian Rice. 22.2a: Marian Rice. 22.2b: Nina Zanetti. 22.2c: Marian Rice. 22.2d: Marian Rice. 22.2e: Biophoto/Science Source. 22.3a: Marian Rice. 22.3b: Marian Rice. 22.3c: Marian Rice. 22.4b: Victor Eroschenko. 22.5a: Judith Morgan. 22.1: LUMEN Histology. 23.9: SPL/Science Source. 24.7: Judith Morgan. 25.5: George von Dassow. 25.6: George von Dassow. 25.7: George von Dassow. 25.8: George von Dassow. 25.9: George von Dassow. 25.10: George von Dassow. 25.11: George von Dassow. 25.13a: Omikron/Science Source. 25.13b: Omikron/Science Source. 25.13c: Omikron/Science Source. 25.13d: Omikron/Science Source. 25.13e: Andrew Ewald. 25.13f: Omikron/Science Source. 25.15: Steve Baskauf. 25.16: Steve Baskauf. 25.17: Judith Morgan. 25.18: Steve Baskauf. 25.19: Judith Morgan. 25.20: Judith Morgan. 25.21: Judith Morgan. 25.22: Steve Baskauf. 25.23: Judith Morgan. 25.25: Pearson Education. 25.26: Pearson Education. 25.27: Pearson Education. 25.28: Oxford Scientific/Getty Images. 25.29: Pearson Education. 26.1: Hartl/blickwinkel/Alamy. 26.2: Danny Smythe/Alamy. 26.3: Eric Isselee/Thinkstock. AppC.1a: Thermo-Fischer. AppC.1b: Thermo-Fischer. AppC.2a: Eloise Carter. AppC.2b: Eloise Carter. AppE.1: Eric Isselee/Fotolia. AppE.2: Eric Isselee/Shutterstock. AppE.3: Eric Isselee/Shutterstock.

Text

Lab Topic 7: "Karyotyping Activity." Department of Biochemistry, University of Arizona, 1996.

Lab Topic 8: Williams, Paul H. "Bee-Sticks." 1980.

Lab Topic 11: Cook, L.M. "The Rise and Fall of the Carbonaria Form of the Peppered Moth." *The Quarterly Review of Biology*, 2003, vol. 78 (4), pp. 339–417.

Lab Topic 11: Sundih, O. H., J. Yank, Y. Li, D. Zhu, J. Hurd, T. Mitchell, E. Silva, and I. Maumenee. "Genetic Basis of Total Colourblindness Among the Pingelapese Islanders." *Nature Genetics*, 2000, vol. 25, pp. 289–293.

LAB TOPIC 1

Scientific Investigation

Before going to lab, read the Introduction and Exercises 1.1 and 1.2. Be prepared to answer all questions and contribute your ideas in a class discussion.

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Identify and characterize questions that can be answered through scientific investigation.
2. Define *hypothesis* and explain what characterizes a good scientific hypothesis.
3. Identify and describe the components of a scientific experiment.
4. Summarize and present results in tables and graphs.
5. Discuss results and critique experiments.
6. Design a scientific experiment.
7. Interpret and communicate results.

Introduction

Biology is the study of the phenomena of life, and biological scientists—researchers, teachers, and students—observe living systems and organisms, ask questions, and propose explanations for those observations. Scientific investigation is a way of testing those explanations. Science assumes that biological systems are understandable and can be explained by fundamental rules or laws. Scientific investigations share some common elements and procedures, which are referred to as the *scientific method*. Not all scientists follow these procedures in a strict fashion, but each of the elements is usually present. Science is a creative human endeavor that involves asking questions, making observations, developing explanatory hypotheses, and testing those hypotheses. Scientists closely scrutinize investigations in their field, and each scientist must present his or her work at scientific meetings or in professional publications, providing evidence from observations and experiments that supports the scientist's explanations of biological phenomena.

In this lab topic, you will not only review the process that scientists use to ask and answer questions about the living world, but you will develop the skills to

conduct and critique scientific investigations. Like scientists, you will work in research teams in this laboratory and others, collaborating as you ask questions and solve problems. Throughout this laboratory manual, you will be investigating biology using the methodology of scientists, asking questions, proposing explanations, designing experiments, predicting results, collecting and analyzing data, and interpreting your results in light of your hypotheses.

EXERCISE 1.1

Questions and Hypotheses

This exercise explores the nature of scientific questions and hypotheses. Before going to lab, read the explanatory paragraphs and then be prepared to present your ideas in the class discussion.

Lab Study A. Asking Questions

Scientists are characteristically curious and creative individuals whose curiosity is directed toward understanding the natural world. They use their study of previous research or personal observations of natural phenomena as a basis for asking questions about the underlying causes or reasons for these phenomena. For a question to be pursued by scientists, the phenomenon must be well defined and testable. The elements must be measurable and controllable.

There are limits to the ability of science to answer questions. Science is only one of many ways of knowing about the world in which we live. Consider, for example, this question: Do excessively high temperatures cause people to behave immorally? Can a scientist investigate this question? Temperature is certainly a well-defined, measurable, and controllable factor, but morality of behavior is not scientifically measurable. We probably could not even reach a consensus on the definition. Thus, there is no experiment that can be performed to test the question. Which of the following questions do you think can be answered scientifically?

1. Does playing football cause Lou Gehrig's disease?
2. Did the consumption of seven cans of "energy drink" cause the heart attack of a motorcyclist in Australia?
3. Will global warming increase the length of allergy seasons?
4. How effective are extracts of marigold and rosemary as insect repellents?
5. Should it be illegal to prolong the life of a terminally ill patient?

How did you decide which questions can be answered scientifically?

Lab Study B. Developing Hypotheses

As questions are asked, scientists attempt to answer them by proposing possible explanations. Those proposed explanations are called **hypotheses.** A hypothesis tentatively explains something observed. It proposes an answer to a question. Consider question 4, preceding. One hypothesis based on this question might be "Marigold and rosemary extracts are more effective than DEET in repelling insects." The hypothesis has suggested a possible explanation that compares the difference in efficacy between these plant extracts and DEET.

A scientifically useful hypothesis must be testable and falsifiable (able to be proved false). To satisfy the requirement that a hypothesis be falsifiable, it must be possible that the test results do not support the explanation. In our example, the experiment might be to spray one arm with plant extracts and the other with DEET. Then place both arms in a chamber with mosquitoes. If mosquitoes bite both arms with equal frequency or the DEET arm has fewer bites, then the hypothesis has been falsified. *Even though the hypothesis can be falsified, it can never be proved true.* The evidence from an investigation can only provide support for the hypothesis. In our example, if there are fewer bites on the arm with plant extracts than on the DEET-treated arm, then the hypothesis has not been proved, but has been supported by the evidence. Other explanations still must be excluded, and new evidence from additional experiments and observations might falsify this hypothesis at a later date. In science, seldom does a single test provide results that clearly support or falsify a hypothesis. In most cases, the evidence serves to modify the hypothesis or the conditions of the experiment.

Science is a way of knowing about the natural world (Moore, 1993) that involves testing hypotheses or explanations. The scientific method can be applied to the unusual and the commonplace. You use the scientific method when you investigate why your once-white socks are now blue. Your hypothesis might be that your blue jeans and socks were washed together, an assertion that can be tested through observations and experimentation.

Students often think that controlled experiments are the only way to test a hypothesis. The test of a hypothesis may include experimentation, additional observations, or the synthesis of information from a variety of sources. Many scientific advances have relied on other procedures and information to test hypotheses. For example, James Watson and Francis Crick developed a model that was their hypothesis for the structure of DNA. Their model could only be supported if the accumulated data from a number of other scientists were consistent with the model. Actually, their first model (hypothesis) was falsified by the work of Rosalind Franklin. Their final model was tested and supported not only by the ongoing work of Franklin and Maurice Wilkins but also by research previously published by Erwin Chargaff and others. Watson and Crick won the Nobel Prize for their scientific work. They did not perform a controlled experiment in the laboratory but tested their powerful hypothesis through the use of existing evidence from other research. Methods other than experimentation are acceptable in testing hypotheses. Think about other areas of science that require comparative observations and the accumulation of data from a variety of sources, all of which must be consistent with and support hypotheses or else be inconsistent and falsify hypotheses.

The information in your biology textbook is often thought of as a collection of facts, well understood and correct. It is true that much of the knowledge of biology has been derived through scientific investigations, has been thoroughly tested, and is supported by strong evidence. However, scientific knowledge is always subject to novel experiments and new technology, any aspect of which may result in modification of our ideas and a better understanding of biological phenomena. The self-correcting nature of science can be seen in newly proposed phylogenetic trees illustrating evolutionary relationships among groups of animals. For many years these relationships were based on studies of animal structure and development. Recently, new tools in molecular biology have provided scientists with the ability to sequence genes and even whole genomes. Results from molecular biology reveal new relationships among animal groups that are the basis for reconstructing evolutionary trees.

Application

Before scientific questions can be answered, they must first be converted to hypotheses, which can be tested. For each of the following questions, write an explanatory hypothesis. Recall that the hypothesis is a statement that explains the phenomenon you are interested in investigating.

1. Can human dander cause allergic reactions in cats?

2. Does the proximity of neighborhood parks have an effect on the health of children?

Scientists often propose and reject a variety of hypotheses before they design a single test. Discuss with your class which of the following statements would be useful as scientific hypotheses and could be investigated using scientific procedures. Give the reason for each answer by stating whether it could possibly be falsified and what factors are measurable and controllable.

1. "Snake oil" from pythons can be used to build heart muscle.
2. Obese women are more likely to have children who develop autism than normal-weight women.
3. Pierced and tattooed people consume more alcohol when visiting bars than persons without piercings and tattoos.
4. Birds evolved from bipedal dinosaurs called theropods.
5. Women who take aspirin have a lower risk of developing melanoma.

EXERCISE 1.2
Designing Experiments to Test Hypotheses

The most creative aspect of science is designing a test of your hypothesis that will provide unambiguous evidence to falsify or support a particular explanation. Scientists often design, critique, and modify a variety of

experiments and other tests before they commit the time and resources to perform a single experiment. In this exercise, you will follow the procedure for experimentally testing hypotheses, but it is important to remember that other methods, including observation and the synthesis of other sources of data, are acceptable in scientific investigations. An experiment involves defining variables, outlining a procedure, and determining controls to be used as the experiment is performed. Once the experiment is defined, the investigator predicts the outcome of the experiment based on the hypothesis.

Read the following description of a scientific investigation of the disease defense mechanisms in monarch butterflies.Then in Lab Study A you will determine the types of variables involved, and in Lab Study B, the experimental procedure for this experiment and for others.

Investigating Adult Monarch Butterfly Preference for Food Plants That Reduce Parasitic Infection in Their Offspring (Lefèvre et al., 2012)

In nature, free-living organisms are constantly infected with a wide range of parasites, and it is not uncommon to discover that many organisms have evolved defensive mechanisms to reduce the negative effects of parasitic infection. The monarch butterfly *(Danaus plexippus)* is regularly infected by the protozoan parasite *Ophryocystis elektroscirrha*, and this infection negatively affects the growth and survivability of these butterflies.

In the life cycle of a monarch butterfly, an adult female lays eggs on a milkweed plant, and after hatching, the larvae feed on the plant. Each larva pupates to an immobile chrysalis, from which a butterfly later emerges. Scientists in the laboratory of Jacobus de Roode of Emory University are studying if monarch butterflies have evolved defense mechanisms to protect offspring or adults against these protozoan parasites. In one experiment performed in de Roode's lab, the researchers asked if adult female butterflies infected with the protozoan parasite *preferentially* lay their eggs on milkweed plants that reduce parasite growth in their offspring, or do they *randomly* deposit eggs, regardless of the type of milkweed plant? They hypothesized that female butterflies would lay more eggs on plants that reduce parasite growth.

To test this hypothesis, they bred monarchs in the lab to obtain larvae. They then infected some larvae with the protozoan parasite, while leaving other larvae uninfected. All larvae were reared in a similar environment. As adult butterflies emerged, *infected* females were transferred to a mating cage. Control female monarchs (*uninfected*) were transferred to another cage. Uninfected males were released into each cage to serve as mating partners. Three days after mating (average time required for egg maturation), 10 infected and 10 uninfected females were released one at a time into one of five cages containing two milkweed plants. One plant, *Asclepias curassavica*, is known to contain high concentrations of chemicals that reduce parasite infection in offspring, and another plant, *Asclepias incarnata*, contains the chemicals but in lower concentrations. After allowing the females to lay eggs on the milkweed plants for 1 hour, they were returned to their holding cage. Two days later the experiment was repeated with the same female butterflies. The investigators then counted the number of eggs laid on each milkweed plant (Figure 1.1).

a. b.

FIGURE 1.1
Butterfly and milkweed experiment. (a) Monarch butterfly laying eggs on a milkweed plant. (b) Infected and uninfected butterflies were allowed to lay eggs in cages containing milkweed plants with different levels of anti-parasite chemicals.

Hypothesis

Infected monarch butterflies preferentially lay their eggs on plants that reduce parasite infection in their offspring.

Lab Study A. Determining the Variables

Read the description of each category of variable, and then identify the variable described in the preceding investigation. The variables in an experiment must be clearly defined and measurable. The investigator must identify and define *dependent*, *independent*, and *controlled* variables for a particular experiment.

The Dependent Variable

Within the experiment, one variable will be measured, counted, or observed in response to the experimental conditions. This variable is the **dependent variable.** For the monarch butterfly experiment as described, one dependent variable was measured. What is this variable?

Although only one dependent variable was measured in this experiment, it is acceptable to measure several dependent variables in an experiment. What additional dependent variables might be measured in this experiment, and why is this acceptable?

It is acceptable to measure more than one dependent variable because any of these may give additional information about the hypothesis being investigated. In this case, the question is whether females prefer to lay their eggs on plants that reduce parasitic infection in their offspring. Therefore, measuring characteristics that indicate the survivability of the next generation will add more evidence to support the hypothesis.

