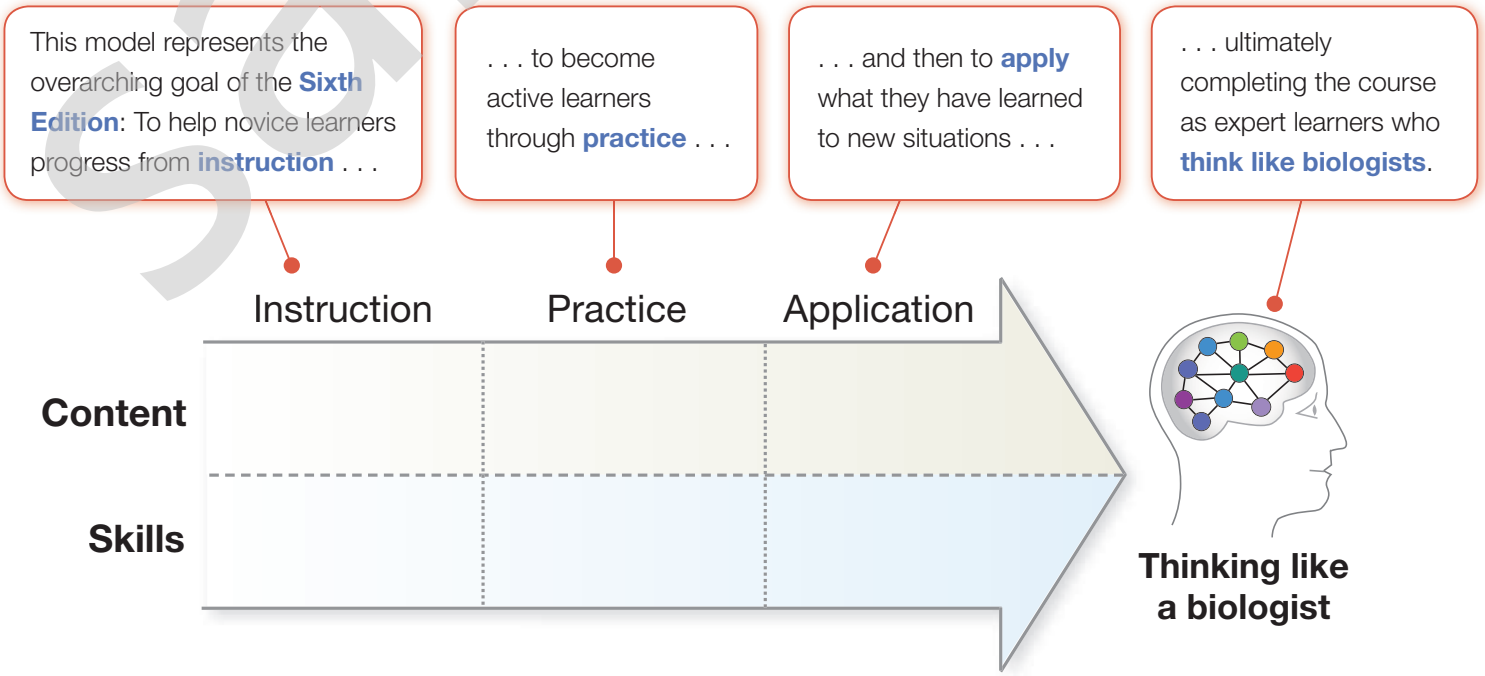
FREEMAN
QUILLIN
ALLISON
BLACK
PODGORSKI
TAYLOR

BIOLOGICAL SCIENCE

SIXTH
EDITION

A Student-Centered Approach to the Study of Biology

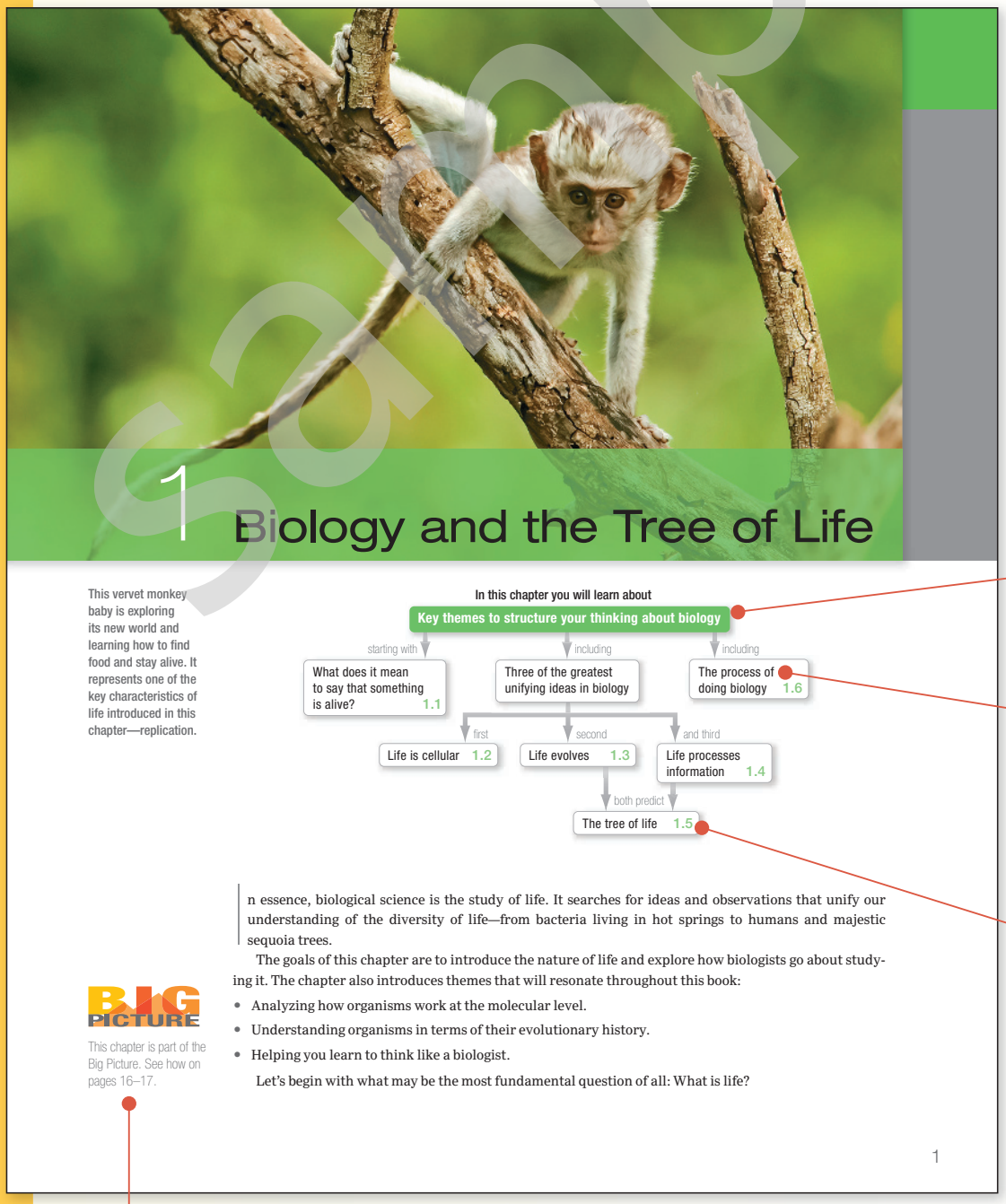
Since its trailblazing First Edition, ***Biological Science*** has delivered numerous biology teaching innovations that emphasize higher-order thinking skills and conceptual understanding rather than an encyclopedic grasp of what is known about biology. With each edition, this approach has grown and improved to better help students make the shift from being novice learners to expert learners. Central to this shift is a student-centered approach that provides deep support for the learning of core content and the development of key skills that help students learn and practice biology.



On the pages that follow, we will show how the text and MasteringBiology resources work together to achieve this goal.

Develop a Conceptual Understanding of Biology

Unique Chapter-opening Roadmaps set the table for learning by visually grouping and organizing information to help students anticipate key ideas as well as recognize meaningful relationships and connections that are explored in the chapter that follows.



1 Biology and the Tree of Life

This vervet monkey baby is exploring its new world and learning how to find food and stay alive. It represents one of the key characteristics of life introduced in this chapter—replication.

In essence, biological science is the study of life. It searches for ideas and observations that unify our understanding of the diversity of life—from bacteria living in hot springs to humans and majestic sequoia trees.

The goals of this chapter are to introduce the nature of life and explore how biologists go about studying it. The chapter also introduces themes that will resonate throughout this book:

- Analyzing how organisms work at the molecular level.
- Understanding organisms in terms of their evolutionary history.
- Helping you learn to think like a biologist.

Let's begin with what may be the most fundamental question of all: What is life?

BIG PICTURE

This chapter is part of the Big Picture. See how on pages 16–17.

1

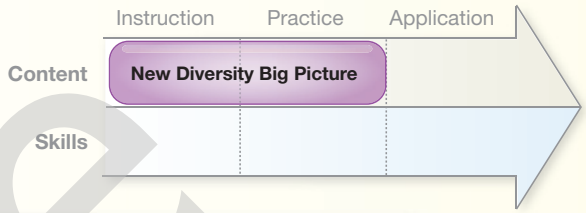
Each **Roadmap** begins with a statement of why the chapter topic is important.

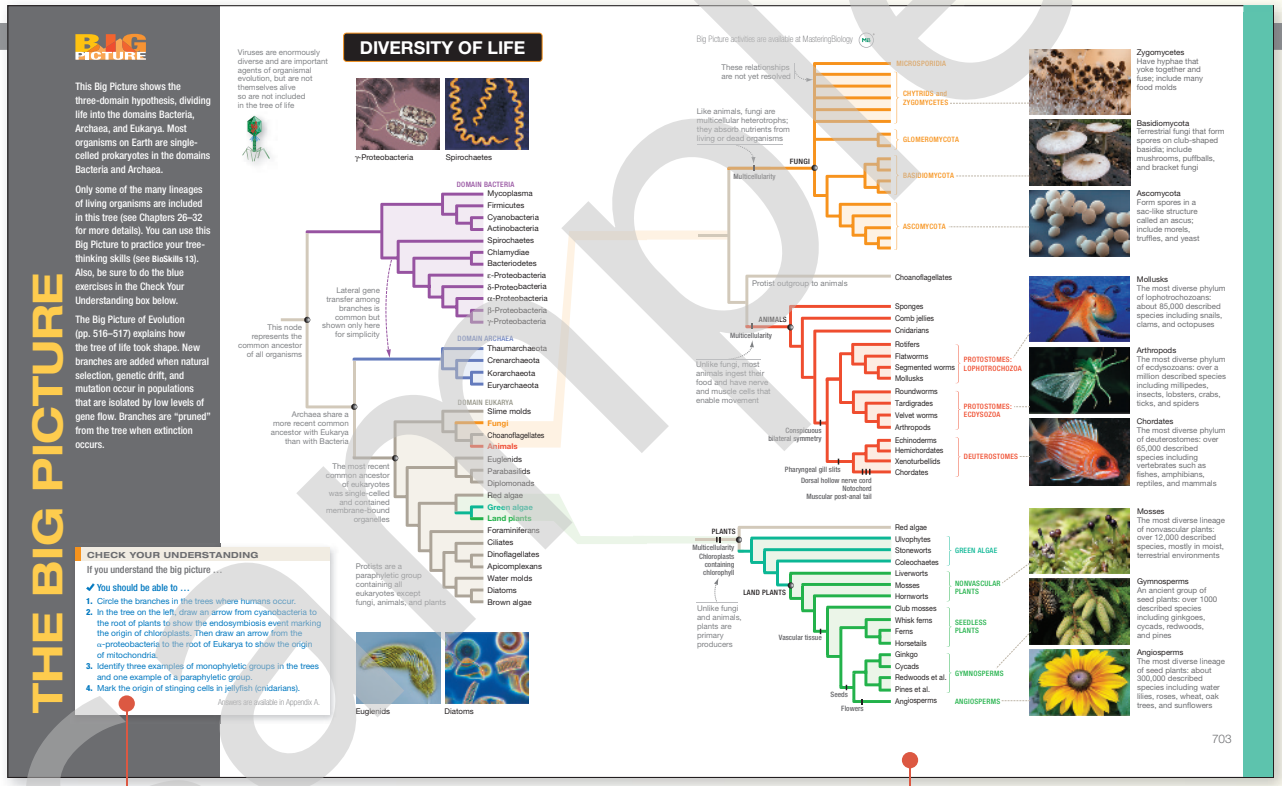
Key topics from each chapter are previewed, and related ideas are connected through **linking words**.

Chapter section numbers help students find key ideas easily in the chapter.

Big Picture Concept Maps are referenced on the opening page of related chapters, pointing students to summary pages that help them synthesize challenging topics.

Big Picture Concept Maps integrate visuals and words to help students synthesize information about challenging topics in biology that span multiple chapters and units.





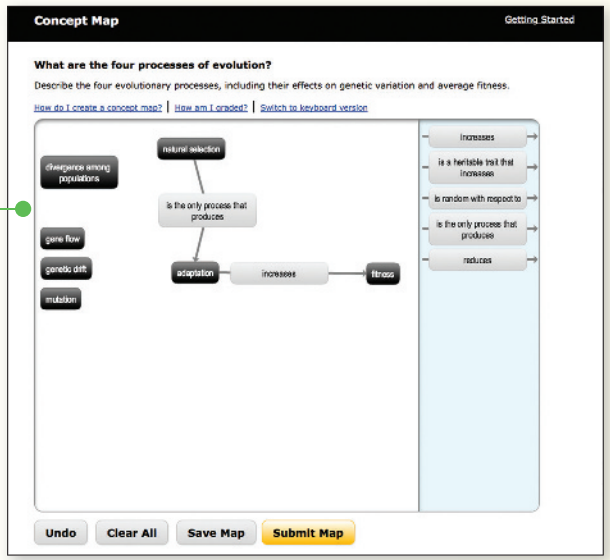
"You should be able to..." activities encourage students to analyze important patterns within each Big Picture concept map.

Big Picture topics include:

- Doing Biology, pp. 16–17
- The Chemistry of Life, pp. 140–141
- Energy for Life, pp. 232–233
- Genetic Information, pp. 396–397
- Evolution, pp. 516–517
- **NEW!** Diversity of Life, pp. 702–703
- Plant and Animal Form and Function, pp. 816–817
- Ecology, pp. 1162–1163

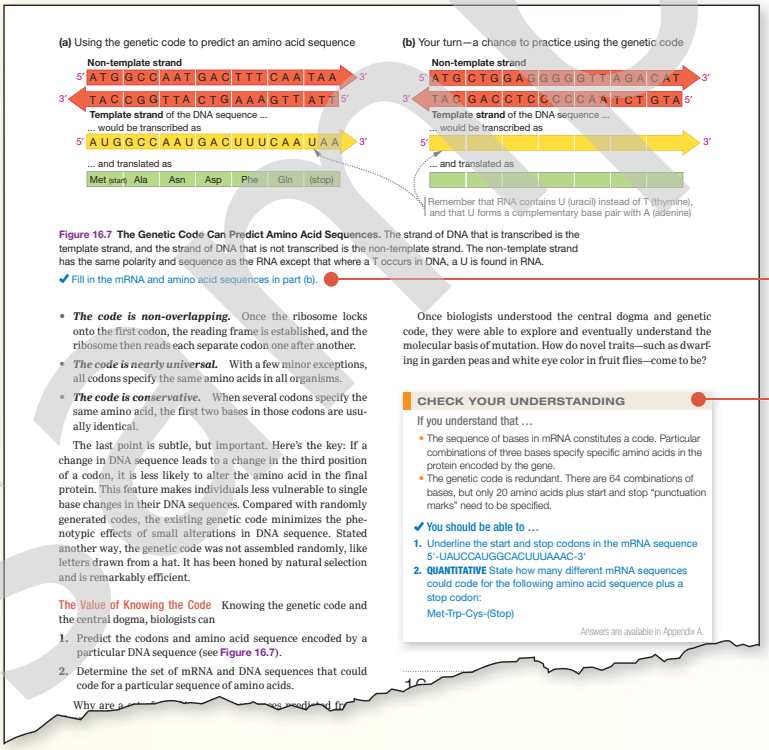
MasteringBiology®

Big Picture concept map tutorials are challenging, higher-level activities that require students to build their own concept map and to answer questions about the content. They are automatically graded to make it easy for professors to assign. New to the **Sixth Edition** are tutorials on diversity.



Engage in Scientific Inquiry and Active Problem-Solving

A wide variety of practice questions and exercises are designed to encourage readers to pause and test their understanding as they proceed through each chapter. All questions and exercises are highlighted in blue throughout the text.



(a) Using the genetic code to predict an amino acid sequence

Non-template strand
5′ ATGGCCAATGACTTTCAATAA 3′
3′ TACCGGTTACTGAAAGTTATT 5′
Template strand of the DNA sequence ...
... would be transcribed as
5′ AUGGCCAAUGACUUUCAAUAA 3′
... and translated as
Met (start) | Ala | Asn | Asp | Phe | Gln | (stop)

(b) Your turn—a chance to practice using the genetic code

Non-template strand
5′ ATGCTGGAGGGGGTTAGACAT 3′
3′ TAC GACCTC CCCCAA ICT GTA 5′
Template strand of the DNA sequence ...
... would be transcribed as
5′ 3′
... and translated as
Remember that RNA contains U (uracil) instead of T (thymine), and that U forms a complementary base pair with A (adenine)

Figure 16.7 The Genetic Code Can Predict Amino Acid Sequences. The strand of DNA that is transcribed is the template strand, and the strand of DNA that is not transcribed is the non-template strand. The non-template strand has the same polarity and sequence as the RNA except that where a T occurs in DNA, a U is found in RNA.