The Independent Variable

The scientist will choose one variable or experimental condition to manipulate or change. This variable is considered the most important variable by which to test the investigator's hypothesis, and is called the **independent variable.** What was the independent variable in the investigation of female butterfly preference of milkweed plants?

Can you suggest other variables that the investigators might have changed that would have had an effect on the dependent variable?

Although other factors such as light, humidity, the source of the larvae, the natural habitat of the butterflies, differences in test plants—any of which might affect the dependent variable—only one independent variable is usually chosen. Why is it important to have only one independent variable?

The Controlled Variable

Consider the variables that you identified as alternative independent variables. Although they are not part of the hypothesis being tested in this investigation, they could have significant effects on the outcome of this experiment. These variables must therefore be kept constant during the course of the experiment. They are known as the **controlled variables.** The underlying assumption in experimental design is that the selected independent variable is the one affecting the dependent variable. This is only true if all other variables are controlled. What are the controlled variables in this experiment?

Lab Study B. Choosing or Designing the Procedure

The **procedure** is the stepwise method, or sequence of steps, to be performed for the experiment. It should be recorded in a laboratory notebook before initiating the experiment, and any exceptions or modifications should be noted during the experiment. The procedures may be designed

from research published in scientific journals, through collaboration with colleagues in the lab or other institutions, or by means of one's own novel and creative ideas. The process of outlining the procedure includes determining levels of treatments, numbers of replications, and control treatment(s).

Level of Treatment

The value set for the independent variable is called the **level of treatment.** This is based on knowledge of the system and the biological significance of the treatment level. For example, if you were investigating the effect of sulfur dioxide (the independent variable) on plants growing near a coal-fired power plant, you would treat plants in your experiment with concentrations of sulfur dioxide that fall below, throughout, and above the range found around the power plant. In some experiments, however, independent variables represent categories that do not have a level of treatment. This is the case in the butterfly experiment, where the independent variable is the presence of parasites in the adult butterflies.

Replication

Scientific investigations are not valid if the conclusions drawn from them are based on only one experiment with one or two individuals. Generally, the same procedure will be repeated several times (replication) and many individuals must be used. Describe replication in this experiment.

Control

The experimental design includes a control in which the independent variable is held at an established level or is omitted. The control or control treatment serves as a benchmark that allows the scientist to decide whether the predicted effect is really due to the independent variable. What was the control in this experiment?

What is the difference between the control and the controlled variables discussed previously?

Lab Study C. Making Predictions

The investigator never begins an experiment without a prediction of its outcome. The **prediction** is always based on the particular experiment designed to test a specific hypothesis. Predictions are written in the form of if/then statements: "If the hypothesis is true, then the results of the experiment will be …"; for example, "**if** extracts of marigold and rosemary are more effective than DEET in repelling insects, **then** there will be fewer bites

on the arm sprayed with the plant extract compared to the arm sprayed with DEET after a 5-minute exposure to mosquitoes." Making a prediction provides a critical analysis of the experimental design. If the predictions are not clear, the procedure can be modified before beginning the experiment. For the butterfly experiment, the hypothesis was: "Infected monarch butterflies preferentially lay their eggs on plants that reduce parasite infection in their offspring." What should the prediction be? State your prediction.

To evaluate the results of the experiment, the investigator always returns to the prediction. If the results match the prediction, then the hypothesis is supported. If the results do not match the prediction, then the hypothesis is falsified. Either way, the scientist has increased knowledge of the process being studied. Many times the falsification of a hypothesis can provide more information than confirmation, as the ideas and data must be critically evaluated in light of new information. In the butterfly experiment, the scientist may learn that the prediction is supported—there is a greater proportion of eggs on the milkweed plant with higher levels of anti-parasite chemicals *(A. curassavica).* As a next step, the investigator may wish to examine the longevity of infected monarchs reared on *A. curassavica* compared with those reared on *A. incarnata*.

Return to page 4 and review your hypotheses for the numbered questions. Consider how you might design an experiment to test the first hypothesis. For example, you might separate a litter of kittens into two groups and raise them in two environments—one with human dander present and another where the air is filtered, removing human dander. The prediction might be:

> **If** human dander causes allergic reactions in cats *(a restatement of the hypothesis)*, **then** the cats raised in the environment with human dander will develop more allergies than those cats reared in a dander-free environment *(predicting the results of the experiment)*.

Now consider an experiment you might design to test the second hypothesis. How will you measure "healthier"?

State a prediction for this hypothesis and experiment. Use the if/then format.

The actual test of the prediction is one of the great moments in research. No matter the results, the scientist is not just following a procedure but truly testing a creative explanation derived from an interesting question.

Discussion

1. From this exercise, list the components of scientific investigations from asking a question to carrying out an experiment.

2. From this exercise, list the variables that must be identified in designing an experiment.

3. What are the components of an experimental procedure?

EXERCISE 1.3
Designing an Experiment

Materials

steps or platform, 8 to 14 inches high
metronome
clock or stopwatch with seconds
optional: iPads or iPhones with metronome app and heart rate app (See Website resources for suggestions)

Introduction

The Centers for Disease Control and Prevention (CDC) as well as the U.S. Department of Health and Human Services (HHS) have developed guidelines to improve health and fitness in response to continued concerns about chronic disease as a result of sedentary lifestyles and obesity. One measure of physical fitness is cardiovascular fitness, the body's ability to provide oxygen-rich blood to actively working tissues during exercise. Both the lungs and heart contribute to cardiovascular fitness, which can be affected by a number of factors, including types or extent of exercise, smoking, weight, and other physiological indicators. The "2008 Physical Activity Guidelines for Americans" (HHS, 2008) recommends 150 minutes a week of moderate (or 75 minutes of vigorous) physical activity, as well as strength-building exercises two or more times a week. A number of questions about the factors that affect cardiovascular fitness, particularly in college students, remain unanswered. Are the young adults who follow the HHS guidelines more fit than those who are sedentary? Can you be fit and obese or thin and less fit? How does sleep affect cardiovascular fitness? Can smokers regain the cardiovascular fitness levels of nonsmokers? If you are a smoker, how long before your cardiovascular fitness is affected?

Cardiovascular fitness can be determined by measuring a person's pulse rate and respiration rate before and after a period of exercise. A person who is more fit may have a relatively slower pulse rate and lower respiratory rate after exercise, and his or her pulse rate should return to the normal (resting rate) more quickly than that of a person who is less fit. Several tests of cardiovascular fitness have been developed, but the easiest to use and for comparing the results with other studies, is the Harvard Step Test (Simon, 2005). In this test, the subject steps up and down at a constant rate for 3 to 5 minutes.

The pulse rate is measured before and after exercise, as well as the recovery rate at 1, 2, and 3 minutes after exercise. These values can then be compared directly or used to calculate a fitness index.

In this exercise you will brainstorm questions about the factors that affect cardiovascular fitness in college students. As a class you will select one of these questions to pursue in today's laboratory. You will develop a hypothesis, design an experiment using the step test, and state your prediction. In the following exercises you will collect data, analyze and discuss your results, and critique your experiment.

In your research teams, take a few minutes to discuss several *specific* questions that you are interested in asking about factors that affect cardiovascular fitness in college students. Write your questions in the margin of the lab manual. Discuss with your teammates a testable hypothesis for each question. Choose one question and hypothesis from your group to add to a class list recorded by the instructor. Consider the characteristics of a question that can be pursued scientifically as you select the class question.

The class selects one question and hypothesis to investigate and then develops the experimental design using the basic step test as a starting point. They then predict the results of the experiments. All teams carry out the same experiment, pooling and analyzing the class data.

Record the ***question*** chosen by the class.

Hypothesis

Record a hypothesis for the question chosen by the class. Consider the characteristics of a testable hypothesis.

The Experiment

A form of the step test has been used to evaluate cardiovascular fitness for decades. There are several different versions of the test that allow the subject to exercise for a specified amount of time, at a given rate, using a standard-height step. The rate and duration of exercise should increase the heart rate without stressing the subject. Heart rate (beats per minute) before and after exercise can be measured. In some procedures the recovery heart rate is also measured at 1, 2, and 3 minutes after exercise. Recovery heart rates can be used to determine a fitness index. You may modify the design for your experiment. *Do not increase the rate or duration beyond those suggested.*

1. *Team Organization:* Select the subjects for the two test groups who will perform the step test. Designate other students to measure pulse rates and record data. *Each team of students should ideally have four students: one student in each test group, with the other students designated to measure pulse rates and record data.*

FIGURE 1.2
The Step Test.
The subject steps up on a platform and then down again, keeping the rate constant.

Students with respiratory or circulatory disorders should not participate as test subjects in the experiment. Do not exceed the rate or duration beyond those suggested.

2. *Step Test Basics:* The subject steps up and down on a platform or step, approximately 20–36 cm (8 to 14 inches) in height, for 3 to 4 minutes at a rate of 24 to 30 steps per minute. Begin by stepping up with one foot and follow with the other. The sequence for one step will be "up, up" and then "down, down" (Figure 1.2). Use a metronome to count steps to ensure that all subjects maintain a constant step rate. For a rate of 30 steps per minute, set the metronome to 120 beats per minute to coincide with the four steps—up and down. Metronome apps are available for the iPhone and iPad (see Websites at end of the lab topic for suggestions).
3. *Pulse Rate:* The subject's pulse rate is measured before the test (resting rate) and 1 minute after the test. The subject should be sitting quietly in a chair when the pulse is counted. To take a manual pulse, use three fingers to find the pulse in the radial artery (the artery on the underside of the wrist above the thumb). Count the beats per minute (bpm). (Count the beats for 30 seconds and multiply by 2). Pulse rate can also be measured using an iPhone or iPad with an app for heart rate (see Websites at end of the lab topic for suggestions).
4. *Recovery Rate:* Additionally, the pulse rate may be measured at 2 and 3 minutes after the test to determine the recovery rate (measure of the return to resting heart rate). Count the pulse from 1 to 1.5 minutes, then 2 to 2.5 minutes, and then 3 to 3.5 minutes. Multiply the minutes by 2 for beats per minute (bpm).
5. *Fitness Index:* You may choose to calculate the fitness index from the recovery pulse rates at 1, 2, and 3 minutes. These can then be compared to the Fitness Index Ratings in Table 1.1 or graphed to compare the two experimental groups.

   Calculate the fitness index (FI) using the following equation:

$$FI = \frac{\text{Duration of test (sec)}}{(t_1 + t_2 + t_3)} \times 100$$

t_1 = bpm 1 min. after exercise; t_2 = bpm at 2 min.; t_3 = bpm at 3 min

List the details of the step test procedure designed by the class. Be sure all team members understand and follow the same procedure.

Number and name of subjects in each test group:

Students designated to measure and record data:

Step rate:

Duration of test:

Height of step:

When are pulse rates (bpm) measured?

How are pulse rates (bpm) measured?

TABLE 1.1 Fitness Index for Step Test Indicating Relative Fitness

Harvard Step Test Ratings	
Fitness Index	**Rating**
<55	Poor
55–64	Low Average
65–79	High Average
80–89	Good
>90	Excellent

(Bird et al., 1998)

What is (are) the dependent variable(s) in your experiment?

What is the independent variable?

Controlled variables:

Control:

Level of treatment:

Replication:

Prediction

Predict the results of each experiment based on your hypotheses (if/then).

Performing the Experiment

Following the procedures established by your class for the step test, perform the experiment and record the results for your team in Table 1.2.

TABLE 1.2 Results of Step Test for Team Members. *Modify the Table for the Measurements Recorded in Your Experiment.*

Download an Excel version of this table from www.masteringbiology.com in the Study Area under Lab Media.

Measurements	Test Group 1:	Test Group 2:
Before step test Pulse rate (bpm)		
1 minute after step test Pulse rate (bpm) = t_1		
2 minutes after step test Pulse rate (bpm) = t_2		
3 minutes after step test Pulse rate (bpm) = t_3		
Fitness Index $FI = \frac{\text{Duration of test (sec)}}{(t_{11} + t_{22} + t_{33})} \times 100$		

Results

1. Customize Table 1.2 to record the results for your team. List the test groups (for example, athlete, nonathlete) above the columns at the top of the table. Cross through or add to the rows to indicate the dependent variables for your experiment.
2. Record the results for your team in Table 1.2.
3. Calculate the fitness index (if selected for your experiment) and record in Table 1.2.
4. Customize Table 1.3 for the *total class data* to include the test groups and the dependent variables measured.
5. Record the class results for all subjects in Table 1.3.
6. Calculate the class averages.
7. Compare your results with the fitness index and ratings in Table 1.1.
8. Analyze and present your results following the directions in Exercise 1.4.

EXERCISE 1.4
Presenting and Analyzing Results

Once the data are collected, they must be organized and summarized so that scientists can determine if the hypothesis has been supported or falsified. In this exercise, you will design **tables** and graphs; the latter are also called **figures.** Tables and figures have two primary functions. They are used (1) to help you analyze and interpret your results and (2) to enhance the clarity with which you present the work to a reader or viewer.

TABLE 1.3 Summary of Class Data for All Subjects. *Modify the Table and Record the Results from the Experiment by Test Groups 1 and 2.*

Download an Excel version of this table from www.masteringbiology.com in the Study Area under Lab Media.

Results for Test Group 1:

Subject	1	2	3	4	5	6	Average
Before step test Pulse rate (bpm)							
1 minute after step test Pulse rate (bpm)							
2 minutes after step test Pulse rate (bpm)							
3 minutes after step test Pulse rate (bpm)							
Fitness Index							

Results for Test Group 2:

Subject	1	2	3	4	5	6	Average
Before step test Pulse rate (bpm)							
1 minute after step test Pulse rate (bpm)							
2 minutes after step test Pulse rate (bpm)							
3 minutes after step test Pulse rate (bpm)							
Fitness Index							

Lab Study A. Tables

You have collected data from your experiment, and the information may appear at first glance to have little meaning. Look at your data. How could you organize the data set to make it easier to interpret? You could *average* the data set for each test group, but even averages can be rather uninformative. Could you use a summary table to convey the data (in this case, averages)?

Table 1.4 is an example of a table using data from an experiment investigating the effects of sulfur dioxide on soybean production. In this experiment, 48 soybean plants in flower were divided into two groups, experimental and control. One group (the experimental group) was exposed to 0.6 ppm (parts per million) of sulfur dioxide for 4 hours. The other group was exposed to filtered air for the same amount of time. The plants were then maintained in the greenhouse, and when the seed pods matured, the investigators counted the number of pods per plant and the number of seeds per pod. Note that the number of replicates and the units of measurement are provided in the table title.

Tables are used to present results that have many data points. They are also useful for displaying several dependent variables and when the quantitative values rather than the trends are the focus. For example, average number of bean pods, average number of seeds per pod, and average weight of pods per plant for treated and untreated plants could all be presented in one table.

The following guidelines will help you construct a table.

- All values of the same kind should read down the column, not across a row. Include only data that are important in presenting the results and for further discussion.
- Information and results that are not essential (for example: test-tube number, simple calculations, or data with no differences) should be omitted.
- The heading of each column should include units of measurement, if appropriate.
- Tables are numbered consecutively throughout a lab report or scientific paper. For example: Table 1.4 would be the fourth table in your report.
- The **title,** which is located at the top of the table, should be clear and concise, with enough information to allow the table to be understandable apart from the text. Capitalize the first and important words in the title. Do not capitalize articles (*a, an, the*), short prepositions, and conjunctions. The title does not need a period at the end.
- Refer to each table in the written text. Summarize the data and refer to the table—for example, "The plants treated with sulfur dioxide produced an average of 1.96 seeds per pod (Table 4)." Do not write, "See the results in Table 4."
- If you are using a database program, such as Excel, you should still sketch your table on paper before constructing it on the computer.

TABLE 1.4 Effects of 4-Hour Exposure to 0.6 ppm Sulfur Dioxide on Average Seed and Pod Production in Soybeans (24 Replicates)

Treatment	Seeds per Pod	Pods per Plant
Control	3.26	16
SO_2	1.96	13

Application

1. Using the data from your experiment, design a summary table to present the results for one of your dependent variables (for example, pulse rate before and one minute after the step test).
2. Label this Table 1.5. Compose a title for your table. Include the number of replications used to calculate the averages. Refer to the guidelines in the previous section of this lab topic for composing titles.

Lab Study B. Figures

Graphs, diagrams, drawings, and photographs are all called *figures*. The results of an experiment usually are presented graphically, showing the relationships among the independent and dependent variable(s). A graph or figure provides a visual summary of the results. Often, characteristics of the data are not apparent in a table but may become clear in a graph. By looking at a graph, then, you can visualize the effect that the independent variable has on the dependent variable and detect trends in your data. Making a graph may be one of the first steps in analyzing your results.

The presentation of your data in a graph will assist you in interpreting and communicating your results. In the final steps of a scientific investigation, you must be able to construct a logical argument based on your results that either supports or falsifies your starting hypothesis. Your graph should be accurately and clearly constructed, easily interpreted, and well annotated.

The following guidelines will help you to construct such a graph:

- Use graph paper and a ruler to plot the values accurately. If using a database program, you should first sketch your axes and data points before constructing the figure on the computer.
- The independent variable is graphed on the *x*-axis (horizontal axis, or abscissa), and the dependent variable on the *y*-axis (vertical axis, or ordinate).
- The numerical range for each axis should be appropriate for the data being plotted. Generally, begin both axes of the graph at zero (the extreme left corner). Then choose your intervals and range to maximize the use of the graph space. Choose intervals that are logically spaced and therefore will allow easy interpretation of the graph, for example, intervals of 5s or 10s. To avoid generating graphs with wasted space, you may signify unused graph space by two perpendicular tic marks between the zero and your lowest number on one or both axes.
- Label the axes to indicate the variable and the units of measurement. Include a legend if colors or shading is used to indicate different aspects of the experiment.

- Choose the type of graph that best presents your data. Line graphs and bar graphs are most frequently used. The choice of graph type depends on the nature of the variable being graphed.
- Compose a title for your figure and write it below your graph. Figures should be numbered consecutively throughout a lab report or scientific paper. Each figure is given a caption or title that describes its contents, giving enough information to allow the figure to be self-contained. Capitalize only the first word in a figure title and place a period at the end.

The Line Graph

Line graphs show changes in the quantity of the chosen variable and emphasize the rise and fall of the values over their range. Use a line graph to present continuous quantitative data. For example, changes in a dependent variable such as changes in weight measured over time would be depicted best in a line graph.

- Whether to connect the dots or draw a best fit curve depends on the type of data and how they were collected. To show trends, draw smooth curves or straight lines to fit the values plotted for any one data set. Connect the points dot to dot when emphasizing meaningful changes in values on the *x*-axis.
- If more than one set of data is presented on a graph, use different colors or symbols and provide a key or legend to indicate which set is which.
- A boxed graph, instead of one with only two sides, makes it easier to see the values on the right side of the graph.

Note the features of a line graph in Figure 1.3, which shows the relationship between wolf and moose abundance over time on Isle Royale in Lake Superior.

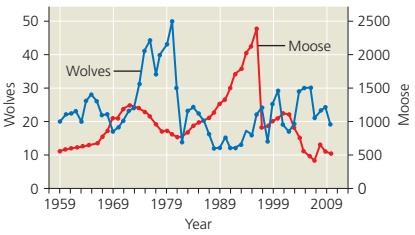


FIGURE 1.3
Five decades of fluctuating wolf and moose populations on Isle Royale, a remote wilderness island in Lake Superior.
Source: From the Isle Royale Wolf-Moose Study

The Bar Graph

Bar graphs are constructed following the same principles as for line graphs, except that vertical bars, in a series, are drawn down to the horizontal axis. Bar graphs are often used for data that represent separate or discontinuous groups or nonnumerical categories, thus emphasizing the discrete differences between the groups. For example, a bar graph might be used to depict differences in the proportion of eggs laid by monarch butterflies on milkweeds with and without chemicals that reduce parasite infection. Bar graphs are also used when the values on the *x*-axis are numerical but grouped together. These graphs are called histograms.

Note the features of a bar graph in Figure 1.4, which shows the percentage of calories from fast food among adults aged 20 and over.

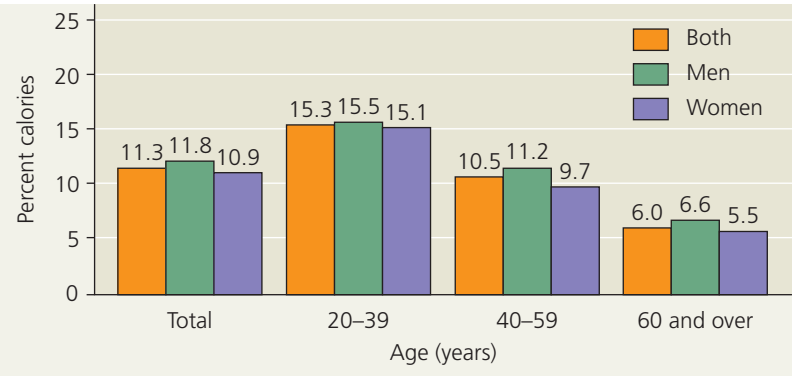


FIGURE 1.4
Percentage of calories from fast food among adults aged 20 and over, by gender and age: United States, 2007–2010.
Source: National Health and Nutrition Survey, 2007–2010

You will be asked to design graphs throughout this laboratory manual. Remember, the primary function of the figure is to present your results in the clearest manner to enhance the interpretation and presentation of your data.

Application

1. Using data from your experiments and the grid provided below, design a *bar graph* that shows the relationship between the dependent and independent variables in your experiment. Discuss with your teammates how to design one figure so that it includes the data for the independent variable and one or more dependent variables.
   a. What was the independent variable for your experiment? On which axis would you graph this?

   b. What was the dependent variable? Write this on the appropriate axis.

2. Add a *legend* to your figure to distinguish the two test groups.
3. Draw, label, and compose a *title* for your figure.
4. Imagine an experiment similar to the one you have performed where it would be appropriate to use a line graph.

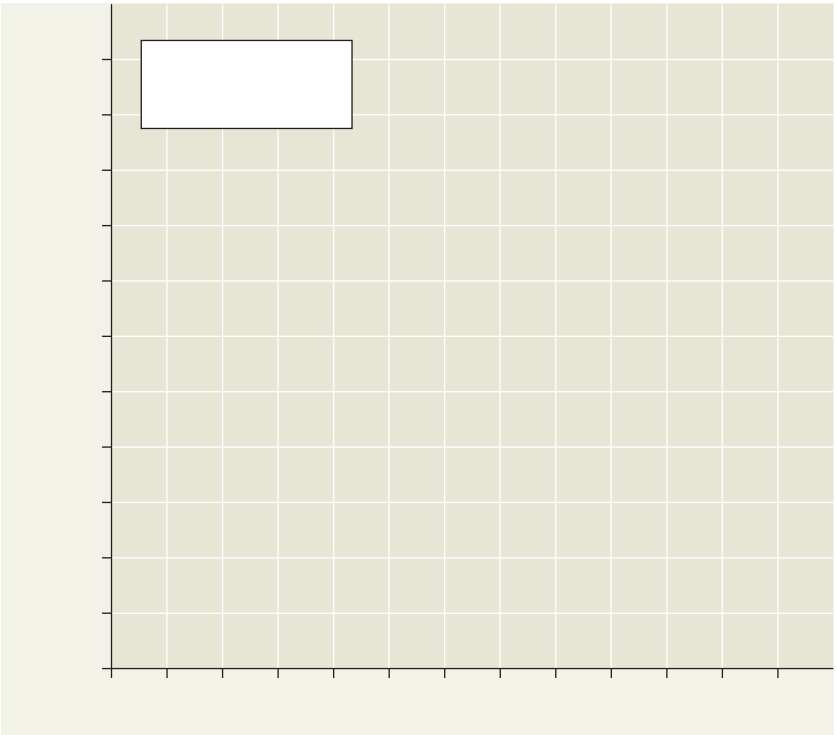


FIGURE 1

_____

_____

EXERCISE 1.5

Interpreting and Communicating Results

The last component of a scientific investigation is to interpret the results and discuss their implications in light of the hypothesis and supporting literature. The investigator studies the results, including tables and figures, and determines if the hypothesis has been supported or falsified. If the hypothesis has been falsified, the investigator must suggest alternate hypotheses for testing. If the hypothesis has been supported, the investigator suggests additional experiments to strengthen the hypothesis, using the same or alternate methods.

Scientists will thoroughly investigate a scientific question, testing hypotheses, collecting data, and analyzing results. In the early stages of a scientific study, scientists review the scientific literature relevant to their topic. They continue to review related published research as they interpret their results and develop conclusions. The final phase of a scientific investigation is the communication of the results to other scientists. Preliminary results may be presented within a laboratory research group and at scientific meetings where the findings can be discussed. Ultimately, the completed project is presented in the form of a scientific paper that is reviewed by scientists within the field and published in a scientific journal. The ideas, procedures, results, analyses, and conclusions of all scientific investigations are critically scrutinized by other scientists. Because of this, science is sometimes described as *self-correcting*, meaning that errors that may occur are usually discovered within the scientific community.

Scientific communication, whether spoken or written, is essential to science. During this laboratory course, you often will be asked to present and interpret your results at the end of the laboratory period. Additionally, you will write components of a scientific paper for many lab topics. In Appendix A at the end of the lab manual, you will find a full description of a scientific paper and instructions for writing each section.

Application

1. Using your tables and figures, analyze your results. What relationships are apparent between variables? Look for trends in your figures and tables. Discuss your conclusions with your group.

2. Write a summary statement for your experiments incorporating evidence from your results. Use your results to support or falsify your hypotheses. Be prepared to present your conclusions to the class.

3. Critique your experiment. What weaknesses do you see in the experiment? Suggest improvements.

Weaknesses in Experiment	Improvement

4. Suggest additional and modified hypotheses that might be tested in the future. Briefly describe your next experiment.

5. Refer to Appendix A "Scientific Writing and Communication," at the end of your lab manual. Briefly describe the four major parts of a scientific paper. What is the abstract? What information is found in a References Cited section? What sections of a scientific paper always include references?

Reviewing Your Knowledge

1. Review the major components of an experiment by matching the following terms to the correct definition: *control, controlled variables, level of treatment, dependent variable, replication, procedure, prediction, hypothesis, independent variable.*

   a. Variables that are kept constant during the experiment (variables not being manipulated)

   b. Tentative explanation for an observation

   c. What the investigator varies in the experiment (for example, time, pH, temperature, concentration)

   d. Process used to measure the dependent variable

e. Appropriate values to use for the independent variable

f. Treatment that eliminates the independent variable or sets it at a standard value

g. What the investigator measures, counts, or records; what is being affected in the experiment

h. Number of times the experiment is repeated

i. Statement of the expected results of an experiment based on the hypothesis

2. Identify the dependent and independent variables in the following investigations.

a. Scientists investigating the effects of decreased rainfall on the occurrence of allergens in urban environments measured the number of days that rapeseed pollen was present in the streets of London and rural fields.

b. Coral reefs compete for light and space with seaweeds that may cover the coral like a lawn. Scientists measure the percent of coral covered with seaweed and the rate of coral growth in test plots with and without goby fish present.

c. Scientists compare the preference for chocolate in rats kept in a warm environment throughout the night, compared with rats kept in a cooler environment.