✔ Fill in the mRNA and amino acid sequences in part (b).

- ***The code is non-overlapping.*** Once the ribosome locks onto the first codon, the reading frame is established, and the ribosome then reads each separate codon one after another.
- ***The code is nearly universal.*** With a few minor exceptions, all codons specify the same amino acids in all organisms.
- ***The code is conservative.*** When several codons specify the same amino acid, the first two bases in those codons are usually identical.

The last point is subtle, but important. Here's the key: If a change in DNA sequence leads to a change in the third position of a codon, it is less likely to alter the amino acid in the final protein. This feature makes individuals less vulnerable to single base changes in their DNA sequences. Compared with randomly generated codes, the existing genetic code minimizes the phenotypic effects of small alterations in DNA sequence. Stated another way, the genetic code was not assembled randomly, like letters drawn from a hat. It has been honed by natural selection and is remarkably efficient.

The Value of Knowing the Code Knowing the genetic code and the central dogma, biologists can

1. Predict the codons and amino acid sequence encoded by a particular DNA sequence (see Figure 16.7).
2. Determine the set of mRNA and DNA sequences that could code for a particular sequence of amino acids.

Why are a

Once biologists understood the central dogma and genetic code, they were able to explore and eventually understand the molecular basis of mutation. How do novel traits—such as dwarfing in garden peas and white eye color in fruit flies—come to be?

CHECK YOUR UNDERSTANDING

If you understand that …

- The sequence of bases in mRNA constitutes a code. Particular combinations of three bases specify specific amino acids in the protein encoded by the gene.
- The genetic code is redundant. There are 64 combinations of bases, but only 20 amino acids plus start and stop "punctuation marks" need to be specified.

✔ You should be able to …

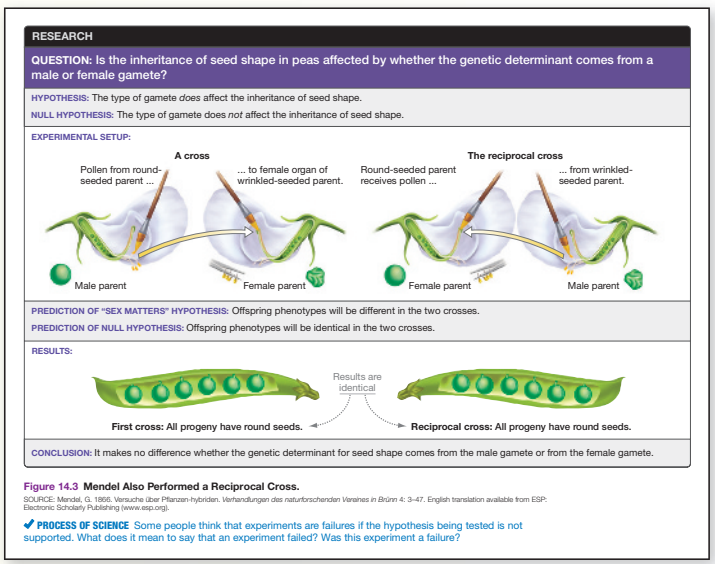
1. Underline the start and stop codons in the mRNA sequence 5′-UAUCCAUGGCACUUUAAAC-3′
2. **QUANTITATIVE** State how many different mRNA sequences could code for the following amino acid sequence plus a stop codon:
   Met-Trp-Cys-(Stop)

Answers are available in Appendix A.

Figure and table caption questions and exercises ask students to critically examine information in figures and tables.

Check Your Understanding activities ask students to work with important concepts in the chapter.

Research boxes teach students how we know what we know about biology by using current and classic research to model the observational and hypothesis-testing process of scientific discovery.



RESEARCH

QUESTION: Is the inheritance of seed shape in peas affected by whether the genetic determinant comes from a male or female gamete?

HYPOTHESIS: The type of gamete *does* affect the inheritance of seed shape.

NULL HYPOTHESIS: The type of gamete does *not* affect the inheritance of seed shape.

EXPERIMENTAL SETUP:

A cross
Pollen from round-seeded parent ...
... to female organ of wrinkled-seeded parent.
Male parent
Female parent

The reciprocal cross
Round-seeded parent receives pollen ...
... from wrinkled-seeded parent.
Female parent
Male parent

PREDICTION OF "SEX MATTERS" HYPOTHESIS: Offspring phenotypes will be different in the two crosses.

PREDICTION OF NULL HYPOTHESIS: Offspring phenotypes will be identical in the two crosses.

RESULTS:

Results are identical

First cross: All progeny have round seeds.

Reciprocal cross: All progeny have round seeds.

CONCLUSION: It makes no difference whether the genetic determinant for seed shape comes from the male gamete or from the female gamete.

Figure 14.3 Mendel Also Performed a Reciprocal Cross.

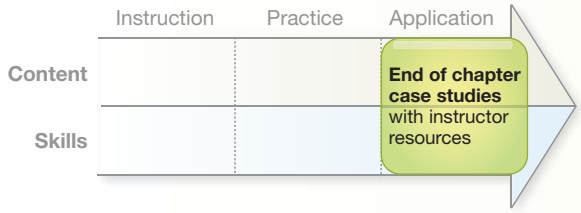
SOURCE: Mendel, G. 1866. Versuche über Pflanzen-hybriden. *Verhandlungen des naturforschenden Vereines in Brünn* 4: 3–47. English translation available from: ESP: Electronic Scholarly Publishing (www.esp.org).

✔ **PROCESS OF SCIENCE** Some people think that experiments are failures if the hypothesis being tested is not supported. What does it mean to say that an experiment failed? Was this experiment a failure?

Each Research box concludes with a **question or exercise** that asks students to think critically about experimental design by predicting outcomes, analyzing the setup used to test a hypothesis, or interpreting data found in experimental results

MasteringBiology®

"Solve It" Tutorials engage learners in a multi-step investigation of a "mystery" or open question in which students must analyze real data.



Steps to Building Understanding

Each chapter ends with three groups of questions that build in difficulty

✓ TEST YOUR KNOWLEDGE

Begin by testing your basic knowledge of new information.

✓ TEST YOUR UNDERSTANDING

Once you're confident with the basics, demonstrate your deeper understanding of the material.

✓ TEST YOUR PROBLEM-SOLVING SKILLS

Work towards mastery of the content by answering questions that challenge you at the highest level of competency.

NEW! "Put It All Together" case studies appear at the end of every chapter and provide a brief summary of contemporary biology research in action. Each case study connects what students learn in class with current, real-world biology research questions. At least one question requires students to **analyze real data** or apply **quantitative skills**.

MasteringBiology®

NEW! Case study questions from the end of chapter are assignable in MasteringBiology.

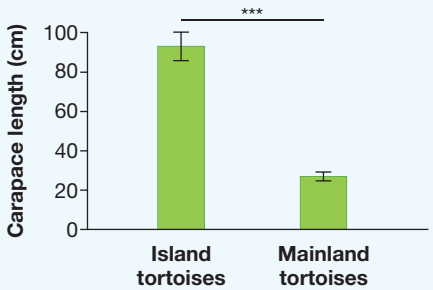
NEW! Classroom activity questions about the case study are available for clickers to help instructors easily incorporate the case studies into their classroom teaching.

✓ PUT IT ALL TOGETHER: Case Study

How does gigantism affect the physiology of animals?

Many species of animals on islands are larger than related species on the mainland. Scientists hypothesize that this phenomenon, called island gigantism, evolved in response to the scarcity of competitors and predators on islands. Reduced competition and predation allows species to exploit more resources and frees them from the need to hide in small refuges.

11. QUANTITATIVE The graph shown here compares the average carapace (shell) length of mainland and island tortoises. Summarize the results (*** means $P < 0.001$, see BioSkills 3), then use the data to predict whether the surface area/volume ratio is higher in mainland or island tortoises.



Source: Jaffe, A. L., G. J. Slater, and M. E. Alfaro. 2011. *Biology Letters* 7: 558–561.

12. Which tortoises, mainland or island, need to eat more food per gram of their body mass?

13. Which of the following might be a trade-off of gigantism experienced by giant island tortoises?

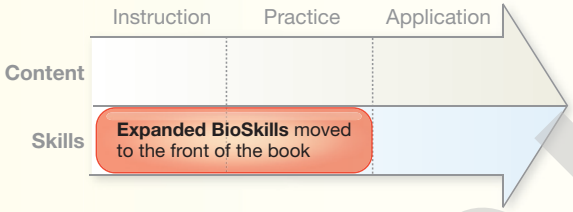
a. They cool very rapidly during cold weather.
b. It would be difficult to sustain their high mass-specific metabolic rates on a diet of plants alone.
c. It could be more difficult to avoid thermally unfavorable conditions.
d. They could hide from nonnative predators more easily.

14. CAUTION True or false: The body temperatures of island tortoises always closely match the temperatures in their environments.

15. Suppose that a small mainland tortoise and a large island tortoise are placed in the same pen at a zoo. Which tortoise will be more poikilothermic, the small or large tortoise? Why?

16. CAUTION On a trip to the Galápagos Islands, you overhear a group of tourists refer to tortoises as "cold blooded." Explain why this word is not accurate to describe a giant tortoise.

Develop Skills for Success in Biology and Beyond...



NEW! Unique BioSkills reference section is now placed earlier in the text to draw attention to key skills students need to succeed in biology. Previously located in an appendix at the end of the text, this easy-to-find reference material now follows Chapter 1 to better support the development of skills throughout the course. Each BioSkill includes practice exercises.

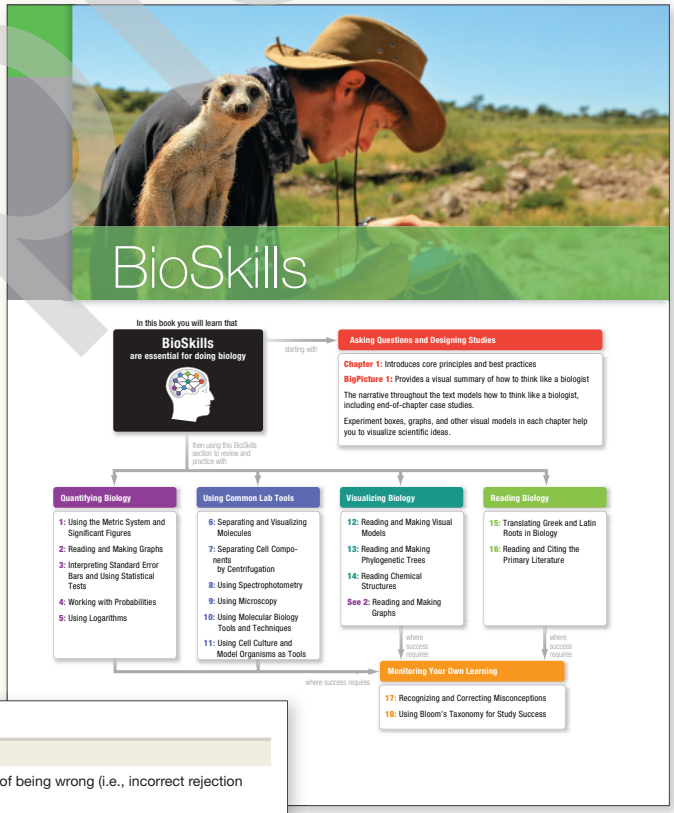
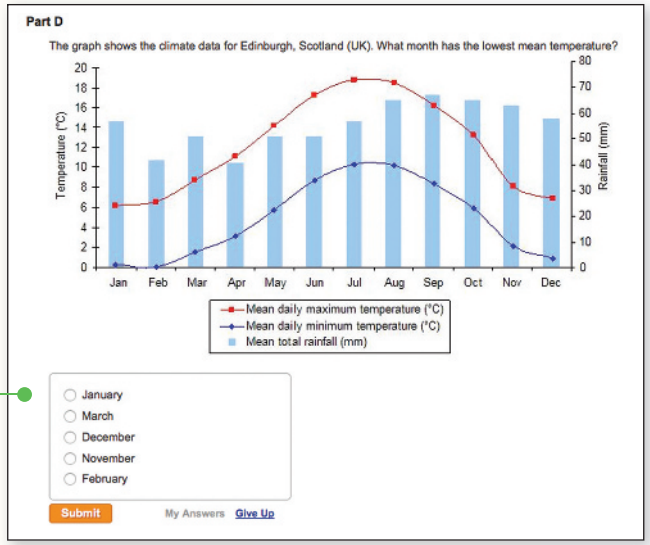


Table B3.1 Asterisk Rating System for *P* Values and Statistical Significance

P Value	Asterisk Rating	Statistical Significance Level	Meaning
$P > 0.05$	None	Not significant	Greater than a 1 in 20 chance of being wrong (i.e., incorrect rejection of the null hypothesis)
$P < 0.05$	*	Statistically significant	Less than a 1 in 20 chance of being wrong
$P < 0.01$	**	Statistically significant	Less than a 1 in 100 chance of being wrong
$P < 0.001$	***	Statistically significant	Less than a 1 in 1000 chance of being wrong

EXPANDED! BioSkill on Interpreting Standard Error Bars and Using Statistical Tests includes a new discussion of commonly used tests, such as chi square, t-test, and analysis of variance (ANOVA). A new section discusses interpreting *P* values and statistical significance.

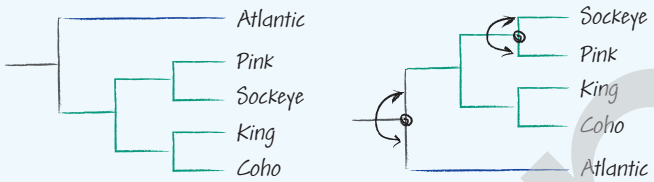


MasteringBiology®

BioSkills review questions are available in the Study Area for self-paced learning and practice. Additional BioSkills questions in the item library are assignable for homework.

Making Models 25.1 Tips on Drawing Phylogenetic Trees

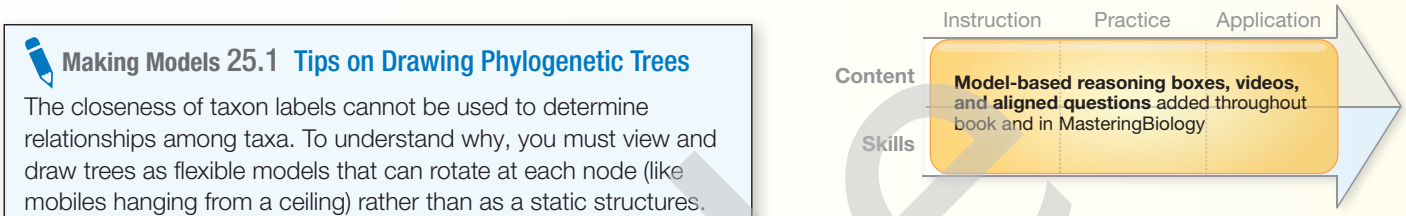
The closeness of taxon labels cannot be used to determine relationships among taxa. To understand why, you must view and draw trees as flexible models that can rotate at each node (like mobiles hanging from a ceiling) rather than as a static structures.



These trees have the same meaning.

MODEL Draw one more "equivalent" tree with the same meaning as the two above, rotating one or more of the nodes.

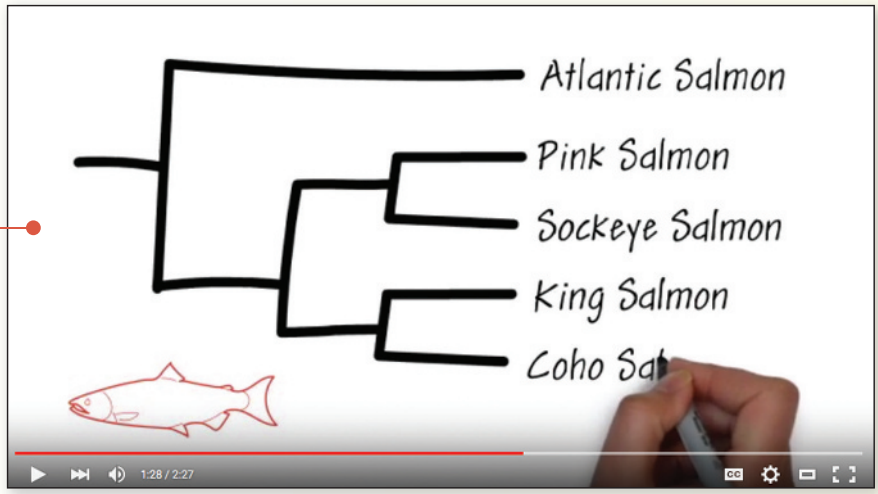
To see this model in action, go to https://goo.gl/mskc9S

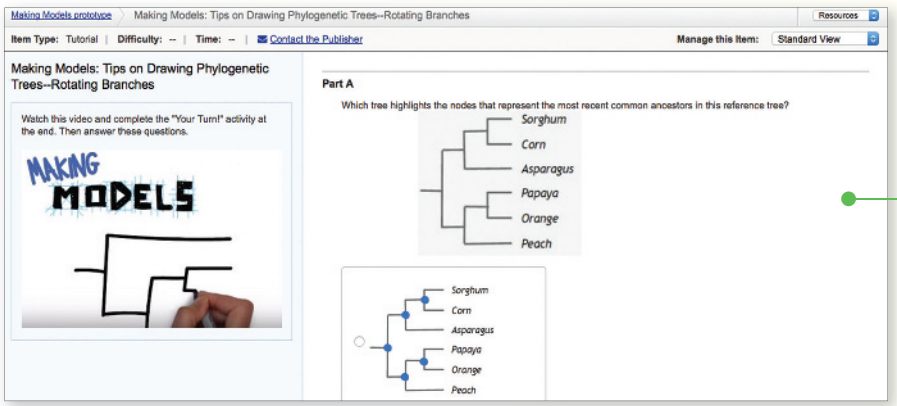


NEW! Unique Making Models boxes appear at strategic points throughout chapters as a guide for developing a deeper understanding of biology concepts by interpreting and creating visual models.