3. Suggest a control treatment for each of the following experiments.

a. Scientists record the levels of cortisol in male blackbirds kept in an environment with minimal noise pollution.

b. To investigate if noise fosters depression, a group of hamsters is exposed to noise throughout the night.

4. Propose an experiment and suggest a control treatment for each of the following questions.

a. Is the mineral zinc effective as a possible emergency treatment for deadly Australian box jellyfish stings?

Experiment:

Control:

b. Does the *fear* of being attacked by predators have a negative impact on reproduction in nesting birds?

Experiment:

Control:

c. Will chewing gum after every meal reduce gum disease?

Experiment:

Control:

5. A recent study of 39,876 Caucasian women aged 45 years or older reports that women who take 100mg of aspirin on alternate days for more than a year are 42% less likely to develop colorectal cancer than women who do not use aspirin. List other variables that would be important to control in this study (controlled variables).

6. A study of the effects of vitamin B (B6 and B12) supplementation on the prevention of cognitive decline in patients with Alzheimer's disease has produced positive results. Can you conclude that vitamin B prevents

Alzheimer's disease? What other possible explanations for this correlation can you suggest?

7. What is the essential feature of science that makes it different from other ways of understanding the natural world?

Applying Your Knowledge

Interpreting Graphed and Tabular Data

1. The use of DDT for malaria control stopped being funded by the World Health Organization (WHO) in the 1980s. The World Bank required a ban on DDT for developing countries seeking loans. At the same time there has been an increase in the resistance of the malarial parasite to the most common antimalarial drugs. Since 2001, WHO has allowed the spraying of DDT in Africa on interior walls to kill mosquitoes. Review the graph in Figure 1.5 and information provided and then answer the following questions.
What is the independent variable?

What is the dependent variable?

Why was a bar graph selected to present these data? Could the authors have used a line graph?

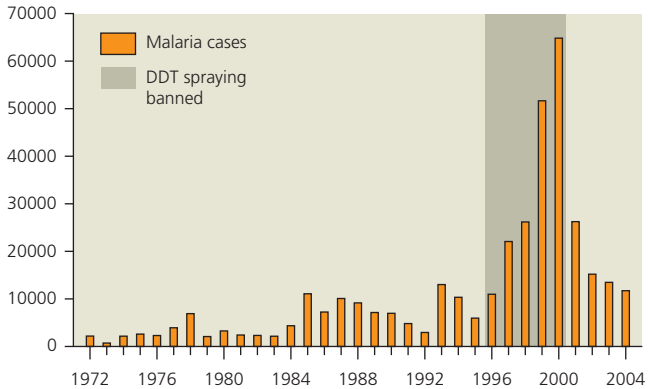


FIGURE 1.5
Malaria cases in South Africa before, during, and after banned DDT spraying.
Source: After Opar, 2006

Write a statement summarizing the results. Specifically address trends from 1972–1992, 1995–2000, and 2001–2004.

2. Review the experimental conditions for the monarch butterfly and milkweed experiment in Exercise 1.2. Recall that adult butterflies lay their eggs on milkweed plants and that the caterpillars that hatch feed on the milkweed leaves. Adult monarch butterflies are often infected by a protozoan parasite that affects their survivability and growth. Some milkweeds *(A. curassavica)* produce high concentrations of anti-parasitic compounds that reduce parasitic infections in monarch butterfly offspring.

Scientists hypothesized that infected monarch butterflies *preferentially* lay their eggs on milkweeds that reduce parasite infection (*A. curassavica*). In the experiment, the investigators measured the proportion of eggs laid on *A. curassavica* by infected and uninfected adult monarch butterflies.

If the butterflies showed no preference for the *A. curassavica* plants, what would be the expected proportion?

The results of this experiment are shown in Figure 1.6. Do the results support or falsify the hypothesis? Explain your answer using the data presented.

In a second experiment scientists hypothesized that parasite-infected caterpillars would consume a greater proportion of their diet from the anti-parasite (*A. curassavica*) milkweed. In the experiment, caterpillars

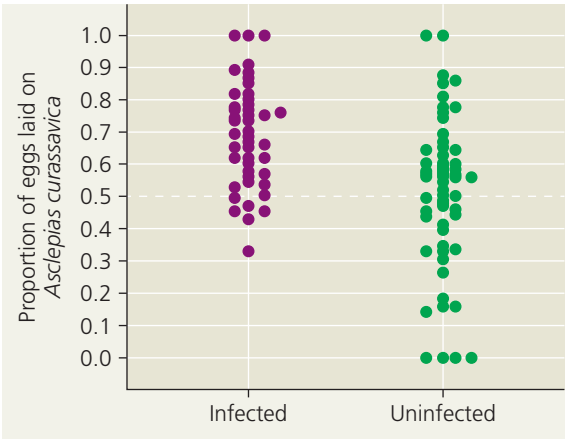
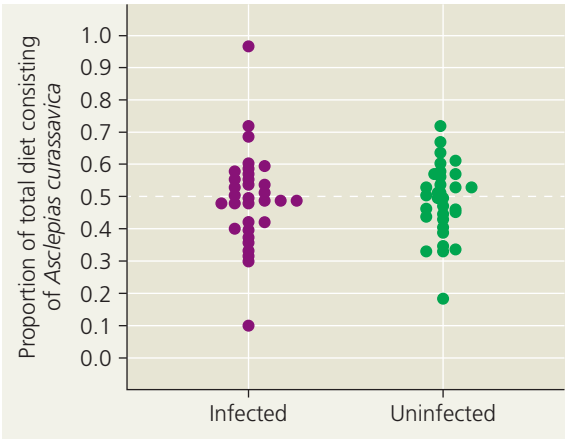


FIGURE 1.6
Proportion of eggs laid on anti-parasitic milkweed (*A. curassavica*) by infected and uninfected monarch butterflies.

Source: Lefèvre et al., 2012

FIGURE 1.7
Proportion of diet from anti-parasitic milkweed (*A. curassavica*) consumed by infected and uninfected caterpillars.
Source: Lefèvre et al., 2012

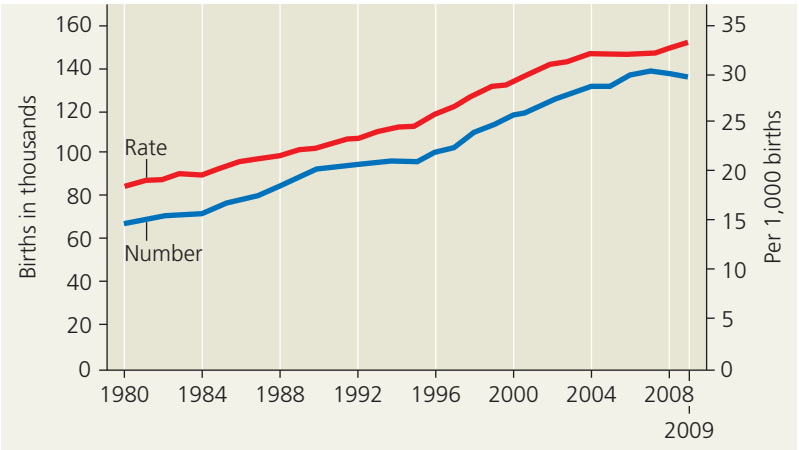


were given the choice of feeding on milkweed leaves with high concentrations of anti-parasite compounds (*A. curassavica*) or another milkweed with low concentrations.

The results of the caterpillar feeding experiment are shown in Figure 1.7. Do these results support of falsify the hypothesis? Explain your answer using the data presented.

3. Review the guidelines for graphs on pages 17–18 earlier in the chapter and critique Figure 1.8. This figure illustrates the number and birth rate for twins born in the United States between 1980 and 2009. Suggest changes that you could make to improve this figure.

FIGURE 1.8
Number and Rate of Twin Births.
Source: Modified from Martin et al., 2012



Practicing Experimental Design

1. In 2006, a spelunker photographing bats in a cave about 40 miles from Albany, New York, noticed a white powdery substance covering the muzzles of the hibernating bats. Five years later the disease had spread into Canada and to Tennessee and North Carolina, and had killed more than a million bats of six different species. Mortality in many bat colonies is 95%, and many biologists fear that in a few years the disease may spread as far as the Pacific Coast. Living and dead bats have a white fungus, identified as *Geomyces destructans*, on their faces, ears, and wings, and thus the disease is known as "white nose syndrome." In addition to harboring the fungus, infected bats demonstrate aberrant behavior. They may come out of hibernation early and during daylight; without insects to eat, they become emaciated or starve to death. Their wings tear easily and lose elasticity. Although it may appear obvious, scientists question if *G. destructans* is the primary cause of death, and they are investigating other explanations. For example, other pathogens (viruses or bacteria) may be the primary infective agents, and the white fungus may be secondary. Scientists also have found that the digestive systems of affected bats have fewer bacteria necessary for the digestion of insects. Therefore, less energy is available to them during hibernation. This could result in starvation and increased susceptibility to the fungus (Zimmerman, 2009). Using the criteria in Lab Study B, Developing Hypotheses, select the hypothesis you would pursue as a scientist and justify your choice.

2. "Grains of paradise" plants grow in the swampy region inhabited by the western lowland gorilla and make up 80–90% of the gorillas' diet, and are even utilized in constructing their nests each night. Captive lowland gorillas in zoos are not fed grains of paradise, but rather have a complex diet of processed vitamin-rich food plus fruits and vegetables available in the marketplace. Recently, scientists identified potent anti-inflammatory and antimicrobial compounds in grains of paradise that may hold the key to a puzzling question: "Why do western lowland gorillas in zoos have an alarmingly high rate of cardiomyopathy (a type of heart disease)?" Hypothesize about the effect of diet (grains of paradise) on rates of heart disease. Describe a simple preliminary experiment to test your hypothesis, and state a prediction.

   Hypothesis:

   Experiment:

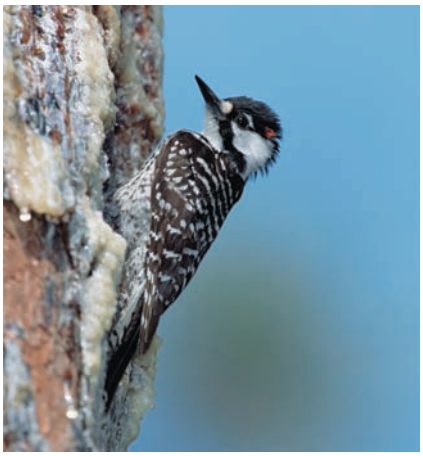


FIGURE 1.9
Red-cockaded woodpecker.
A federally endangered species that lives in old-growth longleaf pine forests of the southeastern United States.

Prediction:

3. Scientists have been studying ways to increase numbers of new populations of the federally endangered red-cockaded woodpecker (Figure 1.9). Previous research has shown that woodpeckers prefer to colonize forests with *naturally* existing nesting holes, rarely moving into new territories with no holes. Scientists wondered if birds would move into new sites if they included trees with *artificially* constructed nesting cavities. They hypothesized that birds *would* colonize these forests with artificially constructed cavities. Describe a possible experiment to test the hypothesis, including a control. State a prediction.

Experiment:

Control:

Prediction:

Student Media: BioFlix, Activities, Investigations, Videos, and Data Tables

www.masteringbiology.com (select Study Area)

Activities—Ch. 1: Graph It! An Introduction to Graphing

Investigations—Ch. 1: How Does Acid Precipitation Affect Trees?

Data Tables—Tables 1.2 and 1.3 can be downloaded in Excel format. Look in the Study Area under Lab Media.

References

Barnard, C., F. Gilbert, and P. McGregor. *Asking Questions in Biology*, 4th ed. Harlow, England: Pearson, 2011.

Bird, S. R., A. Smith, and K. James. *Exercise Benefits and Prescriptions*, Cheltenham, UK: Nelson Thornes Ltd., 1998.

Burch, Druin. "Eat Dirt? Allergies, Autoimmune Disease, and the Hygiene Hypothesis," *Natural History*, 2012, vol. 120, pp. 12–15. Good discussion of correlation vs. causation in scientific research.

Knisely, K. *A Student Handbook for Writing in Biology*, 4th ed. Sunderland, MA: Sinauer Associates, 2013. (Instructions for making graphs using Excel, Appendix 2.)

Lefèvre, T., A. Chiang, M. Kelavkar, H. Li, J. Li, C. Lopez, F. deCastillejo, L. Oliver, Y. Potini, M. Hunter, and J. C. de Roode. "Behavioural Resistance Against a Protozoan Parasite in the Monarch Butterfly." *Journal of Animal Ecology*, 2012, vol. 81, pp. 70–79.

Martin, J. A., B. E. Hamilton, and M. J. K. Osterman. "Three Decades of Twin Births in the United States, 1980–2009." *NCHS Data Brief* , 2012, no. 80. http://www.cdc.gov/nchs/data/databriefs/db80.htm. Accessed March, 2013.

Moore, J. *Science as a Way of Knowing*. Cambridge, MA: Harvard University Press, 1993.

Opar, A. "The Return of DDT." *SEED*, 2006, vol. 2, no. 8, p. 20.

Pechenik, J. *A Short Guide to Writing About Biology*, 8th ed. San Francisco, CA: Addison Wesley Longman, 2012.

Reece, J., et al. *Campbell Biology*, 10th ed. San Francisco, CA: Pearson Education, 2014.

Simon, H. B. *The No Sweat Exercise Plan: Lose Weight, Get Healthy, and Live Longer*. New York: McGraw-Hill, 2005.

Walters, J. R., C. K. Copeyon, and J. H. Carter. "Test of the Ecological Basis of Cooperative Breeding in Red-cockaded Woodpeckers." *The Auk*, 1992, vol. 1009, no. 1, pp. 90–97.

Zimmerman, R. "Biologists Struggle to Solve Bat Deaths." *Science*, 2009, vol. 324, pp. 1134–1135.

Websites

Download iPad/iPhone apps from iTunes for the metronome and heart rate monitor. Suggested apps:

Instant Heart Rate—Heart Rate Monitor by Azumio. Place your fingertip on the camera. Free.

Vital Signs Camera—Philips. Uses a camera to measure heart rate and breathing rate by measuring facial changes. Can measure two people at the same time. Will record a series of heart rates. Small fee.

Pro Metronome—Beat with sound and light. Free.

Isle Royale Wolf-Moose Study. For information on the longest-running study of predator/prey interactions: http://www.isleroyalewolf.org

"2008 Physical Activity Guidelines for Americans" by U.S. Department of Health and Human Services: http://www.health.gov/paguidelines/guidelines/default.aspx

Recommendations for physical activity and diet from the Centers for Disease Control and Prevention: http://www.cdc.gov/physicalactivity

NOTES

LAB TOPIC 2

Microscopes and Cells

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Identify the parts of compound and stereoscopic microscopes and be proficient in their correct use in biological studies.
2. Describe procedures used in preparing materials for electron microscopy and compare these with procedures used in light microscopy.
3. Identify cell structures and organelles from electron micrographs and state the functions of each.
4. Describe features of specific cells and determine characteristics shared by all cells studied.
5. Compare the structure of animal and plant cells as seen in both light and electron microscopy.
6. Distinguish between eukaryotic and prokaryotic cells.
7. Discuss the evolutionary significance of increasing complexity from unicellular to multicellular organization and provide examples from the lab.

Introduction

According to cell theory, the *cell* is the fundamental biological unit, the smallest and simplest biological structure possessing all the characteristics of the living condition. All living organisms are composed of one or more cells, and every activity taking place in a living organism is ultimately related to metabolic activities in cells. Thus, understanding the processes of life necessitates an understanding of the structure and function of the cell.

The earliest known cells found in fossilized sediments 3.5 billion years old (called **prokaryotic** cells) lack nuclei and membrane-bound organelles. Cells with a membrane-bound nucleus and organelles (**eukaryotic** cells) do not appear in the fossil record for another 2 billion years. But the eventual evolution of the eukaryotic cell and its internal compartmentalization led to enormous biological diversity in single cells. The evolution of loose aggregates of cells ultimately to colonies of connected cells provided for specialization, so that groups of cells had specific and different functions. This early division of labor included cells whose primary function was locomotion or reproduction. The evolution of multicellularity appears to have originated more than once in eukaryotes and provided an opportunity for extensive adaptive radiation as organisms specialized and diversified, eventually giving rise to fungi, plants, and animals. This general trend in increasing complexity and specialization seen in the history of life will be illustrated in Lab Topic 2.

Given the fundamental role played by cells in the organization of life, one can readily understand why the study of cells is essential to the study of life. Cells, however, are below the limit of resolution of the human eye. We cannot study them without using a microscope. The microscope has probably contributed more than any other instrument to the development of biology as a science and continues today to be the principal tool used in medical and biological research. There are four types of microscopes commonly used by biologists. You will learn how to use two of these microscopes, the compound microscope and the stereoscopic microscope, in today's laboratory. Both of these microscopes use visible light as the source of illumination and are called light microscopes. Two other microscopes, the scanning electron microscope and the transmission electron microscope, use electrons as the source of illumination. Electron microscopes are able to view objects much smaller than those seen in a light microscope. Although these microscopes are not used in this laboratory, you will be given the opportunity to learn more about them in Exercise 2.4.

Microscopes are used by biologists in numerous subdisciplines: genetics, molecular biology, neurobiology, cell biology, evolution, and ecology. The knowledge and skills you develop today will be used and enhanced throughout this course and throughout your career in biology. It is important, therefore, that you take the time to master these exercises thoroughly.

EXERCISE 2.1
The Compound Light Microscope

Materials

compound microscope

Introduction

The microscope is designed to make objects visible that are too difficult or too small to see with the unaided eye. There are many variations of light microscopes, including phase-contrast, darkfield, polarizing, and UV. These differ primarily in the source and manner in which light is passed through the specimen to be viewed.

The microscopes in biology lab are usually compound binocular or monocular light microscopes, some of which may have phase-contrast attachments. **Compound** means that the scopes have a minimum of two magnifying lenses (the ocular and the objective lenses). **Binocular microscopes** have two eyepieces, **monoculars** have only one eyepiece, and **light** refers to the type of illumination used, that is, visible light from a lamp.

Your success in and enjoyment of a large portion of the laboratory work in introductory biology will depend on how proficient you become in the use of the microscope. When used and maintained correctly, these precision instruments are capable of producing images of the highest quality.

Although there are many variations in the features of microscopes, they are all constructed on a similar plan (Figure 2.1). In this exercise you will be introduced to the common variations found in different models of compound microscopes and asked to identify those features found on your microscope.

FIGURE 2.1a

The compound binocular light microscope. Locate the parts of your microscope described in Exercise 2.1 and label this photograph. Indicate in the margin of your lab manual any features unique to your microscope.

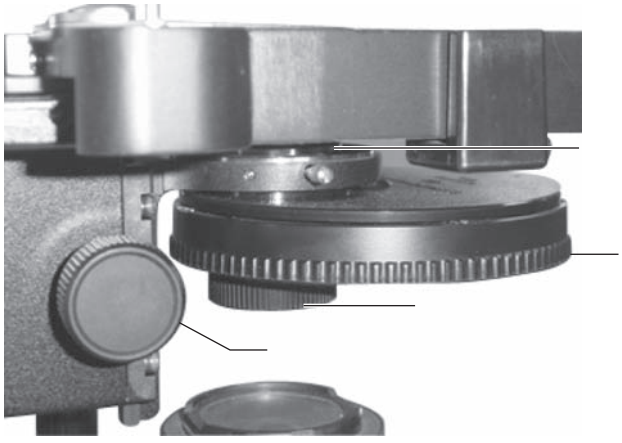


FIGURE 2.1b

Enlarged photo of compound light microscope as viewed from under the stage. This microscope is equipped with phase-contrast optics. Locate the condenser, condenser adjustment knob, phase-contrast revolving turret, and iris diaphragm on your microscope (if present) and label them on the diagram.

Please treat these microscopes with the greatest care!

Procedure

1. Obtain a compound light microscope, following directions from your instructor. To carry the microscope correctly, hold the arm with one hand, and support the base with your other hand. Remove the cover, but do not plug in the microscope.
2. Locate the parts of your microscope, and label Figure 2.1. Refer to the following description of a typical microscope. In the spaces provided, indicate the specific features related to your microscope.
   a. The **head** supports the two sets of magnifying lenses. The **ocular** is the lens in the eyepiece, which typically has a magnification of 10×. If your microscope is binocular, the distance between the eyepieces (**interpupillary distance**) can be adjusted to suit your eyes. Move the eyepieces apart, and look for the scale used to indicate the distance between the eyepieces. Do not adjust the eyepieces at this time. A pointer has been placed in the eyepiece and is used to point to an object in the **field of view,** the circle of light that one sees in the microscope.

      Is your microscope monocular (one eyepiece) or binocular (two eyepieces)?

      What is the magnification of your ocular(s)?

Although the eyepiece may be removable, it should not be removed from the microscope.

   b. **Objectives** are the three lenses on the **revolving nosepiece.** The shortest lens is typically 4× and is called the **scanning lens.** The **intermediate lens** is 10×, and the longest, the **high-power lens,** is 40× (the fourth position on the nosepiece is empty). It is important to clean both the objective and ocular lenses before each use. Dirty lenses will cause a blurring or fogging of the image. Always use lens paper for cleaning! Any other material (including Kimwipes®) may scratch the lenses.

      What is the magnification of each of your objectives? List them in order of increasing magnification.

   c. The **arm** supports the stage and condenser lens. The **condenser lens** is used to focus the light from the **lamp** through the specimen to be viewed. The height of the condenser can be adjusted by an **adjustment knob.** The **iris diaphragm** controls the width of the circle of light and, therefore, the amount of light passing through the specimen.

If your microscope has phase-contrast optics, the condenser may be housed in a **revolving turret.** When the turret is set on 0, the normal optical arrangement is in place. This condition is called **bright-field microscopy.** Another position of the turret sets phase-contrast optics in place. To use phase-contrast, the turret setting must correspond to the magnifying power of the objective being used.

Is your microscope equipped with phase-contrast optics?

The **stage** supports the specimen to be viewed. A mechanical stage can be moved right and left and back and forth by two **stage adjustment knobs.** With a stationary stage, the slide is secured under stage clips and moved slightly by hand while viewing the slide. The distance between the stage and the objective can be adjusted with the **coarse** and **fine focus adjustment knobs**.

Does your microscope have a mechanical or stationary stage?

d. The **base** acts as a stand for the microscope and houses the lamp. In some microscopes, the intensity of the light that passes through the specimen can be adjusted with the **light intensity lever.** Generally, more light is needed when using high magnification than when using low magnification. Describe the light system for your microscope.

EXERCISE 2.2
Basic Microscope Techniques

Materials

clear ruler
coverslips
prepared slides: letter and crossed thread
lens paper
blank slides
Kimwipes®
dropper bottle with distilled water

Introduction

In this exercise, you will learn to use the microscope to examine a recognizable object, a slide of the letter *e*. Recall that microscopes vary, so you may have to omit steps that refer to features not available on your microscope. The following procedure will allow you to practice adjusting your microscope to become proficient in locating a specimen, focusing clearly, and adjusting the light for the best contrast.

Procedure

1. Clean microscope lenses.

Each time you use the microscope, you should begin by cleaning the lenses. Using lens paper moistened with a drop of distilled water, wipe the ocular, objective, and condenser lenses. Wipe them again with a piece of dry lens paper.

Use only lens paper on microscope lenses. Do not use Kimwipes®, tissues, or other papers.

2. Adjust the focus on your microscope.
   a. Plug your microscope into the outlet.
   b. Turn on the light. Adjust the light intensity to mid-range if your microscope has that feature.
   c. Rotate the 4× objective into position using the revolving nosepiece ring, not the objective itself.
   d. Take the letter slide and wipe it with a Kimwipes® tissue. Each time you study a prepared slide, you should first wipe it clean. Place the letter slide on the stage, and center it over the stage opening.

Slides should be placed on and removed from the stage only when the 4x objective is in place. Removing a slide when the higher objectives are in position may scratch the lenses.

   e. Look through the ocular and bring the letter into rough focus by slowly focusing upward using the coarse adjustment.
   f. For binocular microscopes, looking through the oculars, move the oculars until you see only one image of the letter *e*. In this position, the oculars should be aligned with your pupils. In the margin of your lab manual, make a note of the **interpupillary distance** on the scale between the oculars. Each new lab day, before you begin to use the microscope, set this distance.
   g. Raise the condenser to its highest position, and fully close the iris diaphragm.
   h. Looking through the ocular, slowly lower the condenser just until the graininess disappears. Slowly open the iris diaphragm just until the entire field of view is illuminated. This is the correct position for both the condenser and the iris diaphragm.
   i. Rotate the 10× objective into position.
   j. Look through the ocular and slowly focus upward with the coarse adjustment knob until the image is in rough focus. Sharpen the focus using the fine adjustment knob.

Do not turn the fine adjustment knob more than two revolutions in either direction. If the image does not come into focus, return to 10x and refocus using the coarse adjustment.

k. For binocular microscopes, cover your left eye and use the fine adjustment knob to focus the fixed (right) ocular until the letter *e* is in maximum focus. Now cover the right eye and, using the diopter ring on the left ocular, bring the image into focus. The letter *e* should now be in focus for both of your eyes. Each new lab day, as you begin to study your first slide, repeat this procedure.

l. You can increase or decrease the contrast by adjusting the iris diaphragm opening. Note that the maximum amount of light provides little contrast. Adjust the aperture until the image is sharp.

m. Move the slide slowly to the right. In what direction does the image in the ocular move?

n. Is the image in the ocular inverted relative to the specimen on the stage?

o. Center the specimen in the field of view; then rotate the 40× objective into position while watching from the side. *If it appears that the objective will hit the slide, stop and ask for assistance.*

Most of the microscopes have **parfocal** lenses, which means that little refocusing is required when moving from one lens to another. If your scope is *not* parfocal, ask your instructor for assistance.

p. After the 40× objective is in place, focus using the fine adjustment knob.

Never focus with the coarse adjustment knob when you are using the high-power objective.

q. The distance between the specimen and the objective lens is called the **working distance.** Is this distance greater with the 40× or the 10× objective?

3. Compute the total magnification of the specimen being viewed. To do so, multiply the magnification of the ocular lens by that of the objective lens.

a. What is the total magnification of the letter as the microscope is now set?

b. What would be the total magnification if the ocular were 20× and the objective were 100× (oil immersion)? This is the magnification achieved by the best light microscopes.

4. Measure the diameter of the **field of view.** Once you determine the size of the field of view for any combination of ocular and objective lenses, you can determine the size of any structure within that field.

a. Rotate the 4× objective into position and remove the letter slide.

b. Place a clear ruler on the stage, and focus on its edge.

c. The distance between two lines on the ruler is 1 mm. What is the diameter (mm) of the field of view?

d. Convert this measurement to micrometers (μm), a more commonly used unit of measurement in microscopy (1 mm = 1,000 μm).

e. Measure the diameters of the field of view for the 10× and 40× objectives, and enter all three in the spaces below to be used for future reference.

4× = 10× = 40× =

f. What is the relationship between the size of the field of view and magnification?

5. Determine spatial relationships. The **depth of field** is the thickness of the specimen that may be seen in focus at one time. Because the depth of focus is very short in the compound microscope, focus up and down to clearly view all planes of a specimen.

a. Rotate the 4× objective into position and remove the ruler. Take a slide of crossed threads, wipe it with a Kimwipe®, and place the slide on the stage. Center the slide so that the region where the two threads cross is in the center of the stage opening.

b. Focus on the region where the threads cross. Are both threads in focus at the same time?

c. Rotate the 10× objective into position and focus on the cross. Are both threads in focus at the same time?