Readers can access the videos via **QR codes**, through the eText, or in the Study Area of MasteringBiology.

NEW! Interactive whiteboard videos accompany each Making Models box to reinforce learning and to demonstrate how to build visual models.



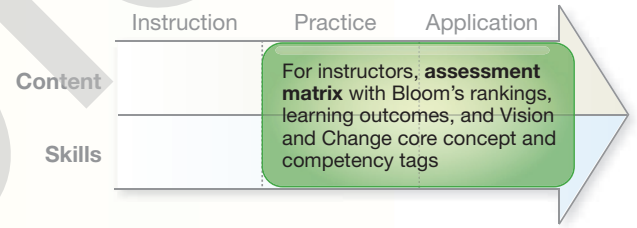


MasteringBiology®

NEW! Making Models activities are assignable for homework and include the whiteboard videos plus application questions that help in developing the skills of interpreting visual models.

For Instructors: Easily Align Assessment with Your Course Goals

Informed by current science education research and curriculum reform strategies, the Sixth Edition instructor resources provide a broad range of easy-to-use assessment options.



NEW! Chapter Assessment Grids help instructors quickly identify suitable assessment questions in the text according to learning outcomes, Bloom's taxonomy ranking, core concepts and core competencies discussed in the *Vision and Change in Undergraduate Biology Education* report, and, when applicable, common student misconceptions.

BLOOMS TAXONOMY RANKING

"Blue Thread" questions, including end-of-chapter problems, are ranked according to **Bloom's taxonomy** and are assignable in MasteringBiology.

LEARNING OUTCOMES

Each question is tagged to a publisher-provided **Learning Outcome**. Instructors may also track their own Learning Outcomes using MasteringBiology.

MISCONCEPTIONS

NEW! When applicable, **common student misconceptions** are addressed and identified with targeted questions.

VISION & CHANGE CORE CONCEPTS

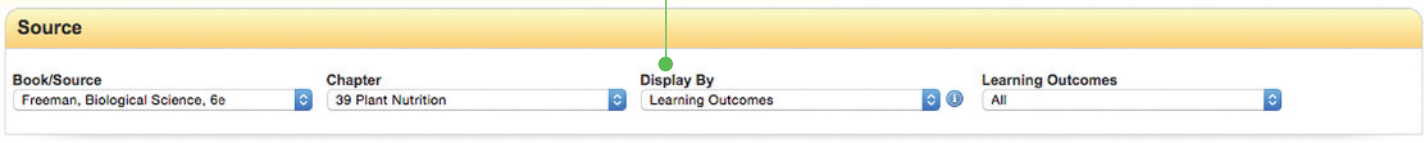
NEW! Each question that covers a **Core Concept** from the *Vision and Change in Undergraduate Biology Education* report is noted in the chapter assessment grid and in MasteringBiology.

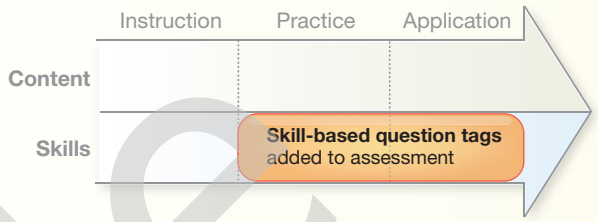
VISION & CHANGE CORE COMPETENCIES

NEW! Core Competencies from the *Vision and Change in Undergraduate Biology Education* report are indicated in the chapter assessment grid and in MasteringBiology.

MasteringBiology®

EXPANDED! Questions, activities, and tutorials are tagged by Bloom's ranking, Learning Outcome, and Vision and Change Core Concepts and Core Competencies.





An extensive selection of mid- and high-level assessment questions are provided throughout each chapter to help students learn, practice, and prepare for tests.

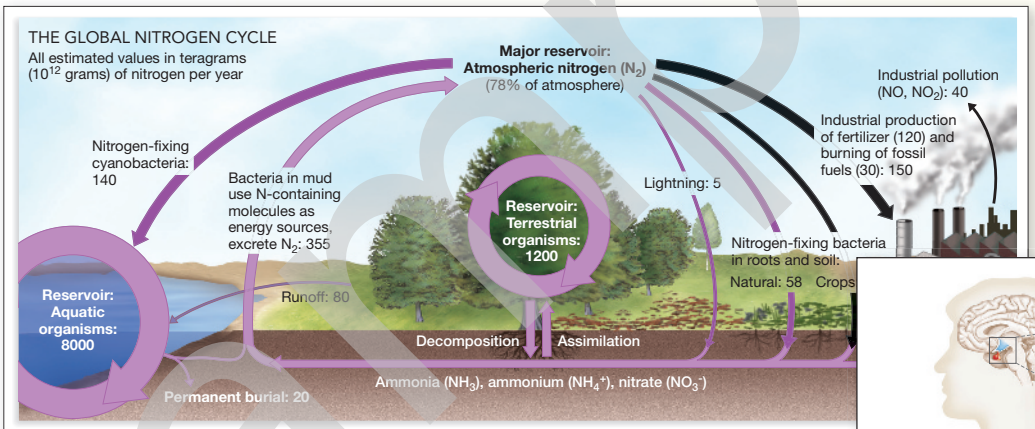


Figure 53.14 The Global Nitrogen Cycle. Nitrogen enters ecosystems as ammonia or nitrate via fixation from atmospheric nitrogen. It is exported in runoff and as nitrogen gas given off by bacteria that use nitrogen-containing compounds as an electron acceptor.

DATA: Fowler, D., et al. 2013. *Philosophical Transactions of the Royal Society B* 368 (1621): 20130165.

✓ **QUANTITATIVE** Calculate the percentage of total nitrogen fixation (all downward-pointing arrows) that is caused by human activities (black arrows).

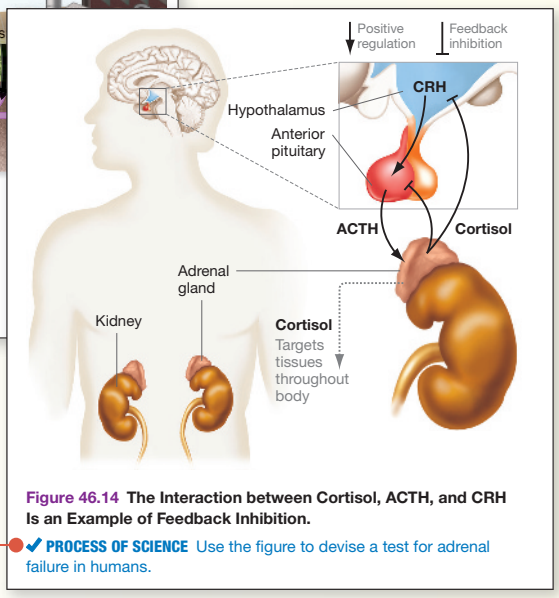


Figure 46.14 The Interaction between Cortisol, ACTH, and CRH Is an Example of Feedback Inhibition.

✓ **PROCESS OF SCIENCE** Use the figure to devise a test for adrenal failure in humans.

NEW! Question labels call attention to questions that require **quantitative skills**, an understanding of the **process of science**, connecting biology and **society**, making **models**, and more.

NEW! Caution questions address topics for which students often hold common misconceptions. Answers to Caution questions include information that addresses the misconception.

5. **CAUTION** According to data presented in this chapter, which one of the following statements is correct?
   - **a.** When individuals change in response to challenges from the environment, their altered traits are passed on to offspring.
   - **b.** Species are created independently of each other and do not change over time.
   - **c.** Populations—not individuals—change when natural selection occurs.
   - **d.** The traits of populations become more perfect over time.

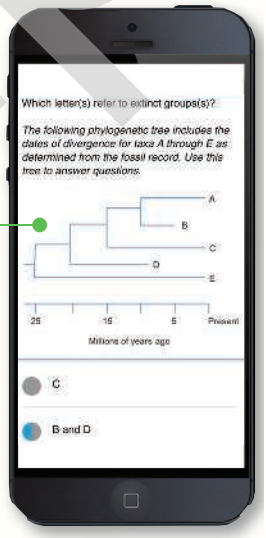
Succeed with MasteringBiology®

MasteringBiology is a powerful online learning and assessment system proven to improve results by engaging students before, during, and after class with a deep library of helpful activities. Mastering brings learning full circle by continuously adapting to each student and making learning more personal than ever—before, during, and after class.

Before Class

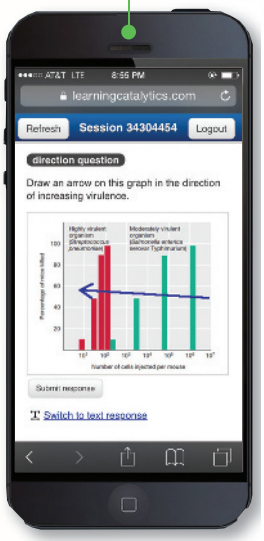
NEW! Dynamic Study Modules provide students with multiple sets of questions with extensive feedback so that they can test, learn, and retest until they achieve mastery of the textbook material.

NEW! More mobile-friendly Pre-class reading quizzes help students pinpoint concepts that they understand and concepts with which they need more help. By identifying topics that are most difficult for them, students are better prepared to ask questions and more likely to listen actively.



During Class

NEW! Learning Catalytics™ allows students to use their smartphone, tablet, or laptop to respond individually or in groups to questions in class. Visit learningcatalytics.com to learn more.



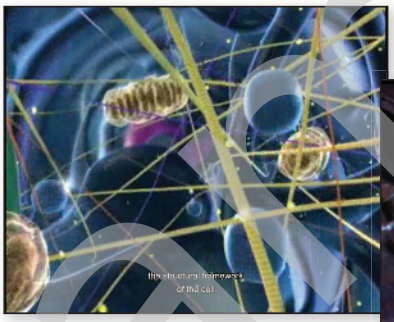
After Class

NEW! Optional Adaptive Follow-up Assignments are based on each student's performance on the original MasteringBiology assignment and provide additional questions and activities tailored to each student's needs.

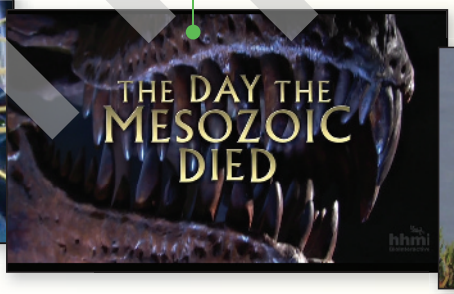
Hundreds of self-paced tutorials and coaching activities provide students with individualized coaching with specific hints and feedback on the toughest topics in the course.

NEW! Pearson eText 2.0 integrates the text with videos and animations, in a format that adapts to the device being used. Features include student and instructor note-taking, highlighting, bookmarking, search, and hotlinked glossary.

MasteringBiology offers a wide variety of tutorials that can be assigned as homework. Examples include:



BioFlix® Tutorials use 3-D, movie-quality animations and coaching exercises to help students master tough topics outside of class. Animations can also be shown in class.



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Detailed Contents

1 Biology and the Tree of Life 1

1.1 What Does It Mean to Say That Something Is Alive? 2

1.2 Life Is Cellular 2
All Organisms Are Made of Cells 2
Where Do Cells Come From? 3
Life Replicates Through Cell Division 4

1.3 Life Evolves 4
What Is Evolution? 4
What Is Natural Selection? 4

1.4 Life Processes Information 5
The Central Dogma 5
Life Requires Energy 6

1.5 The Tree of Life 6
Using Molecules to Understand the Tree of Life 6
How Should We Name Branches on the Tree of Life? 8

1.6 Doing Biology 9
The Nature of Science 9
Why Do Giraffes Have Long Necks? An Introduction to Hypothesis Testing 9
How Do Ants Navigate? An Introduction to Experimental Design 11

CHAPTER REVIEW 14

BIG PICTURE Doing Biology 16

BioSkills 18

B.1 Using the Metric System and Significant Figures 19
Metric System Units and Conversions 19
Significant Figures 20

B.2 Reading and Making Graphs 21
Getting Started 21
Types of Graphs 23
Getting Practice 23

B.3 Interpreting Standard Error Bars and Using Statistical Tests 24
Standard Error Bars 24
Using Statistical Tests 25
Interpreting *P* Values and Statistical Significance 25

B.4 Working with Probabilities 26
The Both-And Rule 26
The Either-Or Rule 26

B.5 Using Logarithms 27

B.6 Separating and Visualizing Molecules 28
Using Electrophoresis to Separate Molecules 28
Using Thin Layer Chromatography to Separate Molecules 29
Visualizing Molecules 29

B.7 Separating Cell Components by Centrifugation 31

B.8 Using Spectrophotometry 33

B.9 Using Microscopy 33
Light and Fluorescence Microscopy 33
Electron Microscopy 34
Studying Live Cells and Real-Time Processes 35
Visualizing Cellular Structures in 3-D 35

B.10 Using Molecular Biology Tools and Techniques 36
Making and Using DNA Libraries 36
Amplifying DNA Using the Polymerase Chain Reaction (PCR) 37
Dideoxy Sequencing 38
Shotgun Sequencing 39
DNA Microarray 40

B.11 Using Cell Culture and Model Organisms as Tools 41
Cell and Tissue Culture Methods 41
Model Organisms 42

B.12 Reading and Making Visual Models 45
Tips for Interpreting Models 45
Tips for Making your Own Models 46
Concept Maps 46

B.13 Reading and Making Phylogenetic Trees 47
Anatomy of a Phylogenetic Tree 47
How to Read a Phylogenetic Tree 48
How to Draw a Phylogenetic Tree 48

B.14 Reading Chemical Structures 49

B.15 Translating Greek and Latin Roots in Biology 50

B.16 Reading and Citing the Primary Literature 50
What Is the Primary Literature? 50
Getting Started 50
Citing Sources 52
Getting Practice 52

B.17 Recognizing and Correcting Misconceptions 52

B.18 Using Bloom's Taxonomy for Study Success 53
Categories of Human Cognition 53
Six Study Steps to Success 53

UNIT 1 THE MOLECULAR ORIGIN AND EVOLUTION OF LIFE 55

2 Water and Carbon: The Chemical Basis of Life 55

2.1 Atoms, Ions, and Molecules: The Building Blocks of Chemical Evolution 56
Basic Atomic Structure 56
How Does Covalent Bonding Hold Molecules Together? 58
Ionic Bonding, Ions, and the Electron-Sharing Continuum 59
Some Simple Molecules Formed from C, H, N, and O 60
The Geometry of Simple Molecules 60
Representing Molecules 60

2.2 Properties of Water and the Early Oceans 61
Why Is Water Such an Efficient Solvent? 62
What Properties Are Correlated with Water's Structure? 62
The Role of Water in Acid–Base Chemical Reactions 65

2.3 Chemical Reactions, Energy, and Chemical Evolution 67
How Do Chemical Reactions Happen? 67
What Is Energy? 68
What Makes a Chemical Reaction Spontaneous? 68

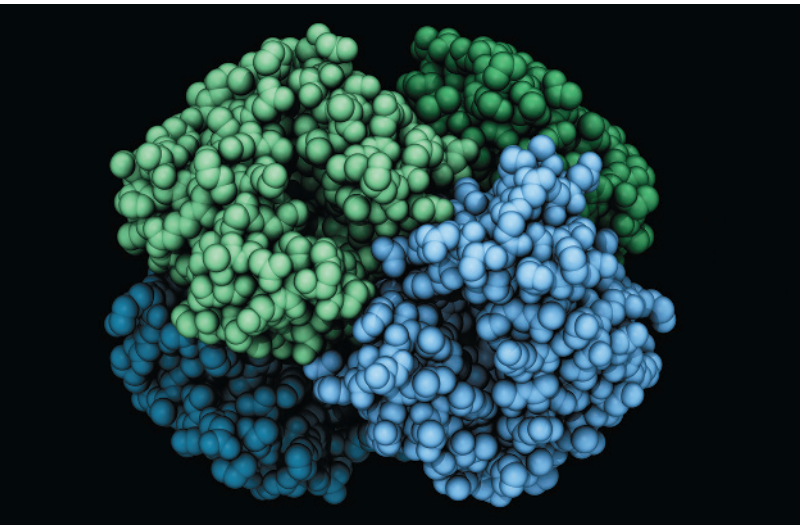
2.4 Model Systems for Investigating Chemical Evolution 70
Early Origin-of-Life Experiments 70
Recent Origin-of-Life Experiments 71

2.5 The Importance of Organic Molecules 73
Linking Carbon Atoms Together 73
Functional Groups 75

CHAPTER REVIEW 75

3 Protein Structure and Function 78

3.1 Amino Acids and Their Polymerization 79
The Structure of Amino Acids 79
The Nature of Side Chains 79
How Do Amino Acids Link to Form Proteins? 81