Does the 4× or the 10× objective have a shorter depth of field?

d. Focus upward (move the stage up) with the coarse adjustment until both threads are just out of focus. Slowly focus down using the fine adjustment. Which thread comes into focus first? Is this thread lying under or over the other thread?

e. Rotate the 40× objective into position and slowly focus up and down, using the fine adjustment only. Does the 10× or the 40× objective have a shorter depth of field?

6. At the end of your microscope session, use the following procedures to store your microscope.

a. Rotate the 4× objective into position.

b. Remove the slide from the stage.

c. Return the phase-contrast condenser to the 0 setting if you have used phase-contrast.

d. Set the light intensity to its lowest setting and turn off the power.

e. Unplug the cord and wrap it around the base of the microscope.

f. Replace the dust cover.

g. Return the microscope to the cabinet using two hands; one hand should hold the arm, and the other should support the base.

These steps should be followed every time you store your microscope.

EXERCISE 2.3

The Stereoscopic Microscope

Materials

stereoscopic microscope
dissecting needles
living *Elodea*
microscope slides
droppers of water
coverslips

Introduction

The stereoscopic (dissecting) microscope has relatively low magnification, 7× to 30×, and is used for viewing and manipulating relatively large objects. The binocular feature creates the stereoscopic effect. The stereoscopic microscope is similar to the compound microscope except in the following ways: (1) The depth of field is much greater than with the compound microscope, so objects are seen in three dimensions, and (2) the light source can be directed down onto as well as up through an object, which permits the viewing of objects too thick to transmit light. Light directed down on

the object is called **reflected** or **incident light.** Light passing through the object is called **transmitted light**.

Procedure

1. Remove your stereoscopic microscope from the cabinet and locate the parts labeled in Figure 2.2. Locate the switches for both incident and transmitted light. In the margin of your lab manual, note any features of your microscope that are not shown in the figure. What is the range of magnification for your microscope?
2. Observe an object of your choice at increasing magnification. Select an object that fits easily on the stage (e.g., ring, coin, fingertip, pen, ruler).
   a. Place the object on the stage and adjust the interpupillary distance (distance between the oculars) by gently pushing or pulling the oculars until you can see the object as a single image.
   b. Change the magnification and note the three-dimensional characteristics of your object.
   c. Adjust the lights, both reflected and transmitted. Which light gives you the best view of your object?

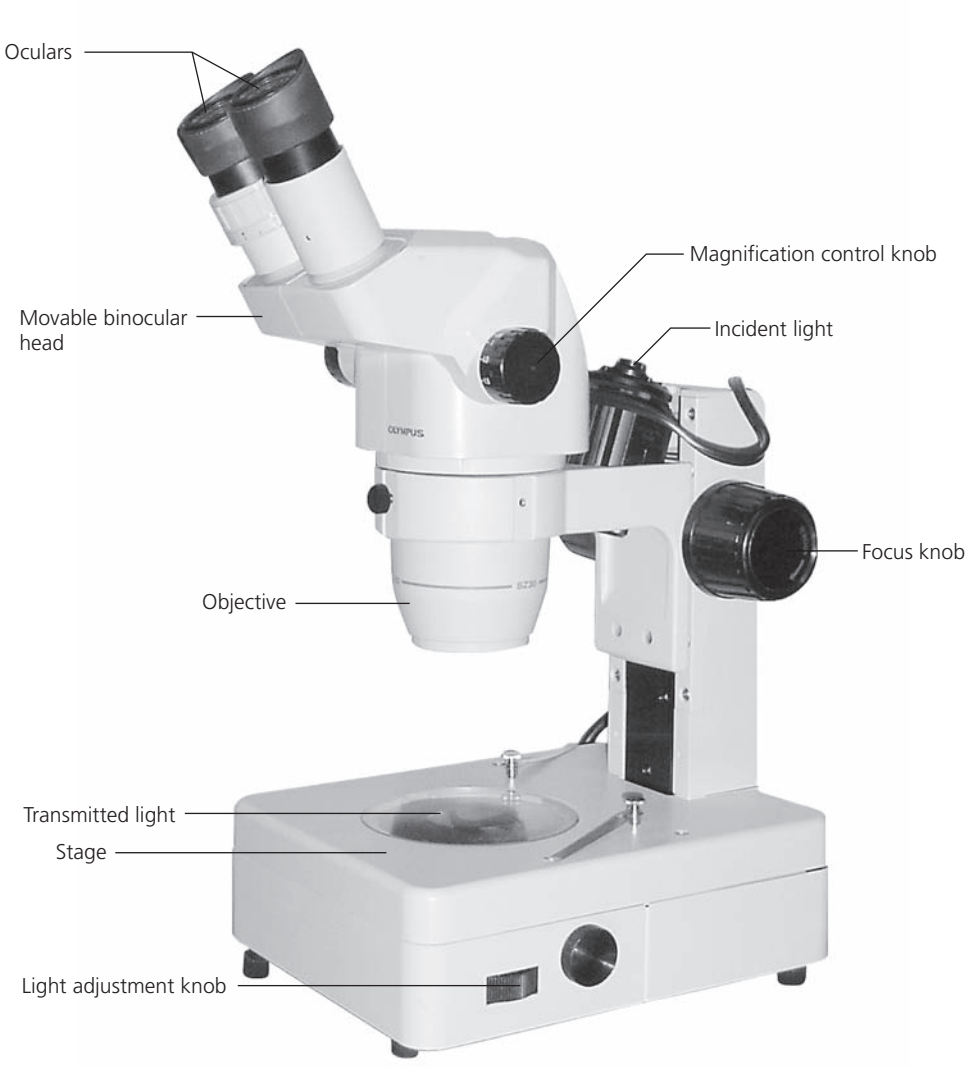


FIGURE 2.2
The stereoscopic (dissecting) microscope. Locate the parts of your microscope by referring to this photograph. Note in the margin any features of your microscope that are not shown in the photograph.

3. Prepare a **wet mount** of *Elodea.* Living material is often prepared for observation using a wet mount. (The material is either in water or covered with water prior to adding a coverslip.) You will use this technique to view living material under the dissecting and compound microscopes (Figure 2.3).
   a. Place a drop of water in the center of a clean microscope slide.
   b. Remove a single leaf of *Elodea,* and place it in the drop of water.
   c. Using a dissecting needle, place a coverslip at a 45° angle above the slide with one edge of the coverslip in contact with the edge of the water droplet, as shown.
   d. Lower the coverslip slowly onto the slide, being careful not to trap air bubbles in the droplet. The function of the coverslip is threefold: (1) to flatten the preparation, (2) to keep the preparation from drying out, and (3) to protect the objective lenses. Over long periods of time, the preparation may dry out, at which point water can be added to one edge of the coverslip.

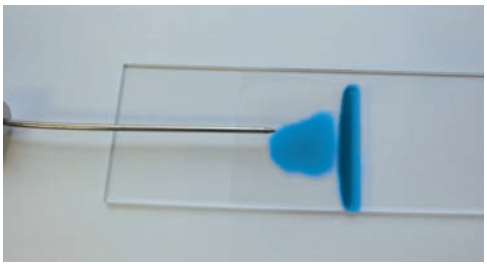


FIGURE 2.3
Preparation of a wet mount.
Place a drop of water and your specimen on the slide. Using a dissecting needle, slowly lower a coverslip onto the slide, being careful not to trap air bubbles in the droplet.

Specimens can be viewed without a coverslip using the stereoscopic microscope, but a coverslip must always be used with the compound microscope.

4. Observe the structure of the *Elodea* leaf at increasing magnification.
   a. Place the leaf slide on the stage and adjust the focus. Change the magnification and note the characteristics of the leaf at increased magnification.
   b. Sketch the leaf in the margin of your lab manual and list, in the space below, the structures that are visible at low and high magnification.

      Low:

      High:

      Is it possible to see cells in the leaf using the stereoscopic microscope?

      Organelles?

   c. Save your slide for later study. In Exercise 2.5, Lab Study C, you will be asked to compare these observations of *Elodea* with those made while using the compound microscope.

EXERCISE 2.4
The Transmission Electron Microscope

Materials

demonstration resources for the electron microscope
electron micrographs

Introduction

The transmission electron microscope (TEM) magnifies objects approximately 1,000× larger than a light microscope can (up to 1,000,000×). This difference depends on the **resolving power** of the electron microscope, which allows the viewer to see two objects of comparable size that are close together and still be able to recognize that they are two objects rather than one. Resolving power, in turn, depends on the wavelength of light passed through the specimen: the shorter the wavelength, the greater the resolution. Because electron microscopes use electrons as a source of illumination and electrons have a much shorter wavelength than does visible light, the resolving power of electron microscopes is much greater than that of light microscopes. Both the electron and light microscopes can be equipped with lenses that allow for tremendous magnification, but only the electron microscope has sufficient resolving power to make these lenses useful.

Procedure

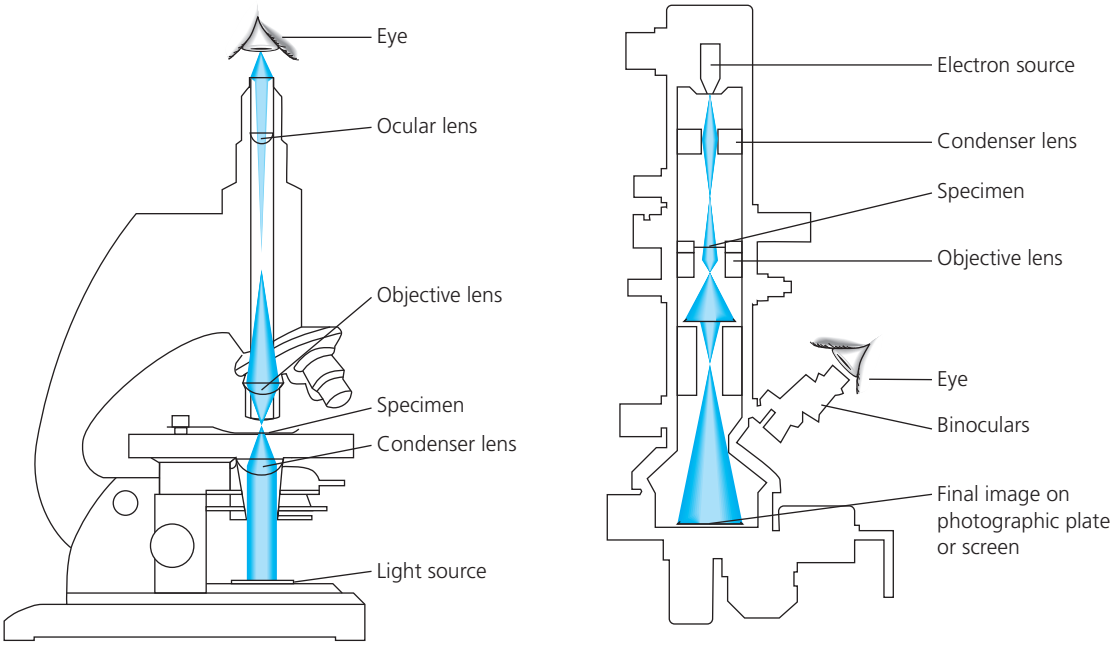
1. Compare the features of the light and electron microscopes (Figure 2.4).
   a. Name three structures found in both microscopes.

   b. What is the energy source for the electron microscope?

      For the compound microscope?

   c. Describe how the lenses differ for the two microscopes.

2. Using the resources provided by your instructor, review the procedures and materials used to prepare specimen for electron microscopy. Websites that describe electron microscopy are listed at the end of this lab topic.
3. Although the magnifying power of an electron microscope is much greater than that of a light microscope, one important disadvantage of studying cells with the transmission electron microscope is that the process for preparing cells and tissues kills the cells. Define the following terms associated with electron microscopy. As you define these terms, you will understand why living cells cannot be studied with a transmission electron microscope.



a. Light microscope

b. Electron microscope

FIGURE 2.4

Comparison of light microscope and electron microscope. The source of illumination is light for the light microscope and electrons for the electron microscope. The image is magnified by glass objectives in light microscopy and by electromagnets in electron microscopy.

fixation:

embedding in plastic:

staining with heavy metals:

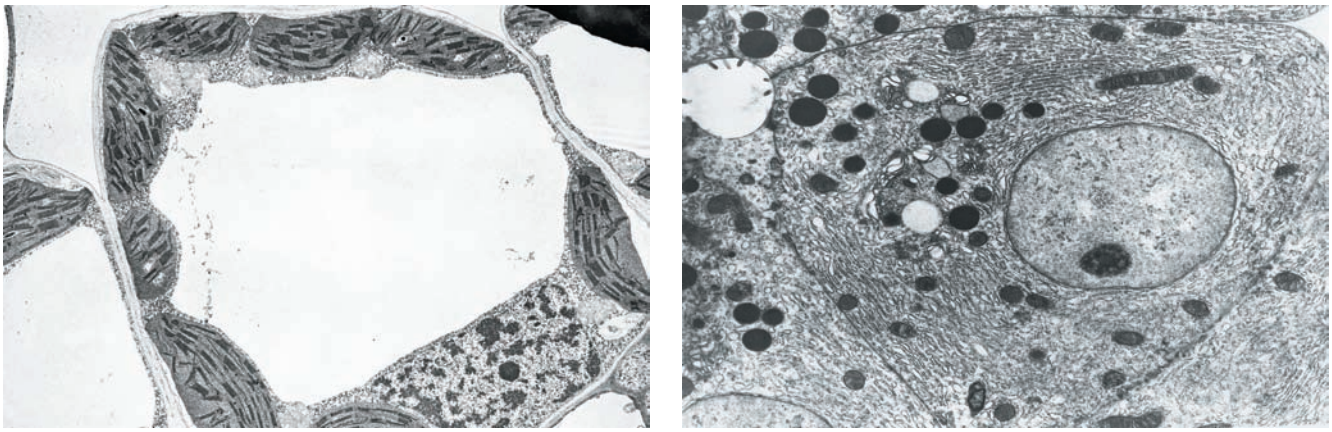
glass or diamond knife:

ultramicrotome:

fluorescent screen:

4. In addition to the *transmission electron microscope* (TEM), there is an additional type of electron microscope that is frequently used for specific purposes when studying cells and tissues, and even whole organisms. This microscope is called a *scanning electron microscope* (SEM). Using your text or the Web, investigate the use of scanning electron microscopy for biological applications.