3.2 What Do Proteins Look Like? 83
Primary Structure 84
Secondary Structure 85
Tertiary Structure 86
Quaternary Structure 87

3.3 Folding and Function 88
Normal Folding Is Crucial to Function 88
Protein Shape Is Flexible 89

3.4 Protein Functions Are as Diverse as Protein Structures 90
Why Are Enzymes Good Catalysts? 90
Did Life Arise from a Self-Replicating Enzyme? 91

CHAPTER REVIEW 91

4 Nucleic Acids and the RNA World 93

4.1 What Is a Nucleic Acid? 94
Could Chemical Evolution Result in the Production of Nucleotides? 95
How Do Nucleotides Polymerize to Form Nucleic Acids? 95

4.2 DNA Structure and Function 97
What Is the Nature of DNA's Secondary Structure? 97
The Tertiary Structure of DNA 99
DNA Functions as an Information-Containing Molecule 99
The DNA Double Helix Is a Stable Structure 100

4.3 RNA Structure and Function 101
Structurally, RNA Differs from DNA 101
RNA's Versatility 102
RNA Can Function as a Catalytic Molecule 102

4.4 In Search of the First Life-Form 103
How Biologists Study the RNA World 104
The RNA World May Have Sparked the Evolution of Life 104

CHAPTER REVIEW 105

5 An Introduction to Carbohydrates 107

5.1 Sugars as Monomers 108
What Distinguishes One Monosaccharide from Another? 108
Can Monosaccharides Form by Chemical Evolution? 109

5.2 The Structure of Polysaccharides 110
Starch: A Storage Polysaccharide in Plants 111
Glycogen: A Highly Branched Storage Polysaccharide in Animals 111
Cellulose: A Structural Polysaccharide in Plants 113
Chitin: A Structural Polysaccharide in Fungi and Animals 113
Peptidoglycan: A Structural Polysaccharide in Bacteria 113
Polysaccharides and Chemical Evolution 113

5.3 What Do Carbohydrates Do? 113
Carbohydrates Can Provide Structural Support 114
The Role of Carbohydrates in Cell Identity 114
Carbohydrates and Energy Storage 115

CHAPTER REVIEW 117

6 Lipids, Membranes, and the First Cells 119

6.1 Lipid Structure and Function 120
How Does Bond Saturation Affect Hydrocarbon Structure? 120
A Look at Three Types of Lipids Found in Cells 121
How Membrane Lipids Interact with Water 122
Were Lipids Present during Chemical Evolution? 123

6.2 Phospholipid Bilayers 123
Artificial Membranes as an Experimental System 124
Selective Permeability of Lipid Bilayers 124
How Does Lipid Structure Affect Membrane Permeability? 125
How Does Temperature Affect the Fluidity and Permeability of Membranes? 126

6.3 How Substances Move across Lipid Bilayers: Diffusion and Osmosis 127
Diffusion 127
Osmosis 128
Membranes and Chemical Evolution 129

6.4 Proteins Alter Membrane Structure and Function 130
Development of the Fluid-Mosaic Model 130
Systems for Studying Membrane Proteins 132
Channel Proteins Facilitate Diffusion 132
Carrier Proteins Facilitate Diffusion 134
Pumps Perform Active Transport 135
Plasma Membranes Define the Intracellular Environment 137

CHAPTER REVIEW 138

BIG PICTURE The Chemistry of Life 140

UNIT 2 CELL STRUCTURE AND FUNCTION 142

7 Inside the Cell 142

7.1 Bacterial and Archaeal Cell Structures and Their Functions 143
A Revolutionary New View 143
Prokaryotic Cell Structures: A Parts List 143

7.2 Eukaryotic Cell Structures and Their Functions 146
The Benefits of Organelles 146
Eukaryotic Cell Structures: A Parts List 146

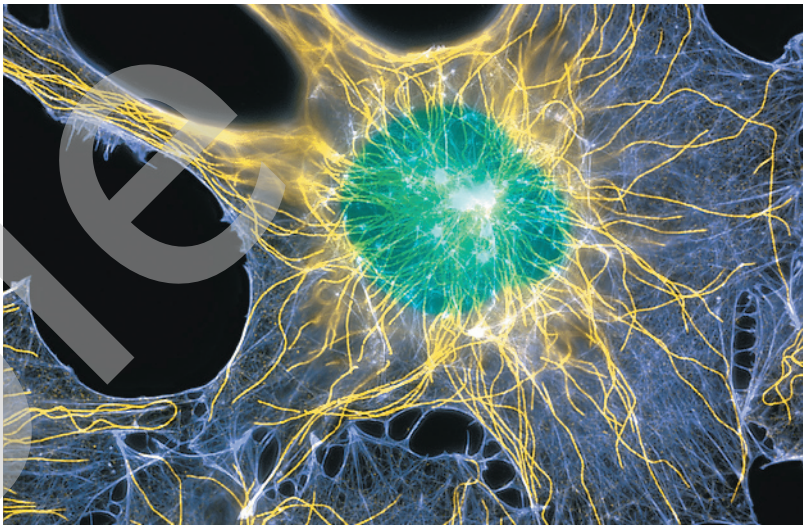
7.3 Putting the Parts into a Whole 154
Structure and Function at the Whole-Cell Level 154
The Dynamic Cell 154

7.4 Cell Systems I: Nuclear Transport 155
Structure and Function of the Nuclear Envelope 155
How Do Molecules Enter the Nucleus? 156

7.5 Cell Systems II: The Endomembrane System Manufactures, Ships, and Recycles Cargo 157
Studying the Pathway through the Endomembrane System 157
Entering the Endomembrane System: The Signal Hypothesis 159
Moving from the ER to the Golgi Apparatus 160
What Happens Inside the Golgi Apparatus? 160
How Do Proteins Reach Their Destinations? 160
Recycling Material in the Lysosome 160

7.6 Cell Systems III: The Dynamic Cytoskeleton 163
Actin Filaments 163
Intermediate Filaments 164
Microtubules 164
Flagella and Cilia: Moving the Entire Cell 166

CHAPTER REVIEW 168



8 Energy and Enzymes: An Introduction to Metabolism 171

8.1 What Happens to Energy in Chemical Reactions? 172
Chemical Reactions Involve Energy Transformations 172
Temperature and Concentration Affect Reaction Rates 173

8.2 Nonspontaneous Reactions May Be Driven Using Chemical Energy 175
Redox Reactions Transfer Energy via Electrons 175
ATP Transfers Energy via Phosphate Groups 177

8.3 How Enzymes Work 179
Enzymes Help Reactions Clear Two Hurdles 179
What Limits the Rate of Catalysis? 181
Do Enzymes Work Alone? 182

8.4 What Factors Affect Enzyme Function? 182
Enzymes Are Optimized for Particular Environments 182
Most Enzymes Are Regulated 183

8.5 Enzymes Can Work Together in Metabolic Pathways 184
Metabolic Pathways Are Regulated 185
Metabolic Pathways Evolve 185

CHAPTER REVIEW 186

9 Cellular Respiration and Fermentation 189

9.1 An Overview of Cellular Respiration 190
What Happens When Glucose Is Oxidized? 190
Cellular Respiration Plays a Central Role in Metabolism 192

9.2 Glycolysis: Oxidizing Glucose to Pyruvate 193
Glycolysis Is a Sequence of 10 Reactions 193
How Is Glycolysis Regulated? 194

9.3 Processing Pyruvate to Acetyl CoA 196

9.4 The Citric Acid Cycle: Oxidizing Acetyl CoA to CO_2 197
How Is the Citric Acid Cycle Regulated? 197
What Happens to the NADH and $FADH_2$? 199

9.5 Electron Transport and Chemiosmosis: Building a Proton Gradient to Produce ATP 200
The Electron Transport Chain 201
The Discovery of ATP Synthase 203
The Chemiosmosis Hypothesis 203
Organisms Use a Diversity of Electron Acceptors 205

9.6 Fermentation 206
Many Different Fermentation Pathways Exist 206
Fermentation as an Alternative to Cellular Respiration 207

CHAPTER REVIEW 208

10 Photosynthesis 210

10.1 Photosynthesis Harnesses Sunlight to Make Carbohydrate 211
Photosynthesis: Two Linked Sets of Reactions 211
Photosynthesis Occurs in Chloroplasts 212

10.2 How Do Pigments Capture Light Energy? 213
Photosynthetic Pigments Absorb Light 213
When Light Is Absorbed, Electrons Enter an Excited State 216

10.3 The Discovery of Photosystems I and II 218
How Does Photosystem II Work? 218
How Does Photosystem I Work? 220
The Z Scheme: Photosystems II and I Work Together 221

10.4 How Is Carbon Dioxide Reduced to Produce Sugars? 223
The Calvin Cycle Fixes Carbon 223
The Discovery of Rubisco 225
How Is Photosynthesis Regulated? 226
Oxygen and Carbon Dioxide Pass through Stomata 227
Mechanisms for Increasing CO_2 Concentration 227
What Happens to the Sugar That Is Produced by Photosynthesis? 229

CHAPTER REVIEW 230

BIG PICTURE Energy for Life 232

11 Cell–Cell Interactions 234

11.1 The Cell Surface 235
The Structure and Function of an Extracellular Layer 235
The Extracellular Matrix in Animals 235
The Cell Wall in Plants 236

11.2 How Do Adjacent Cells Connect and Communicate? 238
Cell–Cell Attachments in Multicellular Eukaryotes 238
Cells Communicate via Cell–Cell Gaps 241

11.3 How Do Distant Cells Communicate? 243
Cell–Cell Signaling in Multicellular Organisms 243
Signal Reception 243
Signal Processing 244
Signal Response 248
Signal Deactivation 248
Crosstalk: Synthesizing Input from Many Signals 248

11.4 Signaling between Unicellular Organisms 249

CHAPTER REVIEW 250

12 The Cell Cycle 253

12.1 How Do Cells Replicate? 254
What Is a Chromosome? 254
Cells Alternate between M Phase and Interphase 255
The Discovery of S Phase 255
The Discovery of the Gap Phases 255
The Cell Cycle 256

12.2 What Happens during M Phase? 257
Events in Mitosis 257
How Do Chromosomes Move during Anaphase? 260
Cytokinesis Results in Two Daughter Cells 262
Bacterial Cell Replication 262

12.3 Control of the Cell Cycle 263
The Discovery of Cell-Cycle Regulatory Molecules 263
Cell-Cycle Checkpoints Can Arrest the Cell Cycle 265

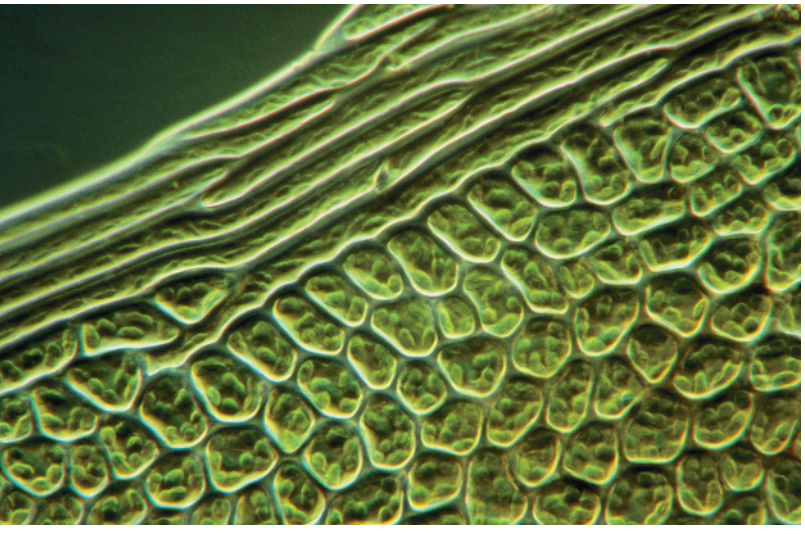
12.4 Cancer: Out-of-Control Cell Division 266
Properties of Cancer Cells 267
Cancer Involves Loss of Cell-Cycle Control 267

CHAPTER REVIEW 269

UNIT 3 GENE STRUCTURE AND EXPRESSION 271

13 Meiosis 271

13.1 How Does Meiosis Occur? 272
Chromosomes Come in Distinct Sizes and Shapes 272
The Concept of Ploidy 273
An Overview of Meiosis 273



The Phases of Meiosis I 277
The Phases of Meiosis II 278
A Closer Look at Synapsis and Crossing Over 279
Mitosis versus Meiosis 279

13.2 Meiosis Promotes Genetic Variation 280
Chromosomes and Heredity 281
The Role of Independent Assortment 281
The Role of Crossing Over 282
How Does Fertilization Affect Genetic Variation? 282

13.3 What Happens When Things Go Wrong in Meiosis? 283
How Do Mistakes Occur? 283
Why Do Mistakes Occur? 284

13.4 Why Does Meiosis Exist? 284
The Paradox of Sex 284
The Purifying Selection Hypothesis 285
The Changing-Environment Hypothesis 285

CHAPTER REVIEW 287

14 Mendel and the Gene 289

14.1 Mendel's Experimental System 290
What Questions Was Mendel Trying to Answer? 290
The Garden Pea Served as the First Model Organism in Genetics 290

14.2 Mendel's Experiments with a Single Trait 292
The Monohybrid Cross 292
Particulate Inheritance 294

14.3 Mendel's Experiments with Two Traits 296
The Dihybrid Cross 296
Using a Testcross to Confirm Predictions 298

14.4 The Chromosome Theory of Inheritance 299
Meiosis Explains Mendel's Principles 300
Testing the Chromosome Theory 300

14.5 Extending Mendel's Rules 302
Linkage: What Happens When Genes Are Located on the Same Chromosome? 303
Quantitative Methods 14.1 Linkage and Genetic Mapping 305
How Many Alleles Can a Gene Have? 306
Are Alleles Always Dominant or Recessive? 306
Does Each Gene Affect Just One Trait? 306
Are All Traits Determined by a Gene? 307
Can Mendel's Principles Explain Traits That Don't Fall into Distinct Categories? 308

14.6 Applying Mendel's Rules to Human Inheritance 310
Identifying Alleles as Recessive or Dominant 310
Identifying Traits as Autosomal or Sex-Linked 311

CHAPTER REVIEW 312

15 DNA and the Gene: Synthesis and Repair 316

15.1 What Are Genes Made Of? 317
The Hershey–Chase Experiment 317
The Secondary Structure of DNA 318

15.2 Testing Early Hypotheses about DNA Synthesis 319
Three Alternative Hypotheses 320
The Meselson–Stahl Experiment 320

15.3 A Model for DNA Synthesis 320
Where Does Replication Start? 322
How Is the Helix Opened and Stabilized? 322
How Is the Leading Strand Synthesized? 324
How Is the Lagging Strand Synthesized? 324

15.4 Replicating the Ends of Linear Chromosomes 327
The End Replication Problem 327
Telomerase Solves the End Replication Problem 328
Effect of Telomere Length on Cell Division 329

15.5 Repairing Mistakes and DNA Damage 329
Correcting Mistakes in DNA Synthesis 330
Repairing Damaged DNA 330
Xeroderma Pigmentosum: A Case Study 331

CHAPTER REVIEW 332

16 How Genes Work 335

16.1 What Do Genes Do? 336
The One-Gene, One-Enzyme Hypothesis 336
An Experimental Test of the Hypothesis 336

16.2 The Central Dogma of Molecular Biology 338
The Genetic Code Hypothesis 338
RNA as the Intermediary between Genes and Proteins 338
Dissecting the Central Dogma 339

16.3 The Genetic Code 341
How Long Is a "Word" in the Genetic Code? 341
How Did Researchers Crack the Code? 342

16.4 What Are the Types and Consequences of Mutation? 343
Point Mutations 344
Chromosome Mutations 345

CHAPTER REVIEW 346

17 Transcription, RNA Processing, and Translation 348

17.1 An Overview of Transcription 349
Initiation: How Does Transcription Begin in Bacteria? 349
Elongation and Termination 351
Transcription in Eukaryotes 351

17.2 RNA Processing in Eukaryotes 353
The Startling Discovery of Split Eukaryotic Genes 353
RNA Splicing 353
Adding Caps and Tails to Transcripts 354

17.3 An Introduction to Translation 355
Ribosomes Are the Site of Protein Synthesis 355
Translation in Bacteria and Eukaryotes 355
How Does an mRNA Triplet Specify an Amino Acid? 356

17.4 The Structure and Function of Transfer RNA 356
What Do tRNAs Look Like? 357
How Are Amino Acids Attached to tRNAs? 358
How Many tRNAs Are There? 358

17.5 The Structure of Ribosomes and Their Function in Translation 359
Initiating Translation 361
Elongation: Extending the Polypeptide 361
Terminating Translation 362
Post-Translational Modifications 363

CHAPTER REVIEW 364

18 Control of Gene Expression in Bacteria 367

18.1 An Overview of Gene Regulation and Information Flow 368
Mechanisms of Regulation 368
Metabolizing Lactose—A Model System 369

18.2 Identifying Regulated Genes 370

18.3 Negative Control of Transcription 372
The Operon Model 373
How Does Glucose Regulate the *lac* Operon? 374
Why Has the *lac* Operon Model Been So Important? 375

18.4 Positive Control of Transcription 375

18.5 Global Gene Regulation 376

CHAPTER REVIEW 377

19 Control of Gene Expression in Eukaryotes 379

19.1 Gene Regulation in Eukaryotes—An Overview 380

19.2 Chromatin Remodeling 380
What Is Chromatin's Basic Structure? 381
Evidence that Chromatin Structure Is Altered in Active Genes 382
How Is Chromatin Altered? 382
Chromatin Modifications Can Be Inherited 383

19.3 Initiating Transcription: Regulatory Sequences and Proteins 384
Promoter-Proximal Elements Are Regulatory Sequences Near the Core Promoter 385
Enhancers Are Regulatory Sequences Far from the Core Promoter 385
The Role of Transcription Factors in Differential Gene Expression 386
How Do Transcription Factors Recognize Specific DNA Sequences? 386
A Model for Transcription Initiation 387

19.4 Post-Transcriptional Control 388
Alternative Splicing of Primary Transcripts 388
How Is Translation Controlled? 389
Post-Translational Control 391

19.5 How Does Gene Expression Compare in Bacteria and Eukaryotes? 391

19.6 Linking Cancer to Defects in Gene Regulation 392
The Genetic Basis of Uncontrolled Cell Growth 392
The p53 Tumor Suppressor: A Case Study 392

CHAPTER REVIEW 393

BIG PICTURE Genetic Information 396

20 The Molecular Revolution: Biotechnology and Beyond 398

20.1 Recombinant DNA Technology 399
Using Plasmids in Cloning 399
Using Restriction Endonucleases and DNA Ligase to Cut and Paste DNA 399
Transformation: Introducing Recombinant Plasmids into Bacterial Cells 401
Using Reverse Transcriptase to Produce cDNAs 401
Biotechnology in Agriculture 401

20.2 The Polymerase Chain Reaction 401
Requirements of PCR 401
DNA Fingerprinting 402
A New Branch of the Human Family Tree 402

20.3 DNA Sequencing 403
Whole-Genome Sequencing 404
Bioinformatics 404
Which Genomes Are Being Sequenced, and Why? 404
Which Sequences Are Genes? 404

20.4 Insights from Genome Analysis 405
The Natural History of Prokaryotic Genomes 406
The Natural History of Eukaryotic Genomes 407
Insights from the Human Genome Project 410

20.5 Finding and Engineering Genes: The Huntington Disease Story 411
How Was the Huntington Disease Gene Found? 411
How Are Human Genes Found Today? 412
What Are the Benefits of Finding a Disease Gene? 412
Can Gene Therapy Provide a Cure? 413

20.6 Functional Genomics, Proteomics, and Systems Biology 414
What Is Functional Genomics? 414
What Is Proteomics? 414
What Is Systems Biology? 414

CHAPTER REVIEW 415

21 Genes, Development, and Evolution 418

21.1 Shared Developmental Processes 419
Cell Division 419
Cell–Cell Interactions 420
Cell Differentiation 420
Cell Movement and Changes in Shape 421
Programmed Cell Death 421

21.2 Genetic Equivalence and Differential Gene Expression in Development 422
Evidence that Differentiated Plant Cells Are Genetically Equivalent 422
Evidence that Differentiated Animal Cells Are Genetically Equivalent 422
How Does Differential Gene Expression Occur? 423

21.3 Regulatory Cascades Establish the Body Plan 424
Morphogens Set Up the Body Axes 424
Regulatory Genes Provide Increasingly Specific Positional Information 426
Regulatory Genes and Signaling Molecules Are Evolutionarily Conserved 428

One Regulator Can Be Used Many Different Ways 429

21.4 Cells Are Determined Before They Differentiate 429
Commitment and Determination 430
Master Regulators of Differentiation and Development 430
Stem Cell Therapy 431

21.5 Changes in Developmental Gene Expression Drive Evolutionary Change 431

CHAPTER REVIEW 432

UNIT 4 EVOLUTIONARY PATTERNS AND PROCESSES 435

22 Evolution by Natural Selection 435

22.1 The Rise of Evolutionary Thought 436
Plato and Typological Thinking 436
Aristotle and the Scale of Nature 436
Lamarck and the Idea of Evolution as Change through Time 437
Darwin and Wallace and Evolution by Natural Selection 437

22.2 The Pattern of Evolution: Have Species Changed, and Are They Related? 437
Evidence for Change through Time 437
Evidence of Descent from a Common Ancestor 440
Evolution's "Internal Consistency"—The Importance of Independent Data Sets 443

22.3 The Process of Evolution: How Does Natural Selection Work? 445
Darwin's Inspiration 445
Darwin's Four Postulates 445
The Biological Definitions of Fitness, Adaptation, and Selection 446

22.4 Evolution in Action: Recent Research on Natural Selection 446
Case Study 1: How Did *Mycobacterium tuberculosis* Become Resistant to Antibiotics? 446
Case Study 2: Why Do Beak Sizes and Shapes Vary in Galápagos Finches? 448

22.5 Debunking Common Myths about Natural Selection and Adaptation 451
Natural Selection Does Not Change Individuals 451
Natural Selection Is Not Goal Directed 452
Natural Selection Does Not Lead to Perfection 453

CHAPTER REVIEW 454

23 Evolutionary Processes 456

23.1 Analyzing Change in Allele Frequencies: The Hardy–Weinberg Principle 457
The Gene Pool Concept 457
Quantitative Methods 23.1 Deriving the Hardy–Weinberg Principle 458
The Hardy–Weinberg Principle Makes Important Assumptions 458
How Do Biologists Apply the Hardy–Weinberg Principle to Real Populations? 459

23.2 Nonrandom Mating 461
How Does Inbreeding Affect Allele Frequencies and Genotype Frequencies? 461

How Does Inbreeding Influence Evolution? 462

23.3 Natural Selection 462
How Does Selection Affect Genetic Variation? 462
Sexual Selection 466

23.4 Genetic Drift 469
Simulation Studies of Genetic Drift 469
Experimental Studies of Genetic Drift 470
What Causes Genetic Drift in Natural Populations? 472

23.5 Gene Flow 473
Measuring Gene Flow between Populations 473
Gene Flow Is Random with Respect to Fitness 474

23.6 Mutation 475
Mutation as an Evolutionary Process 475
Experimental Studies of Mutation 475
Studies of Mutation in Natural Populations 476
Take-Home Messages 477

CHAPTER REVIEW 478

24 Speciation 480

24.1 How are Species Defined and Identified? 481
The Biological Species Concept 481
The Morphospecies Concept 482
The Phylogenetic Species Concept 482
Species Definitions in Action: The Case of the Dusky Seaside Sparrow 484

24.2 Isolation and Divergence in Allopatry 485
Allopatric Speciation by Dispersal 485
Allopatric Speciation by Vicariance 486

24.3 Isolation and Divergence in Sympatry 487
Sympatric Speciation by Disruptive Selection 487
Sympatric Speciation by Polyploidization 489

24.4 What Happens When Isolated Populations Come into Contact? 491
Reinforcement 491
Hybrid Zones 491
New Species through Hybridization 492

CHAPTER REVIEW 493

25 Phylogenies and the History of Life 496

25.1 Tools for Studying History: Phylogenetic Trees 497
How Do Biologists Estimate Phylogenies? 498
How Can Biologists Distinguish Homology from Homoplasy? 500
Whale Evolution: A Case Study 501

25.2 Tools for Studying History: The Fossil Record 503
How Do Fossils Form? 503
Limitations of the Fossil Record 504
Life's Time Line 504

25.3 Adaptive Radiation 507
Why Do Adaptive Radiations Occur? 507
The Cambrian Explosion 509

25.4 Mass Extinction 511
How Do Mass Extinctions Differ from Background Extinctions? 511
The End-Permian Extinction 511
The End-Cretaceous Extinction 512
The Sixth Mass Extinction? 513

CHAPTER REVIEW 513

BIG PICTURE Evolution 516

UNIT 5 THE DIVERSIFICATION OF LIFE 518

26 Bacteria and Archaea 518

26.1 Why Do Biologists Study Bacteria and Archaea? 519
Biological Impact 519
Some Prokaryotes Thrive in Extreme Environments 519
Medical Importance 520
Role in Bioremediation 522

26.2 How Do Biologists Study Bacteria and Archaea? 523
Using Enrichment Cultures 523
Using Metagenomics 524
Investigating the Human Microbiome 524
Evaluating Molecular Phylogenies 524

26.3 What Themes Occur in the Diversification of Bacteria and Archaea? 525
Genetic Variation through Gene Transfer 525
Morphological Diversity 526
Metabolic Diversity 528
Ecological Diversity and Global Impacts 531

26.4 Key Lineages of Bacteria and Archaea 534
Bacteria 534
Archaea 534

CHAPTER REVIEW 537

27 Protists 539

27.1 Why Do Biologists Study Protists? 540
Impacts on Human Health and Welfare 540
Ecological Importance of Protists 542

27.2 How Do Biologists Study Protists? 543
Microscopy: Studying Cell Structure 544
Evaluating Molecular Phylogenies 544
Discovering New Lineages via Direct Sequencing 545

27.3 What Themes Occur in the Diversification of Protists? 546
What Morphological Innovations Evolved in Protists? 546
How Do Protists Obtain Food? 551
How Do Protists Move? 552
How Do Protists Reproduce? 552

27.4 Key Lineages of Protists 556
Amoebozoa 556
Excavata 556
Plantae 556
Rhizaria 556
Alveolata 558
Stramenopila (Heterokonta) 558

CHAPTER REVIEW 558

28 Green Algae and Land Plants 561

28.1 Why Do Biologists Study Green Algae and Land Plants? 562
Plants Provide Ecosystem Services 562
Plants Provide Humans with Food, Fuel, Fiber, Building Materials, and Medicines 563

28.2 How Do Biologists Study Green Algae and Land Plants? 564
Analyzing Morphological Traits 564
Using the Fossil Record 565
Evaluating Molecular Phylogenies 566

28.3 What Themes Occur in the Diversification of Land Plants? 568
The Transition to Land, I: How Did Plants Adapt to Dry Conditions with Intense Sunlight? 568
Mapping Evolutionary Changes on the Phylogenetic Tree 571
The Transition to Land, II: How Do Plants Reproduce in Dry Conditions? 571
The Angiosperm Radiation 580

28.4 Key Lineages of Green Algae and Land Plants 581
Green Algae 581

Nonvascular Plants 582
Seedless Vascular Plants 582
Seed Plants: Gymnosperms and Angiosperms 582
CHAPTER REVIEW 588

29 Fungi 590

29.1 **Why Do Biologists Study Fungi?** 591
Fungi Have Important Economic and Ecological Impacts 591
Mycorrhizal Fungi Provide Nutrients for Land Plants 592
Saprophytic Fungi Accelerate the Carbon Cycle on Land 593

29.2 **How Do Biologists Study Fungi?** 593
Analyzing Morphological Traits 594
Evaluating Molecular Phylogenies 596

29.3 **What Themes Occur in the Diversification of Fungi?** 598
Fungi Often Participate in Symbioses 599
What Adaptations Make Fungi Such Effective Decomposers? 602
Variation in Reproduction 603
Four Major Types of Life Cycles 605

29.4 **Key Lineages of Fungi** 608
Microsporidia 608
Chytrids 609
Zygomycetes 609
Glomeromycota 610
Basidiomycota 610
Ascomycota 610
CHAPTER REVIEW 610

30 An Introduction to Animals 613

30.1 **What Is an Animal?** 614

30.2 **What Key Innovations Occurred during the Origin of Animal Phyla?** 615
Origin of Multicellularity 616
Origin of Embryonic Tissue Layers and Muscle 618
Origin of Bilateral Symmetry, Cephalization, and the Nervous System 619
Origin of the Coelom 621
Origin of Protostomes and Deuterostomes 622
Origin of Segmentation 623

30.3 **What Themes Occur in the Diversification of Animals within Phyla?** 623
Sensory Organs 624
Feeding 625
Movement 626
Reproduction 628
Life Cycles 629

30.4 **Key Lineages of Animals: Non-Bilaterian Groups** 630
Porifera (Sponges) 630
Ctenophora (Comb Jellies) 631
Cnidaria (Jellyfish, Corals, Anemones, Hydroids) 631
CHAPTER REVIEW 632

31 Protostome Animals 634

31.1 **What Is a Protostome?** 635
The Water-to-Land Transition 636
Modular Body Plans 637

31.2 **What Is a Lophotrochozoan?** 637
What Is a Flatworm? 640
What Is a Segmented Worm? 641
What Is a Mollusk? 641

31.3 **What Is an Ecdysozoan?** 644
What Is a Roundworm? 645
What Are Tardigrades and Velvet Worms? 645
What Is an Arthropod? 645
Arthropod Diversity 648
Arthropod Metamorphosis 652
CHAPTER REVIEW 653

32 Deuterostome Animals 655

32.1 **What Is an Echinoderm?** 656
The Echinoderm Body Plan 656
Echinoderms Are Important Consumers 657

32.2 **What Is a Chordate?** 659
The Cephalochordates 660
The Urochordates 660
The Vertebrates 661

32.3 **What Is a Vertebrate?** 661

32.4 **What Key Innovations Occurred during the Evolution of Vertebrates?** 662
Urochordates: Outgroup to Vertebrates 662
First Vertebrates: Origin of the Cranium and Vertebrae 664
Gnathostomes: Origin of the Vertebrate Jaw 665
Origin of the Bony Endoskeleton 667
Tetrapods: Origin of the Limb 667
Amniotes: Origin of the Amniotic Egg 668
Mammals: Origin of Lactation and Fur 669
Reptiles: Origin of Scales and Feathers Made of Keratin 670
Parental Care 672
Take-Home Messages 673

32.5 **The Primates and Hominins** 673
The Primates 673
Fossil Humans 675
The Out-of-Africa Hypothesis 678
Have Humans Stopped Evolving? 679
CHAPTER REVIEW 680

33 Viruses 682

33.1 Why Do Biologists Study Viruses? 683
Viruses Shape the Evolution of Organisms 683
Viruses Cause Disease 683
Current Viral Pandemics in Humans: AIDS 683

33.2 How Do Biologists Study Viruses? 685
Analyzing Morphological Traits 686
Analyzing the Genetic Material 686
Analyzing the Phases of Replicative Growth 687
Analyzing How Viruses Coexist with Host Cells 693

33.3 What Themes Occur in the Diversification of Viruses? 694
Where Did Viruses Come From? 694
Emerging Viruses, Emerging Diseases 694

33.4 Key Lineages of Viruses 696

CHAPTER REVIEW 700

BIG PICTURE Diversity of Life 702

UNIT 6 HOW PLANTS WORK 704

34 Plant Form and Function 704

34.1 Plant Form: Themes with Many Variations 705
The Importance of Surface Area/Volume Relationships 706
The Root System 707
The Shoot System 708
The Leaf 710

34.2 Plant Cells and Tissue Systems 713
The Dermal Tissue System 714
The Ground Tissue System 714
The Vascular Tissue System 716

34.3 Primary Growth Extends the Plant Body 718
How Do Apical Meristems Produce the Primary Plant Body? 718
How Is the Primary Root System Organized? 720
How Is the Primary Shoot System Organized? 720

34.4 Secondary Growth Widens Shoots and Roots 721
What Is a Cambium? 721
How Does a Cambium Initiate Secondary Growth? 722
What Do Vascular Cambia Produce? 723
What Do Cork Cambia Produce? 724
The Structure of Tree Trunks 724

CHAPTER REVIEW 725

35 Water and Sugar Transport in Plants 727

35.1 Water Potential and Water Movement 728
What Is Water Potential? 728
What Factors Affect Water Potential? 728
Working with Water Potentials 729
Water Potentials in Soils, Plants, and the Atmosphere 730

35.2 How Does Water Move from Roots to Shoots? 732
Movement of Water and Solutes into the Root 732
Water Movement via Root Pressure 733
Water Movement via Capillary Action 734
The Cohesion-Tension Theory 734

35.3 Plant Features That Reduce Water Loss 737
Limiting Water Loss 737
Obtaining Carbon Dioxide under Water Stress 738

35.4 Translocation of Sugars 738
Tracing Connections between Sources and Sinks 738
The Anatomy of Phloem 740
The Pressure-Flow Hypothesis 740
Phloem Loading 741
Phloem Unloading 744

CHAPTER REVIEW 745

36 Plant Nutrition 747

36.1 Nutritional Requirements of Plants 748
Which Nutrients Are Essential? 748
What Happens When Key Nutrients Are in Short Supply? 750

36.2 Soil: A Dynamic Mixture of Living and Nonliving Components 751
The Importance of Soil Conservation 751
What Factors Affect Nutrient Availability? 752

36.3 Nutrient Uptake 754
Mechanisms of Nutrient Uptake 754
Mechanisms of Ion Exclusion 756

36.4 Nitrogen Fixation 758
The Role of Symbiotic Bacteria 759
How Do Nitrogen-Fixing Bacteria Infect Plant Roots? 759

36.5 Nutritional Adaptations of Plants 760
Parasitic Plants 760
Epiphytic Plants 761
Carnivorous Plants 762

CHAPTER REVIEW 762

37 Plant Sensory Systems, Signals, and Responses 765

37.1 Information Processing in Plants 766

How Do Cells Receive and Process an External Signal? 766
How Do Cells Respond to Cell–Cell Signals? 766

37.2 Blue Light: The Phototropic Response 768
Phototropins as Blue-Light Receptors 768
Auxin as the Phototropic Hormone 769

37.3 Red and Far-Red Light: Germination, Stem Elongation, and Flowering 772
The Red/Far-Red "Switch" 772
Phytochrome Is a Red/Far-Red Receptor 772
Signals That Promote Flowering 773