   When would it be appropriate to use the SEM rather than the TEM?



a. b.

FIGURE 2.5

Cells as seen in a transmission electron microscope. (a) Electron micrograph of a plant cell. (b) Electron micrograph of a rat pancreas cell. The large dark bodies in the cytoplasm are secretory globules.

5. When a transmission electron microscope is used, cells are usually studied using electron micrographs, photographs taken of the image seen on the fluorescent screen. Observe the electron micrographs in Figure 2.5a and b, respectively, a plant cell and an animal cell. Other micrographs may be on demonstration in the laboratory, and also check your lecture text for examples. Working with your lab partner, see if you can identify and label the following organelles and structures in Figure 2.5 and in other micrographs on demonstration in the laboratory.

   plasma membrane, cell wall, nucleus, chloroplast, mitochondria, large central vacuole, Golgi apparatus, lysosome, endoplasmic reticulum, ribosome

   Predict which of these organelles will *not* be visible when studying plant and animal cells using the *light* microscope. *Underline* those structures in the above list. Return to this activity after you have completed Exercise 2.5, Lab Study C, to confirm your predictions.

EXERCISE 2.5
The Organization of Cells

In this exercise, you will examine the features common to all eukaryotic cells that are indicative of their common ancestry. However, you will observe that all cells are not the same. Some organisms are **unicellular** (single-celled), with all living functions (respiration, digestion, reproduction, and excretion) handled by that one cell. Others form random, temporary **aggregates,** or clusters, of cells. Clusters composed of a consistent and predictable number of cells are called **colonies.** Simple colonies are clusters of cells of similar types with a predictable structure, but the cells

have no physiological connections. More complex colonies have cells of different types. In some colonial algae the cells are called *somatic cells* (cells that are not reproductive) and *reproductive cells* (cells that specialize in reproduction). In these colonies, if *either* type of cell is isolated from the colony, it may be reproductive, dividing and producing new colonies.

Other algae may contain both cell types, somatic and reproductive, but their somatic cells *never* become reproductive, even when isolated. Furthermore, their reproductive cells cannot persist independently, but must be associated with somatic cells to live. These algae are described as **multicellular.** They demonstrate the following two defining features:

- Multicellular organisms consist of two or more types of cells with specialized structure and function.
- If any one of the cell types of the organism is isolated, it is not capable of perpetuating the species in nature.

In more complex algae, fungi, plants, and animals, specialized cell types may be organized into *tissues* that perform particular functions for the organism. Tissues, in turn, may combine to form *organs*, and tissues and organs combine to form a coordinated single *organism*.

In this exercise, you will examine selected unicellular, aggregate, colonial, and multicellular organisms.

Lab Study A. Unicellular Organisms

Materials

microscope slides
culture of *Amoeba*
living termites
forceps
coverslips
dissecting needles
insect Ringers

Introduction

Unicellular eukaryotic organisms may be **autotrophic** (photosynthetic) or **heterotrophic** (deriving food from other organisms or their by-products). These diverse organisms, called protists, will be studied in detail in Lab Topic 14.

Procedure

1. Examine a living *Amoeba* (Figure 2.6) under the compound microscope. Amoebas are aquatic organisms commonly found in ponds. To transfer a specimen to your slide, follow these procedures:
   a. Place the culture dish containing the amoeba under the dissecting microscope, and focus on the bottom of the dish. The amoeba will appear as a whitish, irregularly shaped organism attached to the bottom.

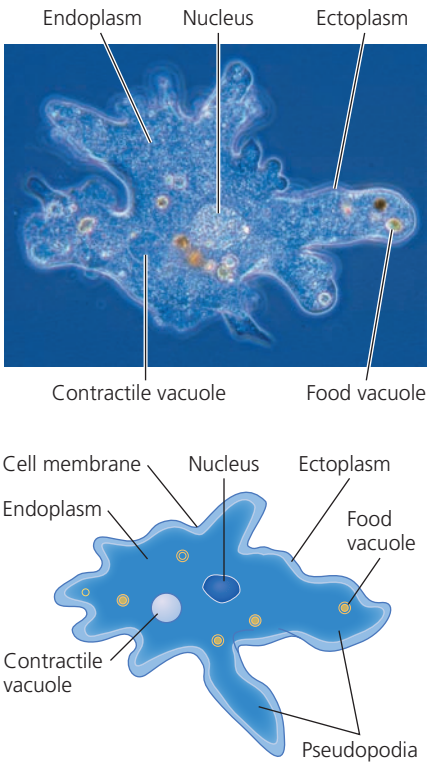
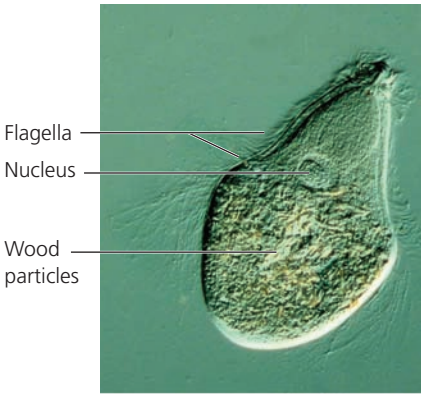


FIGURE 2.6
Amoeba. An amoeba moves using pseudopodia. Observe the living organisms using the compound microscope.



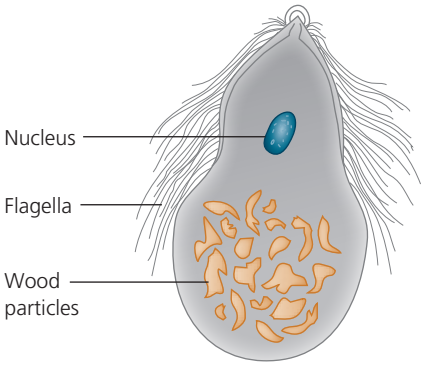


FIGURE 2.7
Trichonympha. A community of microorganisms, including *Trichonympha*, inhabits the intestine of the termite. Following the procedure in Exercise 2.5, Lab Study A, disperse the microorganisms and locate the cellular structures in *Trichonympha*.

b. Using a clean pipette (it is important not to interchange pipettes between culture dishes), transfer a drop with several amoebas to your microscope slide. To do this, squeeze the pipette bulb *before* you place the tip under the surface of the water. Disturbing the culture as little as possible, pipette a drop of water with debris from the *bottom* of the culture dish. You may use your stereoscopic microscope to scan the slide to locate amoebas before continuing.

c. Cover your preparation with a clean coverslip.

d. Under low power on the compound scope, scan the slide to locate an amoeba. Center the specimen in your field of view; then switch to higher powers.

e. Identify the following structures in the amoeba:

The **cell membrane** is the boundary that separates the organism from its surroundings.

Ectoplasm is the thin, transparent layer of cytoplasm directly beneath the cell membrane.

Endoplasm is the granular cytoplasm containing the cell organelles.

The nucleus is the grayish, football-shaped body that is somewhat granular in appearance. This organelle, which directs the cellular activities, will often be seen moving within the endoplasm.

Contractile vacuoles are clear, spherical vesicles of varying sizes that gradually enlarge as they fill with excess water. Once you've located a vacuole, watch it fill and then empty its contents into the surrounding environment. These vacuoles serve an excretory function for the amoeba.

Food vacuoles are small, dark, irregularly shaped vesicles within the endoplasm. They contain undigested food particles.

Pseudopodia ("false feet") are fingerlike projections of the cytoplasm. They are used for locomotion as well as for trapping and engulfing food in a process called **phagocytosis**.

MB Student Media Videos—Ch. 28: Amoeba; Amoeba Pseudopodia

2. Examine *Trichonympha* under a compound microscope. You will first have to separate the *Trichonympha* (Figure 2.7) from the termite with which it lives in a symbiotic relationship. *Trichonympha* and other organisms occupy the gut of the termites, where they digest wood particles eaten by the insect. Termites lack the enzymes necessary to digest wood and are dependent on *Trichonympha* to make the nutrients in the wood available to them. *Trichonympha* has become so well adapted to the environment of the termite's gut that it cannot survive outside of it.

To obtain a specimen:

a. Place a couple of drops of **insect Ringers** (a saline solution that is isotonic to the internal environment of insects) on a clean microscope slide.

b. Using forceps or your fingers, transfer a termite into the drop of Ringers.

c. Place the slide under the dissecting microscope.
d. Place the tips of dissecting needles at either end of the termite and pull in opposite directions.
e. Locate the long tube that is the termite's intestine. Remove all the larger parts of the insect from the slide.
f. Using a dissecting needle, mash the intestine to release the *Trichonympha* and other protozoa and bacteria.
g. Cover your preparation with a clean coverslip.
h. Transfer your slide to the compound microscope and scan the slide under low power. Center several *Trichonympha* in the field of view and switch to higher powers.

Several types of protozoans and bacteria will be present in the termite gut.

i. Locate the following structures under highest power.

Flagella are the long, hairlike structures on the outside of the organism. The function of the flagella is not fully understood. Within the gut of the termite, the organisms live in such high density that movement by flagellar action seems unlikely and perhaps impossible.

The **nucleus** is a somewhat spherical organelle near the middle of the organism.

Wood particles may be located in the posterior region of the organism.

Lab Study B. Aggregate and Colonial Organisms

Materials

microscope slides
dissecting needles
forceps
coverslips
cultures of *Protococcus* and *Scenedesmus*

Introduction

Unlike unicellular organisms, which live independently of each other, colonial organisms are cells that live in groups and are to some degree dependent on one another. The organisms studied in this exercise show an increasing degree of interaction among cells.

Procedure

1. Examine *Protococcus* under the compound microscope. *Protococcus* (Figure 2.8) is a terrestrial green alga that grows on the north sides of trees and is often referred to as "moss."
   a. To obtain a specimen, use a dissecting needle to brush off a small amount of the green growth on the piece of tree bark provided into a drop of water on a clean microscope slide. Avoid scraping bark onto the slide. Cover the preparation with a clean coverslip.

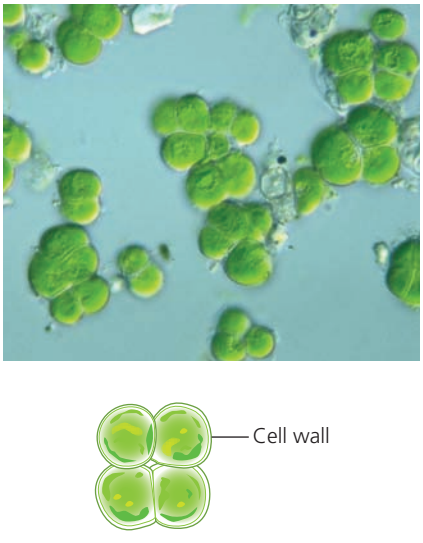


FIGURE 2.8
Protococcus. *Protococcus* is a terrestrial green alga that forms loose aggregates on the bark of trees.

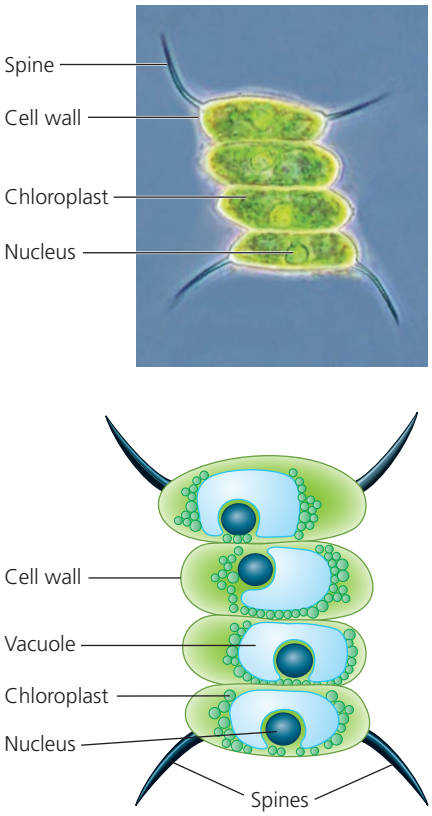


FIGURE 2.9
Scenedesmus. *Scenedesmus* is an aquatic alga that usually occurs in simple colonies of four cells connected by the cell wall.

b. Observe at highest power that these cells are **aggregates:** The size of the cell groupings is random, and there are no permanent connections between cells. Each cell is surrounded by a cell membrane and an outer **cell wall**.

c. Observe several small cell groupings and avoid large clumps of cells. Cellular detail may be obscure.

2. Examine living *Scenedesmus* under the compound microscope. *Scenedesmus* (Figure 2.9) is an aquatic green alga that is common in aquaria and polluted water.

a. To obtain a specimen, place a drop from the culture dish (using a clean pipette) onto a clean microscope slide, and cover it with a clean coverslip.

b. Observe that the cells of this organism form a **simple colony:** The cells always occur in groups of from four to eight cells, and they are permanently united.

c. Identify the following structures.

The **nucleus** is the spherical organelle in the approximate middle of each cell.

Vacuoles are the transparent spheres that tend to occur at either end of the cells.

Spines are the transparent projections that occur on the two end cells.

Cell walls surround each cell.

Lab Study C. Multicellular Organisms

Materials

microscope slides
dropper bottles of water
toothpicks
coverslips
Elodea
methylene blue
finger bowl with disinfectant
broken glass chips
Volvox cultures

Introduction

Review the criteria for characterizing an organism as multicellular in the introduction of Exercise 2.5 on pp. 74–75. Recall that multicellular organisms have two or more cell types with specialized structure and function that cannot persist when isolated from other cells in the organism. If these cells are isolated, they are not capable of perpetuating the species. In this lab study, you will examine an example of a green alga, a plant, and an animal to investigate the criteria for multicellularity and observe cells that compose basic tissue types.

Procedure

Volvox

Volvox (Figure 2.10) is an aquatic green alga that is common in aquaria, ponds, and lakes. In older literature this organism was described as colonial and was not considered to be multicellular. Today, however, scientists have concluded that it is more accurate to call *Volvox* multicellular. In this activity you will look for evidence that supports this conclusion.

1. Examine living *Volvox* under the compound microscope. To obtain a specimen, prepare a wet mount as you did for *Scenedesmus* with the following addition: Before placing a drop of the culture on your slide, place several glass chips on the slide. This will keep the coverslip from crushing these spherical organisms.
2. Observe that the cells of this organism lie in a transparent matrix forming a large hollow sphere. The approximately 500 to 50,000 (depending on the species) nonreproductive somatic cells are permanently united by cytoplasmic connections. These cells have chloroplasts for photosynthesis and flagella that beat in a coordinated motion to move the colony like a ball. During asexual reproduction, certain cells in the sphere (reproductive cells) enlarge and migrate inward to become daughter colonies.
3. Identify the following structures: **somatic cells** with **cytoplasmic connections** and **flagella.** Depending on the magnification of your microscope, you may be able to distinguish **cell walls** and **nuclei** in the cells. **Daughter colonies** are smaller spheres within the larger colony. These are released when the parent colony disintegrates.

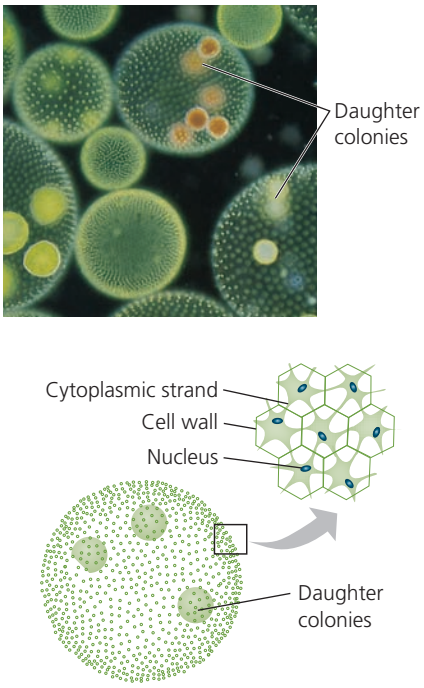


FIGURE 2.10
Volvox. In this organism, the individual cells are interconnected by cytoplasmic strands to form a sphere. Small clusters of cells, called daughter colonies, are specialized for reproduction.

MB

Student Media Video—Ch. 28: *Volvox* Colony

Plant Cells

1. The major characteristics of a typical plant cell are readily seen in the leaf cells of *Elodea,* a common aquatic plant (Figure 2.11). Prepare a wet mount and examine one of the youngest (smallest) leaves from a sprig of *Elodea* under the compound microscope.
2. Identify the following structures.

   The **cell wall** is the rigid outer framework surrounding the cell. This structure gives the cell a definite shape and support. It is not found in animal cells.

   Protoplasm is the organized contents of the cell, exclusive of the cell wall.

   Cytoplasm is the protoplasm of the cell, exclusive of the nucleus.

   The **central vacuole** is a membrane-bound sac within the cytoplasm that is filled with water and dissolved substances. This structure serves to store metabolic wastes and gives the cell support by means of turgor pressure. Animal cells also have vacuoles, but they are not as large and conspicuous as those found in plants.

   Chloroplasts are the green, spherical organelles often seen moving within the cytoplasm. These organelles carry the pigment chlorophyll that is involved in photosynthesis. As the microscope light heats up the cells, cytoplasm and chloroplasts may begin to move around the central vacuole in a process called *cytoplasmic streaming,* or *cyclosis.*

   The **nucleus** is the usually spherical, transparent organelle within the cytoplasm. This structure controls cell metabolism and division.
3. What three structures observed in *Elodea* are unique to plants?

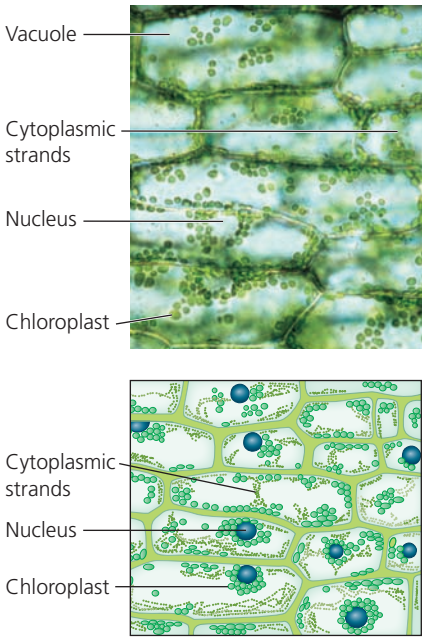


FIGURE 2.11

Elodea. *Elodea* is an aquatic plant commonly grown in freshwater aquaria. The cell structures may be difficult to see because of the three-dimensional cell shape and the presence of a large central vacuole.

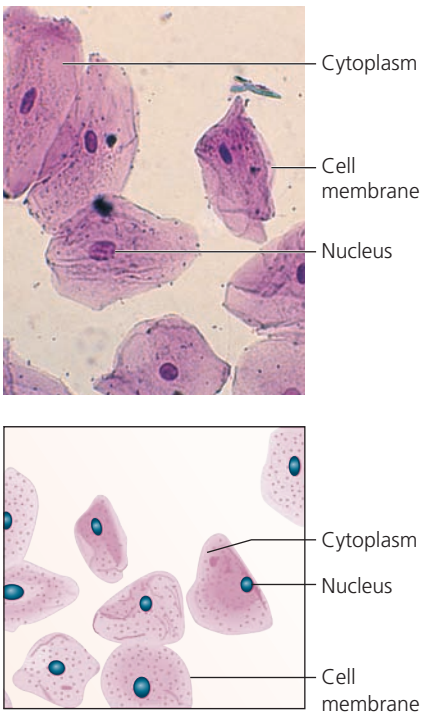


FIGURE 2.12

Human epithelial cells. The epithelial cells that line your cheek are thin, flat cells that you can remove easily from your cheek by scraping it with a toothpick.

4. Compare your observations of *Elodea* using the compound scope with those made in Exercise 2.3 using the stereoscopic scope. List the structures seen with each:

   Stereoscopic:

   Compound:

MB Student Media Video—Ch. 7: Cytoplasmic Streaming

Animal Cells

1. Animals are multicellular heterotrophic organisms that ingest organic matter. They are composed of cells that can be categorized into four major tissue groups: epithelial, connective, muscle, and nervous tissue. In this lab study, you will examine epithelial cells. Similar to the epidermal cells of plants, **epithelial cells** occur on the outside of animals and serve to protect the animals from water loss, mechanical injury, and foreign invaders. In addition, epithelial cells line interior cavities and ducts in animals. Examine the epithelial cells (Figure 2.12) that form the lining of your inner cheek. To obtain a specimen, follow this procedure:
   a. With a clean toothpick, gently scrape the inside of your cheek several times.
   b. Roll the scraping into a drop of water on a clean microscope slide, add a small drop of methylene blue, and cover with a coverslip. Discard the used toothpick in disinfectant.
   c. Using the compound microscope, view the cells under higher powers.
2. Observe that these cells are extremely flat and so may be folded over on themselves. Attempt to locate several cells that are not badly folded, and study their detail.
3. Identify the following structures.

   The **cell membrane** is the boundary that separates the cell from its surroundings.

   The **nucleus** is the large, circular organelle near the middle of the cell.

   Cytoplasm is the granular contents of the cell, exclusive of the nucleus.

Lab Study D. Unknowns

Materials

microscope slides
coverslips
pond water or culture of unknowns

Introduction

Use this lab study to see if you have met the objectives of this lab topic. As you carry out this lab study, (1) think carefully about using correct

microscopic techniques; (2) distinguish organisms with different cellular organization or configuration (unicellular, colonial, etc.); (3) note how the different organisms are similar yet different; and (4) note cell differences.

All of the cells studied to this point in this lab topic have been examples of **eukaryotic** cells. As you examine drops of pond water as described in the following procedure, you may observe examples of prokaryotic cells in colonies or filaments. Eukaryotic cells have a true nucleus containing chromosomes with genetic material separated from the remainder of the cell by a nuclear envelope. All cellular organelles are also bound by membranes. In prokaryotic cells genetic material is not bound by a nuclear envelope, and no membrane-bound organelles are present. Prokaryotic cells will be studied in more detail in Lab Topic 13 Bacteriology.

Procedure

1. Examine several drops of the culture of pond water that you collected, or examine the unknown culture provided by the instructor.
2. Record in Table 2.1 the characteristics of at least four different organisms.
3. Determine if a well-defined nucleus and organelles are present (eukaryote).

TABLE 2.1 Characteristics of Organisms Found in Pond Water

Unknown	Means of Locomotion	Cell Wall (+/–)	Chloroplasts (+/–)	Cellular Organization	Eukaryote (yes or no)
1					
2					
3					
4					
5					

Reviewing Your Knowledge

1. Describe at least two types of materials or observations that would necessitate the use of the stereoscopic microscope.

2. What characteristics do all prokaryotic cells have in common?

3. a. Do animal cells have vacuoles? If yes, what is their function?

   b. How are the structures that are unique to plants important to their success?

4. Return to Step 5, p. 74. Based on your observations in today's laboratory, *circle* those organelles that are visible in the light microscope. Compare your observations with your initial predictions.
5. Review the criteria used to distinguish between colonial and multicellular organisms. Why is *Volvox* now considered multicellular?

Applying Your Knowledge

1. Where do you think unicellular, multicellular, and colonial organisms fit in the order of evolution? Suggest examples of each type from this lab to illustrate your answer.

2. What are the main advantages of being a multicellular organism rather than a unicellular organism? Can you think of any disadvantages?

3. Following is a list of tissues that have specialized functions and demonstrate corresponding specialization of subcellular structure. Match the tissue with the letter of the cell structures and organelles listed to the right that would be abundant in these cells. Use your text for a description of any organelles or structures that are unfamiliar to you.

Tissues

- Cardiac muscle cells in the heart
- Salivary glands of a snake that produces venom
- Cells lining the oviducts of the female reproductive system
- White blood cells that engulf and destroy invading bacteria
- Leaf cells of an oak tree

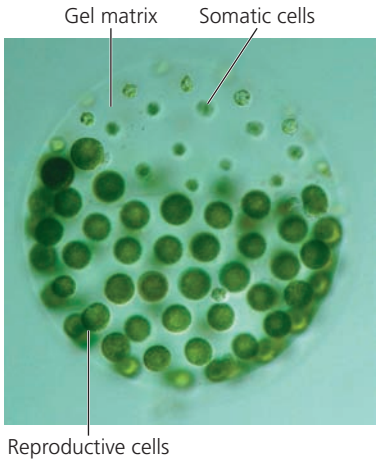
Cell Structures and Organelles

a. plasma membrane
b. mitochondria
c. Golgi apparatus
d. chloroplast
e. endoplasmic reticulum
f. cilia and flagella
g. vacuole
h. ribosome
i. lysosome

4. One organism found in a termite's gut is *Mixotricha paradoxa.* This strange creature looks like a single-celled swimming ciliate under low magnification. However, the electron microscope reveals that it contains spherical bacteria rather than mitochondria and has on its surface, rather than cilia, hundreds of thousands of spirilla and bacilla bacteria. You are the scientist who first observed this organism. How would you describe this organism—single-celled? aggregate? colony? multicellular? Review definitions of these terms on pp. 74–75. Can the structure of this organism give you any insight into the evolution of eukaryotic cells? (*Hint:* See the discussion of the endosymbiosis hypothesis in your text.)

5. *Pleodorina* is an aquatic green alga that is common in ponds, lakes, and roadside ditches. This organism is made up of 32 to 128 cells that are embedded in a gel-like matrix. In mature colonies two types of cells can be distinguished, small somatic cells and larger reproductive cells that divide to form new colonies. Somatic cells carry on photosynthesis, but may become reproductive if isolated from the colony.

   Review the criteria used to determine multicellularity, and decide if *Pleodorina* should be classified as multicellular or colonial.



Investigative Extensions

1. Survey bodies of water surrounding your campus and assess the environmental conditions. Obtain samples of organisms in ponds, lakes, or rivers from several sites with different environmental conditions. Are some sites more polluted than others? Are there temperature differences? If a fountain is available on campus, compare this site with water in a pond that has no fountain (less water movement). You may find a site near the power-generating facility of your campus.

   Measure and note as many independent variables as possible—temperature of the water, relative levels of pollution, surroundings (near an open space, between buildings, etc.).

   List types of organisms observed. Describe the characteristics of organisms. Note differences in density and diversity of organisms in samples taken from different sources.

2. Investigate additional organelles in plants. Plants have specific cellular structures related to their lives as plants. For example, plants use chlorophyll pigments bound in the membranes of **chloroplasts** to absorb wavelengths of light that fuel photosynthesis. The starch synthesized in photosynthesis is later stored in special organelles called **leucoplasts** or **amyloplasts**. Plants are colorful (think of the many functions of color in plants). Some of these colorful pigments are water soluble and stored in vacuoles, but others, including some of the brightly colored carotenoids (reds, yellows, oranges) are membrane bound in organelles called **chromoplasts.** Chloroplasts, chromoplasts, and leucoplasts are three kinds of **plastids,** all of which are similar in structure and typically found in plant cells.