37.4 Gravity: The Gravitropic Response 775
The Statolith Hypothesis 775
Auxin as the Gravitropic Signal 776

37.5 How Do Plants Respond to Wind and Touch? 777
Changes in Growth Patterns 777
Movement Responses 777

37.6 Youth, Maturity, and Aging: The Growth Responses 778
Auxin and Apical Dominance 778
Cytokinins and Cell Division 779
Gibberellins and ABA: Growth and Dormancy 779
Brassinosteroids and Body Size 782
Ethylene and Senescence 783
An Overview of Plant Growth Regulators 784

37.7 Pathogens and Herbivores: The Defense Responses 786
How Do Plants Sense and Respond to Pathogens? 786
How Do Plants Sense and Respond to Herbivore Attack? 788

CHAPTER REVIEW 790

38 Plant Reproduction and Development 793

38.1 An Introduction to Plant Reproduction 794
Asexual Reproduction 794
Sexual Reproduction and the Plant Life Cycle 795

38.2 Reproductive Structures 796
The General Structure of the Flower 796
How Are Female Gametophytes Produced? 797
How Are Male Gametophytes Produced? 798

38.3 Pollination and Fertilization 799
Pollination 799
Fertilization 802

38.4 Seeds and Fruits 802
The Role of Drying in Seed Maturation 803
Fruit Development and Seed Dispersal 803
Seed Dormancy 805
Seed Germination 806

38.5 Embryogenesis and Vegetative Development 807
Embryogenesis 807
Meristem Formation 808
Which Genes Determine Body Axes in the Plant Embryo? 809
Which Genes Determine Leaf Structure and Shape? 810

38.6 Reproductive Development 810
The Floral Meristem and the Flower 811
The Genetic Control of Flower Structures 811

CHAPTER REVIEW 813

BIG PICTURE Plant and Animal Form and Function 816

UNIT 7 HOW ANIMALS WORK 818

39 Animal Form and Function 818

39.1 Form, Function, and Adaptation 819
The Role of Fitness Trade-Offs 819
Adaptation and Acclimatization 821

39.2 Tissues, Organs, and Systems: How Does Structure Correlate with Function? 821
Structure–Function Relationships at the Molecular and Cellular Levels 822
Tissues Are Groups of Cells That Function as a Unit 822
Organs and Organ Systems 825

39.3 How Does Body Size Affect Animal Physiology? 826
Surface Area/Volume Relationships: Theory 826
Surface Area/Volume Relationships: Data 827
Adaptations That Increase Surface Area 828

39.4 Homeostasis 829
Homeostasis: General Principles 829
The Role of Regulation and Feedback 830

39.5 Thermoregulation: A Closer Look 831
Mechanisms of Heat Exchange 831
Thermoregulatory Strategies 832
Comparing Endothermy and Ectothermy 832
Countercurrent Heat Exchangers 833

CHAPTER REVIEW 834

40 Water and Electrolyte Balance in Animals 836

40.1 Osmoregulation and Excretion 837
What Is Osmotic Stress? 837
Osmotic Stress in Seawater, in Freshwater, and on Land 837
How Do Electrolytes and Water Move across Cell Membranes? 839
Types of Nitrogenous Wastes: Impact on Water Balance 839

40.2 Water and Electrolyte Balance in Marine Fishes 840

Osmoconformation versus Osmoregulation in Marine Fishes 840
How Do Sharks Excrete Salt? 840

40.3 Water and Electrolyte Balance in Freshwater Fishes 841
How Do Freshwater Fishes Osmoregulate? 841

40.4 Water and Electrolyte Balance in Terrestrial Insects 842
How Do Insects Minimize Water Loss from the Body Surface? 843

40.5 Water and Electrolyte Balance in Terrestrial Vertebrates 844
The Structure of the Mammalian Kidney 844
The Function of the Mammalian Kidney: An Overview 845
Filtration: The Renal Corpuscle 846
Reabsorption: The Proximal Tubule 846
Creating an Osmotic Gradient: The Loop of Henle 847
Regulating Water and Electrolyte Balance: The Distal Tubule and Collecting Duct 850
Urine Formation in Nonmammalian Vertebrates 851

CHAPTER REVIEW 852

41 Animal Nutrition 855

41.1 Nutritional Requirements 856

41.2 Capturing Food: The Structure and Function of Mouthparts 858
Mouthparts as Adaptations 858
A Case Study: The Cichlid Throat Jaw 858

41.3 How Are Nutrients Digested and Absorbed? 859
An Introduction to the Digestive Tract 859
An Overview of Digestive Processes 860
The Mouth and Esophagus 861
The Stomach 862
The Small Intestine 864
The Large Intestine 868

41.4 Nutritional Homeostasis—Glucose as a Case Study 869
The Discovery of Insulin 869
Insulin's Role in Homeostasis 870
Diabetes Mellitus Has Two Forms 870
The Type 2 Diabetes Mellitus Epidemic 870

CHAPTER REVIEW 871

42 Gas Exchange and Circulation 874

42.1 The Respiratory and Circulatory Systems 875

42.2 Air and Water as Respiratory Media 875
How Do Oxygen and Carbon Dioxide Behave in Air? 875
How Do Oxygen and Carbon Dioxide Behave in Water? 876

42.3 Organs of Gas Exchange 877
Physical Parameters: The Law of Diffusion 877
How Do Gills Work? 878
How Do Insect Tracheae Work? 879
How Do Vertebrate Lungs Work? 881
Homeostatic Control of Ventilation 883

42.4 How Are Oxygen and Carbon Dioxide Transported in Blood? 884
Structure and Function of Hemoglobin 884
CO_2 Transport and the Buffering of Blood pH 887

42.5 Circulation 888
What Is an Open Circulatory System? 889
What Is a Closed Circulatory System? 889
How Does the Heart Work? 891
Patterns in Blood Pressure and Blood Flow 895

CHAPTER REVIEW 897

43 Animal Nervous Systems 899

43.1 Principles of Electrical Signaling 900
Types of Neurons 900
The Anatomy of a Neuron 901
An Introduction to Membrane Potentials 901
How Is the Resting Potential Maintained? 902
Using Electrodes to Measure Membrane Potentials 903
What Is an Action Potential? 903

43.2 Dissecting the Action Potential 904
Distinct Ion Currents Are Responsible for Depolarization and Repolarization 904
How Do Voltage-Gated Channels Work? 904
How Is the Action Potential Propagated? 905

43.3 The Synapse 908
Synapse Structure and Neurotransmitter Release 908
What Do Neurotransmitters Do? 910
Postsynaptic Potentials 910

43.4 The Vertebrate Nervous System 912
What Does the Peripheral Nervous System Do? 912
Functional Anatomy of the CNS 913
How Do Learning and Memory Work? 917

CHAPTER REVIEW 919

44 Animal Sensory Systems 922

44.1 How Do Sensory Organs Convey Information to the Brain? 923
Sensory Transduction 923
Transmitting Information to the Brain 924

44.2 Mechanoreception: Sensing Pressure Changes 924
How Do Sensory Cells Respond to Sound Waves and Other Forms of Pressure? 924
Hearing: The Mammalian Ear 925

The Lateral Line System in Fishes and Amphibians 928

44.3 Photoreception: Sensing Light 929
The Insect Eye 929
The Vertebrate Eye 930

44.4 Chemoreception: Sensing Chemicals 934
Taste: Detecting Molecules in the Mouth 934
Olfaction: Detecting Molecules in the Air 935

44.5 Other Sensory Systems 937
Thermoreception: Sensing Temperature 937
Electroreception: Sensing Electric Fields 938
Magnetoreception: Sensing Magnetic Fields 939

CHAPTER REVIEW 939

45 Animal Movement 942

45.1 How Do Muscles Contract? 943
Early Muscle Experiments 943
The Sliding-Filament Model 943
How Do Actin and Myosin Interact? 944
How Do Neurons Initiate Contraction? 946

45.2 Muscle Tissues 947
Smooth Muscle 947
Cardiac Muscle 948
Skeletal Muscle 948

45.3 Skeletal Systems 950
Hydrostatic Skeletons 951
Endoskeletons 952
Exoskeletons 953

45.4 Locomotion 954
How Do Biologists Study Locomotion? 954
Size Matters 957

CHAPTER REVIEW 958

46 Chemical Signals in Animals 961

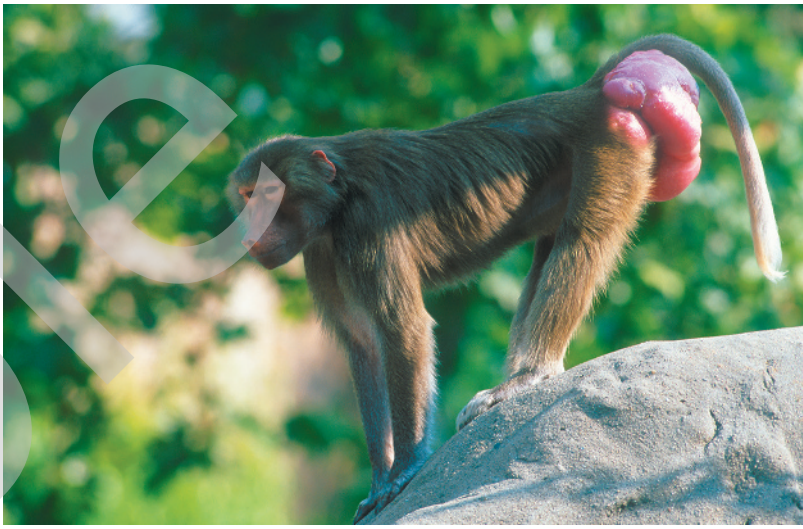
46.1 Cell-to-Cell Signaling: An Overview 962
Major Categories of Chemical Signals 962
Hormone Signaling Pathways 963
What Makes Up the Endocrine System? 964
How Do Researchers Identify a Hormone? 965
A Breakthrough in Measuring Hormone Levels 965

46.2 How Do Hormones Act on Target Cells? 966
Hormone Concentrations Are Low, but Their Effects Are Large 966
Three Chemical Classes of Hormones 967
Steroid Hormones Bind to Intracellular Receptors 967
Polypeptide Hormones Bind to Receptors on the Plasma Membrane 968
Why Do Different Target Cells Respond in Different Ways? 970

46.3 What Do Hormones Do? 971
How Do Hormones Direct Developmental Processes? 971
How Do Hormones Coordinate Responses to Stressors? 973
How Are Hormones Involved in Homeostasis? 975

46.4 How Is the Production of Hormones Regulated? 976
The Hypothalamus and Pituitary Gland 976
Control of Epinephrine by Sympathetic Nerves 978

CHAPTER REVIEW 978



47 Animal Reproduction and Development 981

47.1 Asexual and Sexual Reproduction 982
How Does Asexual Reproduction Occur? 982
Switching Reproductive Modes: A Case History 982
Mechanisms of Sexual Reproduction: Gametogenesis 984

47.2 Reproductive Structures and Their Functions 986
The Male Reproductive System 986
The Female Reproductive System 987

47.3 Fertilization and Egg Development 989
External Fertilization 989
Internal Fertilization 989
The Cell Biology of Fertilization 991
Why Do Some Females Lay Eggs While Others Give Birth? 992

47.4 Embryonic Development 994
Cleavage 994
Gastrulation 995
Organogenesis 996

47.5 The Role of Sex Hormones in Mammalian Reproduction 999
Which Hormones Control Puberty? 999
Which Hormones Control the Menstrual Cycle in Humans? 1000

47.6 Pregnancy and Birth in Mammals 1002
Gestation and Development in Marsupials 1003
Major Events during Human Pregnancy 1003
How Does the Mother Nourish the Fetus? 1004
Birth 1005

CHAPTER REVIEW 1005

48 The Immune System in Animals 1008

48.1 Innate Immunity 1009
Barriers to Entry 1009
The Innate Immune Response 1010

48.2 Adaptive Immunity: Recognition 1013
An Introduction to Lymphocytes 1013

Lymphocytes Recognize a Diverse Array of Antigens 1014
How Does the Immune System Distinguish Self from Nonself? 1017

48.3 Adaptive Immunity: Activation 1018
The Clonal Selection Theory 1018
T-Cell Activation 1019
B-Cell Activation and Antibody Secretion 1020

48.4 Adaptive Immunity: Response and Memory 1022
How Are Extracellular Pathogens Eliminated? 1022
How Are Intracellular Pathogens Eliminated? 1023
Why Does the Immune System Reject Foreign Tissues and Organs? 1024
Responding to Future Infections: Immunological Memory 1024

48.5 What Happens When the Immune System *Doesn't* Work Correctly? 1025
Allergies 1026
Autoimmune Diseases 1026
Immunodeficiency Diseases 1026

CHAPTER REVIEW 1027

UNIT 8 ECOLOGY 1029

49 An Introduction to Ecology 1029

49.1 Levels of Ecological Study 1030
Organismal Ecology 1030
Population Ecology 1031
Community Ecology 1031
Ecosystem Ecology 1031
Global Ecology 1031
Conservation Biology Applies All Levels of Ecological Study 1031

49.2 What Determines the Distribution and Abundance of Organisms? 1031
Abiotic Factors 1032
Biotic Factors 1032
History Matters: Past Abiotic and Biotic Factors Influence Present Patterns 1033
Biotic and Abiotic Factors Interact 1034

49.3 Climate Patterns 1036
Why Are the Tropics Warm and the Poles Cold? 1036

Why Are the Tropics Wet? 1036
What Causes Seasonality in Weather? 1036
What Regional Effects Do Mountains and Oceans Have on Climate? 1038

49.4 Types of Terrestrial Biomes 1039
Natural Biomes 1039
Anthropogenic Biomes 1040
How Will Global Climate Change Affect Terrestrial Biomes? 1042

49.5 Types of Aquatic Biomes 1043
Salinity 1043
Water Depth and Sunlight Availability 1044
Water Flow 1045
Nutrient Availability 1045
How Are Aquatic Biomes Affected by Humans? 1048

CHAPTER REVIEW 1048

50 Behavioral Ecology 1051

50.1 An Introduction to Behavioral Ecology 1052
Proximate and Ultimate Causation 1052
Types of Behavior: An Overview 1053

50.2 Choosing What, How, and When to Eat 1054
Proximate Causes: Foraging Alleles in *Drosophila melanogaster* 1054
Ultimate Causes: Optimal Foraging 1054

50.3 Choosing a Mate 1056
Proximate Causes: How Is Sexual Activity Triggered in *Anolis* Lizards? 1056
Ultimate Causes: Sexual Selection 1057

50.4 Choosing a Place to Live 1058
Proximate Causes: How Do Animals Navigate? 1058
Ultimate Causes: Why Do Animals Migrate? 1060

50.5 Communicating with Others 1061
Proximate Causes: How Do Honeybees Communicate? 1061
Ultimate Causes: Why Do Honeybees Communicate the Way They Do? 1062
When Is Communication Honest or Deceitful? 1063

50.6 Cooperating with Others 1064
Kin Selection 1064
Quantitative Methods 50.1 Calculating the Coefficient of Relatedness 1065
Manipulation 1066
Reciprocal Altruism 1067
Cooperation and Mutualism 1067
Individuals Do Not Act for the Good of the Species 1067

CHAPTER REVIEW 1068

51 Population Ecology 1070

51.1 Distribution and Abundance 1071
Geographic Distribution 1071
Sampling Methods 1072

51.2 Demography 1072
Life Tables 1072
Quantitative Methods 51.1 Mark–Recapture Studies 1073
The Role of Life History 1075
Quantitative Methods 51.2 Using Life Tables to Calculate Population Growth Rates 1075

51.3 Population Growth 1076
Exponential Growth 1077
Quantitative Methods 51.3 Using Growth Models to Predict Population Growth 1078
Logistic Growth 1079
What Factors Limit Population Size? 1080

51.4 Population Dynamics 1081
Why Do Some Populations Cycle? 1081
How Do Metapopulations Change through Time? 1083

51.5 Human Population Growth 1084
Age Structure in Human Populations 1084
Analyzing Change in the Growth Rate of Human Populations 1085

51.6 How Can Population Ecology Help Conserve Biodiversity? 1087
Using Life-Table Data 1087
Preserving Metapopulations 1088

CHAPTER REVIEW 1090

52 Community Ecology 1092

52.1 Species Interactions 1093
Commensalism 1093
Competition 1094
Consumption 1098
Mutualism 1101

52.2 Community Structure 1103
Why Are Some Species More Important than Others in Structuring Communities? 1104
How Predictable Are Communities? 1105

52.3 Community Dynamics 1107
Disturbance and Change in Ecological Communities 1107
Succession: The Development of Communities after Disturbance 1108

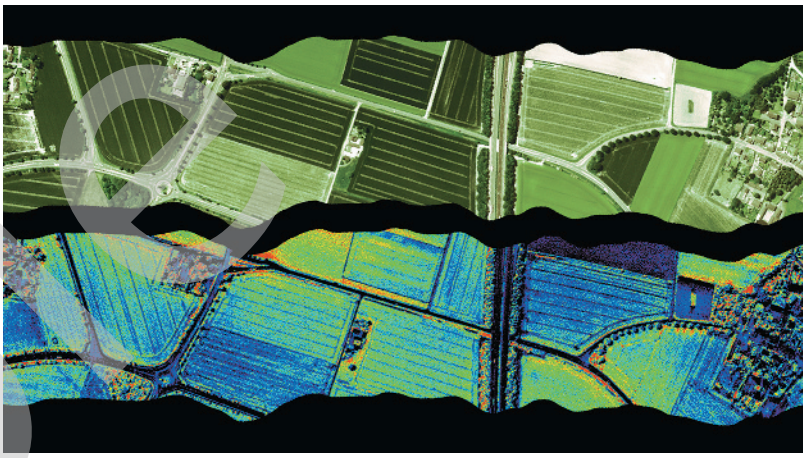
52.4 Patterns in Species Richness 1111
Quantitative Methods 52.1 Measuring Species Diversity 1111
Predicting Species Richness: The Theory of Island Biogeography 1112
Global Patterns in Species Richness 1113

CHAPTER REVIEW 1114

53 Ecosystems and Global Ecology 1116

53.1 How Does Energy Flow through Ecosystems? 1117
How Efficient Are Autotrophs at Capturing Solar Energy? 1117
What Happens to the Biomass of Autotrophs? 1118
Energy Transfer between Trophic Levels 1119
Global Patterns in Productivity 1121

53.2 How Do Nutrients Cycle through Ecosystems? 1123
Nutrient Cycling within Ecosystems 1123
Global Biogeochemical Cycles 1125



53.3 Global Climate Change 1129
What Is the Cause of Global Climate Change? 1130
How Much Will the Climate Change? 1131
Biological Effects of Climate Change 1133
Consequences to Net Primary Productivity 1135

CHAPTER REVIEW 1137

54 Biodiversity and Conservation Biology 1139

54.1 What Is Biodiversity? 1140
Biodiversity Can Be Measured and Analyzed at Several Levels 1140
How Many Species Are Living Today? 1142
Where Is Biodiversity Highest? 1142

54.2 Threats to Biodiversity 1145
Multiple Interacting Threats 1145
How Will These Threats Affect Future Extinction Rates? 1149
Quantitative Methods 54.1 Species–Area Plots 1150

54.3 Why Is Biodiversity Important? 1152
Biological Benefits of Biodiversity 1152
Ecosystem Services: Economic and Social Benefits of Biodiversity and Ecosystems 1154
An Ethical Dimension 1155

54.4 Preserving Biodiversity and Ecosystem Function 1156
Addressing the Ultimate Causes of Loss 1156
Conservation Strategies to Preserve Genetic Diversity, Species, and Ecosystem Function 1157
Take-Home Message 1159

CHAPTER REVIEW 1159

BIG PICTURE Ecology 1162

APPENDIX A Answers A:1
APPENDIX B Periodic Table of Elements B:1
Glossary G:1
Credits Cr:1
Index I:1

About the Authors

A Letter from Scott:

I started working on *Biological Science* in 1997 with a simple goal: To help change the way biology is taught. After just shy of 20,000 hours of work on four editions of this text, that goal still gets me out of bed in the morning. But instead of focusing my energies on textbook writing, I've decided to devote myself full-time to research on student learning and developing new courses for undergraduate and graduate students at the University of Washington.

I have passed the torch to an all-star cast of leading scientists and educators who have enthusiastically taught from, and contributed to, previous editions of *Biological Science*. The new team brings their passion, talent, and creativity to the book, with expertise that spans the breadth of the life sciences. Just as important, they work beautifully together because they think alike. They are driven by a shared concern for student learning, a commitment to the craft of writing, and a background in evidence-based teaching.

These pages provide a brief introduction to Liz Allison, Michael Black, Greg Podgorski, Kim Quillin, Jeff Carmichael, and Emily Taylor. As a group, they've built on the book's existing strengths and infused this edition with fresh energy, perspective, and ideas. I'm full of admiration for what they have accomplished, and excited about the impact this edition will have on biology students from all over the world.

—Scott Freeman

Scott Freeman received a Ph.D. in Zoology from the University of Washington and was subsequently awarded an Alfred P. Sloan Postdoctoral Fellowship in Molecular Evolution at Princeton University. He has done research in evolutionary biology on topics ranging from nest parasitism to the molecular systematics of the blackbird family and is coauthor, with Jon Herron, of the standard-setting undergraduate text *Evolutionary Analysis*. Scott is the recipient of a Distinguished Teaching Award from the University of Washington and is currently a Principal Lecturer in the UW Department of Biology, where he teaches introductory biology for majors, a writing-intensive course for majors called The Tree of Life, and a graduate seminar in college science teaching. Scott's current research focuses on how active learning affects student learning and academic performance.

Lizabeth A. Allison is Chancellor Professor of Biology at the College of William & Mary. She received her Ph.D. in Zoology from the University of Washington, specializing in molecular and cellular biology. Before coming to William & Mary, she spent eight years as a faculty member at the University of Canterbury in New Zealand. Liz teaches introductory biology for majors and upper-division molecular biology courses. She has mentored graduate students and more than 100 undergraduate research students, many of them coauthoring papers with her on intracellular trafficking of the thyroid hormone receptor in normal and cancer cells. The recipient of numerous awards, including a State Council for Higher Education in Virginia (SCHEV) Outstanding Faculty Award in 2009, Liz received one of the three inaugural Arts & Sciences Faculty Awards for Teaching Excellence in 2011, and a Plumeri Award for Faculty Excellence in 2012. In addition to her work on this text, she is author of *Fundamental Molecular Biology*, now in its second edition, with a third edition underway.
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Michael Black received his Ph.D. in Microbiology & Immunology from Stanford University School of Medicine as a Howard Hughes Predoctoral Fellow. After graduation, he studied cell biology as a Burroughs Wellcome Postdoctoral Fellow at the MRC Laboratory of Molecular Biology in Cambridge, England. His current research focuses on the use of molecules to identify and track the transmission of microbes in the environment. Michael is a professor of Cell & Molecular Biology at California Polytechnic State University in San Luis Obispo, where he teaches introductory and advanced classes for majors in cell biology and microbiology. In addition to his teaching and research activities, Michael serves as the director of the Undergraduate Biotechnology Lab, where he works alongside undergraduate technicians to integrate research projects and inquiry-based activities into undergraduate classes.
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Jeff Carmichael received his B.S. in Biology from Slippery Rock University in Pennsylvania and his Ph.D. in Plant Biology from the University of Georgia. As an undergraduate student, he spent some time studying enzyme kinetics through a fellowship at Oak Ridge National Laboratory in Tennessee. His graduate work focused on sexual reproduction in an intriguing group of seed plants. He has been teaching and coordinating Introductory Biology at the University of North Dakota (UND) for more than 20 years. He also serves in the Office of Instructional Development where he helps other faculty members incorporate evidence-based best teaching practices in their courses. He has received excellence in teaching awards at UND and as a graduate student in Georgia. His revision of Unit 6 and part of Unit 5 of the Sixth Edition is his first foray into textbook writing.

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Kim Quillin received her B.A. in Biology at Oberlin College *summa cum laude* and her Ph.D. in Integrative Biology from the University of California, Berkeley as a National Science Foundation Graduate Fellow. Kim has worked in the trenches with Scott Freeman on every edition of *Biological Science*, starting with the ground-up development of the illustrations in the first edition in 1999 and expanding her role in each edition, always with the focus of helping students to think like biologists. Kim currently teaches introductory biology at Salisbury University, a member of the University System of Maryland, where she is actively involved in the ongoing student-centered reform of the concepts-and-methods course for biology majors. Her current research focuses on the scholarship of teaching and learning with an emphasis on visual model-based reasoning as a science process skill.

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Emily Taylor earned a B.A. in English at the University of California, Berkeley followed by a Ph.D. in Biological Sciences from Arizona State University, where she conducted research in the field of environmental physiology as a National Science Foundation Graduate Research Fellow. She is currently an associate professor of Biological Sciences at the California Polytechnic State University in San Luis Obispo. Her student-centered research program focuses on the endocrine and reproductive physiology of free-ranging reptiles, especially rattlesnakes. She teaches numerous undergraduate and graduate courses, including introductory biology, anatomy and physiology, endocrinology, and herpetology, and received the California Faculty Association's Distinguished Educator Award in 2010 and Cal Poly's Distinguished Teaching Award in 2012.

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Preface to Instructors

From the very first edition, *Biological Science's* unique emphasis on the process of scientific discovery and guiding students to think like biologists has placed this book at the forefront of change in the way we teach biology. The Sixth Edition embraces this legacy and continues to exemplify the principles outlined in the recent *Vision and Change in Undergraduate Biology Education* report. As in previous editions, the cutting-edge biology in the Sixth Edition is pitched at the right level for introductory students, and is as accurate and as exciting as ever for instructors and students alike. New findings from education research continue to inform and inspire the coauthor team's thinking about *Biological Science*—we know more today than ever before about how students learn. These findings demand that we constantly look for new ways to increase student engagement in the learning process. Innovative features new to this edition offer students even more opportunities to actively apply concepts in new situations; evaluate experimental design, hypotheses, and data; synthesize results; and make and interpret models. For instructors, additional resources are provided to help align course activities and learning goals with their assessment strategies.

Core Values

In the Sixth Edition, the coauthor team has strived to extend the vision and maintain the core values of *Biological Science*—to provide a book and online resources for instructors who embrace the challenge of boosting students to higher levels of learning, and to provide a book that helps students each step of the way in learning to think like scientists, regardless of their starting point in the process. Dedicated instructors have high expectations of their students. The Sixth Edition provides tools to help students build their cognitive mastery in both biology content and transferrable skills—to learn at the level called for by the National Academy of Sciences, the Howard Hughes Medical Institute, the American Association of Medical Academies, and the National Science Foundation. Reports such as *Biology 2010, Scientific Foundations for Future Physicians*, and *Vision and Change* all place a premium on fundamental concepts and skills as well as connecting core ideas across all levels of biology.

What's New in This Edition

The Sixth Edition contains many new or expanded features, all of them designed to provide students with initial instruction in content and skills, followed by opportunities for lots of practice in applying knowledge and skills to new contexts. The ultimate goal is for students to learn to construct their own knowledge and think like biologists.

- **Relocated and Expanded BioSkills Section** Instructors recognize that biology students need to develop foundational science skills in addition to content knowledge. Since the Third Edition, *Biological Science* has provided a unique, robust set of materials and activities in an appendix to guide students who need extra help with the skills emphasized in the book. In the Sixth Edition, the BioSkills materials have been placed between Chapters 1 and 2 to emphasize their importance as a resource for success in doing biology, and to make it easier for students to access them throughout the course. The BioSkills are grouped within five broad categories depicted in a new opening road map: Quantifying Biology, Using Common Lab Tools, Visualizing Biology, Reading Biology, and Monitoring Your Own Learning. Four new BioSkills have been added: Using Spectrophotometry, Using Molecular Biology Tools and Techniques, Reading and Making Visual Models, and Recognizing and Correcting Misconceptions. Existing BioSkills have been updated to support new features in the Sixth Edition. For example, the explanation of statistical tests has been expanded, and *P* values are introduced to provide students with essential quantitative skills for interpreting data in the end-of-chapter case studies. BioSkills include practice questions, are cross-referenced throughout the text, and can be assigned online in MasteringBiology®.
- **Making Models Boxes** Reports like *Vision and Change* cite the importance of developing model-based reasoning skills. To help attain this goal, Making Models boxes have been added throughout the book to explicitly teach students how to use visual models to learn and do biology. Each Making Models box has three components: instruction in interpreting or creating a specific type of model, an example of that type of model, and an application question so that students can immediately practice their skills. In addition to the guidance in the text, online video versions are accessible via QR code so students can watch and interact with a dynamic presentation of modeling. Lastly, the video version is also included in an assignable MasteringBiology activity that tests students with higher-level questions.
- **Put It All Together Case Studies** The end-of-chapter question sets for every chapter now include a case study. Case studies briefly introduce contemporary biology research in action, followed by questions that ask students to apply the chapter's content and skills to the research topic. Instructor resources include clicker questions to give instructors the opportunity to use the case studies as discussion prompts in the classroom. A constant hallmark of this text is its emphasis on experimental evidence—on teaching how we know what we know. The case studies expand this emphasis, requiring students to evaluate real data and to see how ongoing scientific research is related to core biological ideas.
- **Big Picture on Biological Diversity** Introduced in the Fourth Edition, Big Picture concept maps integrate words and visuals to help students synthesize information about challenging topics

that span multiple chapters and units. In response to requests from instructors and students, a new Big Picture has been added on the Diversity of Life, illustrating the relationships among the major taxonomic groups in the tree of life.

- **Integrated Chapters** Three newly consolidated chapters reorganize and integrate information to better serve instructors and students. Chapter 20 (The Molecular Revolution: Biotechnology and Beyond) merges the most essential information on genome analysis that was previously discussed in separate chapters, while moving details of fundamental techniques to the BioSkills. Core material on the general principles of development, particularly those related to genetics and evolution, now forms the closing chapter of a streamlined unit on Gene Structure and Expression (Chapter 21). Content on plant and animal development has been moved from the former developmental biology unit to the respective reproduction and development chapters of the How Plants Work (Chapter 38) and How Animals Work (Chapter 47) units.
- **Skill-Based Question Tags** *Biological Science* has long emphasized skill development, and reports like *Vision and Change* also encourage this focus for introductory majors. To help students and instructors identify opportunities to practice key skills, questions are tagged to indicate the following: *Process of Science* questions explore the application of the scientific process; *Model* questions ask students to interpret or construct visual models; *Society* questions explore the relationship between science and society; *Quantitative* questions help students perform quantitative analysis and mathematical reasoning; and *Caution* questions address topics for which students often hold common misconceptions. Answers to *Caution* questions include information that addresses the misconception.
- **Detailed Assessment Matrix** At the beginning of the revision process, we thoroughly evaluated the assessment program and focused on revising it throughout the creation of the Sixth Edition. To aid our analysis, we looked at the question data collected in MasteringBiology, and we created an assessment matrix for each chapter that identifies how each question is related to learning outcomes, Bloom's level, common misconceptions, and *Vision and Change* core concepts and competencies. We hope the tool will assist instructors in selecting the most appropriate assessment items to align with the goals of their course.
- **Expanded Use of Summary Tables** The art program is further enhanced in this edition by additional illustrated summary tables that deliver content in a streamlined way and facilitate comparison and analysis by students. For example, the diversity boxes from the Fifth Edition's The Diversification of Life unit have been redesigned as photographic summary tables. These tables make subject areas more accessible to visual learners and reinforce a chapter's key concepts.

Hallmark Features of the Text

We are excited to introduce new features to the Sixth Edition. At the same time, we are committed to strengthening the hallmark features that make this book unique.

- **Road Maps** Starting with the Fifth Edition, each chapter opens with a concept map that visually groups and organizes information to help students anticipate key ideas as well as recognize meaningful relationships and connections among ideas. While the Road Maps help students look forward as they engage with a chapter, **Big Picture** concept maps integrate words and visuals to help students synthesize information about challenging topics that span multiple chapters or units. Together, these two features help students navigate chapter content and see the forest for the trees.
- **Opportunities for Practice** "Blue Thread" questions, integrated throughout the text, are designed to help students identify what they do and do not understand. The idea is that if students really understand a piece of information or a concept, they should be able to do something with it. As in the Fifth Edition, all questions in the text are assigned a Bloom's taxonomy level to help both students and instructors understand whether a question requires higher-order or lower-order cognitive skills.
  - **In-text "You Should Be Able To" questions** focus on topics and concepts that professors and students have identified as most key or difficult in each chapter.
  - **Caption questions and exercises** challenge students to examine the information in a figure or table critically—not just absorb it.
  - **Check Your Understanding boxes** present two to three tasks that students should be able to complete in order to demonstrate a mastery of summarized key ideas.
  - **End-of-chapter questions** are organized in three levels of increasing difficulty so students can build from lower- to higher-order cognitive levels of assessment.
- **Focus on Real Data** Students now have expanded opportunities to develop skills at working with real data from the primary literature. Sources of the data presented in Research Boxes, graphs, and end-of-chapter Case Studies are cited to model good practice for students and to provide a resource for students and instructors who wish to evaluate the original data more deeply.

Integration of Media

The textbook continues to be supported by MasteringBiology, the most powerful online homework, tutorial, and assessment system available. Tutorials follow the Socratic method, coaching students to the correct answer by offering feedback specific to a student's errors or misconceptions as well as supplying hints that students can access if they get stuck. Instructors can associate content with publisher-provided learning outcomes or create their own. Content highlights include the following:

- **Making Models Activities** Whiteboard videos—accessible online via QR code or the Study Area in MasteringBiology, bring the Making Models feature from the book to life to help students develop their visual modeling skills. The videos are also included in assignable activities that allow students to practice modeling and to apply their understanding to new situations.

- **Case Study Questions** Put It All Together Case Study questions are assignable in MasteringBiology. Additional clicker questions are also provided in instructor resources to facilitate classroom activities.
- **Solve It Tutorials** These activities allow students to act like scientists in simulated investigations. Each tutorial presents an interesting, real-world question that students will answer by analyzing and interpreting data.
- **Experimental Inquiry Tutorials** The call to teach students about the process of science has never been louder. To support such teaching, there are 10 interactive tutorials on classic scientific experiments—ranging from Meselson–Stahl on DNA replication to the Grants' work on Galápagos finches and Connell's work on competition. Students who use these tutorials should be better prepared to think critically about experimental design and evaluate the wider implications of the data—preparing them to do the work of real scientists in the future.
- **BioFlix® Animations and Tutorials** BioFlix are movie-quality, 3-D animations that focus on the most difficult core topics and are accompanied by in-depth, online tutorials that provide hints and feedback to guide student learning. Eighteen BioFlix animations and tutorials tackle topics such as meiosis, mitosis, DNA replication, photosynthesis, homeostasis, and the carbon cycle.
- **HHMI Short Films Activities** Documentary-quality movies from HHMI are available in MasteringBiology with assignable questions to make sure students understand key ideas.
- **Galápagos Evolution Video Activities** These incredible videos, filmed on the Galápagos Islands by Peter and Rosemary Grant, bring to life the dynamic evolutionary processes that have an impact on Darwin's finches on Daphne Major Island. Six videos explore important concepts and data from the Grants' field research, and assignable activities keep students focused on the important take-away points.
- **End-of-Chapter Questions** A broad range of end-of-chapter questions are available to assign in MasteringBiology.
- **Blue Thread Questions** Over 500 questions based on the Blue Thread questions in the textbook are assignable in MasteringBiology.
- **Big Picture Concept Map Tutorials** A new, more engaging concept mapping tool is the basis for highly interactive, challenging concept map activities based on the Big Picture figures in the textbook. Students build their own concept maps, which are auto-graded, and then answer questions to make sure they understand key ideas and make important connections.
- **BioSkills Activities** Activities based on the BioSkills content in the textbook are assignable in MasteringBiology, including activities to support the new BioSkills.
- **Reading Quiz Questions** Every chapter includes reading quiz questions that can be assigned to ensure students read the textbook and understand the basics. These quizzes are perfect as a pre-lecture assignment to get students into the content before class, allowing instructors to use class time more effectively.

Serving a Community of Teachers

All of us on the coauthor team are motivated by a deep commitment to students and to supporting the efforts of dedicated teachers. Our passion in life is doing and teaching biology. At various points along our diverse paths, we have been inspired by our own teachers when we were students, and now are inspired by our colleagues as we strive to become even better teacher-scholars. In the tradition of all previous editions of *Biological Science*, we have tried to infuse this textbook with the spirit and practice of evidence-based teaching. We welcome your comments, suggestions, and questions.

Many thanks for all you do for your students.

Content Highlights of the Sixth Edition

As discussed in the preface, a major focus of this revision is to introduce unique, innovative features designed to provide students with initial instruction in content and skills, as well as lots of practice in applying knowledge and skills to new contexts—with the ultimate goal of helping students learn to construct their own knowledge and think like biologists. As in each edition, to ensure that the content reflects the current state of science and is accurate, the author team has scrutinized every chapter to add new, relevant content, update descriptions when appropriate, and adjust the approach to certain topics to enhance student comprehension. New content emphasizes overarching themes—including how advances in understanding gene expression and genome structure inform all of biology, from development to evolution to physiology to ecology, and the profound impact of global climate change on life on Earth. In this section, some of the key content improvements to the textbook are highlighted.

Chapter 1 Biology and the Tree of Life New section titles emphasize the theme of five characteristics of life, within a framework of three unifying theories: the cell theory, the theory of evolution, and new coverage of the chromosome theory of inheritance. A brief introduction to the central dogma of molecular biology is added to provide students with a framework for understanding the connections between genes and phenotype early on in the book.

Chapter 2 Water and Carbon: The Chemical Basis of Life A more thorough explanation of chemical energy is included, covering the role of electronegativity, bond strength, and position of shared electrons with respect to the atomic nuclei. An expanded discussion addresses how molecular shape influences polarity and how changes in entropy are responsible for hydrophobic interactions between nonpolar molecules in a polar solvent.

Chapter 3 Protein Structure and Function The presentation of how electron sharing gives peptide bonds characteristics similar to double bonds is improved. Updated art more clearly illustrates how protein folding forms a substrate-specific active site in an enzyme. The introduction of prions is revised to describe how changes in protein structure may lead to cell death.

Chapter 4 Nucleic Acids and the RNA World The description of ATP hydrolysis is revised to avoid the common misconception that breaking phosphate bonds releases energy. The art and text are updated to present the geometry of nitrogenous bases relative to the sugar–phosphate backbone in double-stranded DNA. The role of hydrophobic interactions in shaping and stabilizing the DNA double helix is explained.

Chapter 5 An Introduction to Carbohydrates The impact of carbohydrate structure is emphasized by comparing the cleavage of maltose and lactose and exploring the basis of lactose intolerance that occurs in adults. The glycolipids and glycoproteins that serve as the ABO blood group antigens are introduced.

Chapter 6 Lipids, Membranes, and the First Cells Illustrations of fats and phospholipids are revised to emphasize similarity in structure. The description of osmosis is updated to include the effect of pressure on water transport and the concentration of solutes across a membrane at equilibrium.

Chapter 7 Inside the Cell Updated content highlights the differences in cell structure in eukaryotes, bacteria, and archaea. A revised description of receptor-mediated endocytosis, phagocytosis, and autophagy includes a new figure that illustrates how these pathways are involved in recycling components via lysosomes.

Chapter 8 Energy and Enzymes: An Introduction to Metabolism The introduction to potential and kinetic energy is expanded. The description of chemical energy is revised to focus on chemical bonds, support changes in Chapter 2, and address a common misconception that individual electrons carry energy. Illustrations of chemical bonds are updated to more accurately represent the correlation between bond length and chemical energy. The role of energetic coupling in converting endergonic reactions into exergonic reactions is clarified.

Chapter 9 Cellular Respiration and Fermentation Figures and text are updated to track the number of intermediates and products in each of the metabolic pathways. Redox potential is introduced as a measure of the ability of molecules to be reduced in redox reactions. The description of the fermentation pathways is expanded.

Chapter 10 Photosynthesis Greater emphasis is placed on the events responsible for converting the kinetic energy in light to potential energy stored in chemical bonds. Content is revised to address the misconceptions that the products of photosynthesis are used only to manufacture carbohydrates and that chloroplasts supply the ATP necessary for all other cellular functions. Figures and text are updated to more easily track the inputs and outputs in the photosynthetic reactions.

Chapter 11 Cell–Cell Interactions New content is added to the discussion of lipid-soluble signaling molecules and how second messengers in a signal transduction pathway can lead to many diverse cellular responses. A new quantitative question that addresses signal amplification is added. The discussion of the yeast pheromone response is expanded to draw connections between cell signaling and remodeling of the cell wall.

Chapter 12 The Cell Cycle Figures are updated to clearly distinguish differences between replicated and unreplicated chromosomes. New questions are added that address the application of a pulse–chase assay and common misconceptions associated with chromosome number during mitosis. New content is added covering the role of microtubules in chromosome movement and cell-cycle checkpoints.

Chapter 13 Meiosis Increased attention is paid to topics students are known to struggle with, such as the distinction between sister chromatids and homologous chromosomes, and the number of chromosomes and DNA molecules present in each daughter cell at the end of meiosis I compared with the end of meiosis II. The How Do Mistakes Occur? section is streamlined to focus on general themes of how aneuploidy arises during meiosis.

Chapter 14 Mendel and the Gene There is a sharper focus on challenging concepts, including the relationship between genotype and phenotype, the ability to consider phenotypes at levels that range from the molecular to the organismal, the meaning of dominance relationships, the significance of genetic mapping, and the importance of the chromosome theory of inheritance.

Chapter 15 DNA and the Gene: Synthesis and Repair Coverage is expanded on the Okazaki experiment and on the Nobel Prize–winning experiments of Greider and colleagues on telomeres and telomerase, so that students can more easily understand these investigations and their significance.

Chapter 16 How Genes Work Greater emphasis is placed on illustrating how the central dogma links genotype to phenotype. A stronger point is made that mutations can occur anywhere in the genome, not just in protein-coding sequences.

Chapter 17 Transcription, RNA Processing, and Translation New content helps students better understand polarity relationships among DNA, mRNA, and polypeptides. Three existing figures and one table are modified to improve clarity.

Chapter 18 Control of Gene Expression in Bacteria The discussion of the mechanism for glucose-mediated control of the *lac* operon is revised to highlight the continuing debate over the way catabolite repression works. The chapter is streamlined to allow students to focus on the fundamentals of how gene regulatory molecules control gene expression.

Chapter 19 Control of Gene Expression in Eukaryotes The material on control of translation is updated and reorganized, including a new example of global regulation of translation by mTor. Discussion of RNA interference is expanded, including a significantly modified figure showing how microRNAs are processed and how they function, and new discussion of how RNA interference can control chromatin condensation. The discussion of transcription initiation and the accompanying figure are updated.

Chapter 20 The Molecular Revolution: Biotechnology and Beyond Material previously spread across two chapters is merged to provide a more focused overview of major aims and techniques of genomics and related fields, including recent innovations such as CRISPR-Cas9 genome editing. Specific details of fundamental techniques are relocated to the BioSkills section for students and instructors who desire this level of coverage.

Chapter 21 Genes, Development, and Evolution Essential concepts previously spread across several chapters are brought together in this chapter, and it now links the Gene Structure and Expression unit to the Evolutionary Patterns and Processes unit by using molecular and cellular aspects of developmental biology as a bridge. New material on determination, induced pluripotent stem cells (iPS cells), and de-differentiation in cancer cells is included.

Chapter 22 Evolution by Natural Selection The historical introduction is simplified and illustrated in a new figure that compares different conceptual models of life's diversity. The homology section is updated to include developmental processes, and the three levels of homology are highlighted in a new summary table. More practice is provided in applying Darwin's postulates and reading phylogenetic trees. There is increased focus on overcoming common evolutionary misconceptions throughout the chapter. More plant examples are included. Focus on the ecological context of evolution is also increased.

Chapter 23 Evolutionary Processes The introduction to the Hardy–Weinberg principle is simplified and updated with some new examples. Increased attention is given to students' struggle to distinguish gene flow and genetic drift, and there are new follow-up questions. The summary table on evolutionary processes now includes icons to help students distinguish evolutionary processes, effect on genetic variation, and effect on fitness.

Chapter 24 Speciation New examples emphasize the origin of biodiversity, variation in rate of speciation, and biogeography, and illustrate the role of sexual selection and genetic mechanisms in speciation. Icons are now included in three summary tables to help students visualize mechanisms of reproductive isolation, species concepts, and outcomes of secondary contact between populations.

Chapter 25 Phylogenies and the History of Life The terms "microevolution" and "macroevolution" are now defined in the introduction. The phylogenetics section is updated to include more diverse examples. There is increased emphasis on avoiding common misconceptions in interpreting and drawing trees. The fossil review is reorganized into a photographic summary table. Dates in the history of life time line are updated. New evidence regarding causes of the end-Cretaceous extinction is introduced.

Chapter 26 Bacteria and Archaea New content is included on the role of endospores in the prokaryote life cycle, and recent studies on the human microbiome are highlighted. The section on themes in diversification is expanded to include mechanisms of gene transfer (e.g., transformation, transduction, and conjugation). Recent ideas that call into question the traditional three-domain tree of life hypothesis are presented.

Chapter 27 Protists Discussion of the role of endosymbiosis in the origin of mitochondria and chloroplasts is streamlined to focus on key concepts. The coverage of euglenids now includes a description of the flexible pellicle of this group, to underscore the point that most protist lineages are characterized by distinct microscopic features. Coverage of slime molds is expanded to include more on the structure and movement of plasmodial slime molds. Greater attention is paid to guiding students step-by-step through complex protist life cycles.

Chapter 28 Green Algae and Land Plants The updated discussion of the origin of plants now recognizes the conjugating algae (Zygnematophyceae) as one of the closest living relatives to land plants. Alternation of generations—the fundamental life cycle of all land plants—is now emphasized and presented with greater clarity.

Chapter 29 Fungi Content is updated to emphasize the important role of asexual spores (conidia) in the reproductive cycle of fungi. The unique relationship between a fungus and ants resulting in "zombie ants" is highlighted to illustrate the diversity of fungal lifestyles.

Chapter 30 An Introduction to Animals The chapter is updated to include insights gleaned from new genetic and developmental data, emphasizing that evolution is not a straightforward march from simple to complex.

Chapter 31 Protostome Animals The revised introduction is organized as a walk-through of a phylogeny to provide context from the previous chapter. Characteristics traditionally used to distinguish protostome development are deemphasized in light of recent research showing many exceptions. A new figure shows the phylogeny of arthropods, including insects within the Crustacea.

Chapter 32 Deuterostome Animals The echinoderm section has an increased emphasis on ecology and process of science, including Paine's keystone predator study. The invertebrate chordate section is expanded to include ascidians, thalaceans, and larvaceans. The key innovations section is revised and streamlined as a walk-through of the chordate phylogeny. The human evolution section is updated, including reference to new hominin species and an image of a Neanderthal woman.

Chapter 33 Viruses A new section on the role of viruses in shaping the evolution of organisms is introduced. A discussion of the SARS-CoV and MERS-CoV outbreaks is included to illustrate the international network of researchers that works to identify and control emerging viral infections. New content on how viruses impact society is included, along with new material covering recent discoveries on how the Ebola virus infects cells.

Chapter 34 Plant Form and Function The chapter is reorganized to discuss the structure and function of cells and tissues before placing them in the context of primary and secondary growth. Practice is provided on calculating and comparing the relationship between surface area and volume in different types of plant structures. Content on secondary growth is expanded to emphasize how trees make the transition from primary to secondary growth.

Chapter 35 Water and Sugar Transport in Plants The discussion of water potential and water movement is streamlined to bring key concepts into sharper focus. Recent work on the role of the SWEET genes in sugar transport is introduced.

Chapter 36 Plant Nutrition Discussion of parasitic plants is broadened and now includes dodder and ghost plants as examples.

Chapter 37 Plant Sensory Systems, Signals, and Responses The discussion of phototropins is streamlined to focus on key concepts. The role of phytochrome in circadian rhythms and etiolation is introduced. A summary table on key plant growth regulators is now illustrated with photographs to show the impact of hormones on plant growth and development.

Chapter 38 Plant Reproduction and Development The chapter is reorganized to merge essential information previously spread across several chapters and bring flowering plant reproduction and development together in a single, integrated story. Discussions of flower structure, pollination, fertilization, the formation of seeds and fruits, and embryogenesis are updated and streamlined. Coverage of vegetative development emphasizes the roles of apical meristems and genes involved in embryogenesis and leaf formation.

Chapter 39 Animal Form and Function The discussion of mammalian thermoregulation is moved into the section on homeostasis. In the introduction to animal tissue types, more explicit structure–function examples are given for each tissue type. The section on regulatory homeostasis is updated, and the idea that regulation and conformation are two ends of a spectrum is introduced. The expressions "warm-blooded" and "cold-blooded" are addressed to explain why these terms are problematic to use in biology. The section on countercurrent multipliers is simplified.

Chapter 40 Water and Electrolyte Balance in Animals The material on reabsorption in insect Malpighian tubules is streamlined. There is a discussion of how the vasa recta absorbs water and ions without disrupting the interstitial fluid gradient. A brief statement about how aldosterone functions in pH regulation of body fluids is added.

Chapter 41 Animal Nutrition The section on diabetes is expanded, and the importance of low cell glucose in addition to high blood glucose in untreated diabetes is stressed. A new figure addresses the relationship between obesity and type 2 diabetes.

Chapter 42 Gas Exchange and Circulation Oxygen–hemoglobin dissociation figures are redrawn more accurately, and new content helps students understand the meaning of a sigmoidal curve. The open circulatory system common to most invertebrates is illustrated with a new figure showing circulation in a spider.

Chapter 43 Animal Nervous Systems A new figure shows the relationships among sensory neurons, motor neurons, and interneurons. Review of material from earlier chapters on how ions are transported across membranes is streamlined. The discussion of the magnitude of action potentials and how action potentials propagate down an axon is clarified. Revisions emphasize that new action potentials are continuously generated along the entire length of an axon, addressing the misconception that a single action potential travels from one end to the other. Updated information is included on the hippocampus, the enteric nervous system, and the technique of optogenetics, a major breakthrough in neuroscience.

Chapter 44 Animal Sensory Systems The section on taste is updated to reflect new knowledge about the structure and function of gustation, and the likely existence of more than just five taste sensations. The role of mechanoreception in taste—by providing information about texture—is introduced. New content highlights one of the chapter's key ideas: Animals do not rely on senses independently and instead integrate information from multiple sensory modalities.

Chapter 45 Animal Movement A new figure shows examples of hydrostatic skeletons, endoskeletons, and exoskeletons. A brief section is added addressing the misconception that muscles grow

by adding new cells during weight-lifting/training (in fact, the cells simply grow). A new section discusses the role of bone in calcium storage and the process of bone remodeling. Osteoblasts and osteoclasts are introduced, and osteoporosis is discussed briefly.

Chapter 46 Chemical Signals in Animals Content is rearranged to flow more logically: first introducing cell signaling, next discussing how hormones stimulate cells, then giving examples of what hormones can do, and finally describing how hormones are regulated overall. Discussion of the discovery of hormones is updated for historical accuracy and includes a new research box on Berthold's classic experiment on roosters, which shows that a chemical blood-borne messenger (later characterized as testosterone) can affect behavior and anatomy. Control of blood-glucose levels by insulin and glucagon is now used to illustrate how hormones maintain homeostasis.

Chapter 47 Animal Reproduction and Development Material previously spread across several chapters is merged to bring reproduction and development together to tell a single, integrated story. Coverage of fertilization is now integrated with egg development; coverage of cleavage, gastrulation, and organogenesis is combined into a new, descriptive section on embryonic development. New content covers formation of the central nervous system from the neural tube. The chapter now focuses more on the physiology of reproduction in mammals, but retains a comparative approach by including examples ranging from insects to marsupials.

Chapter 48 The Immune System in Animals Content is updated on the activation of B cells and allergens that are involved in mast-cell activation in allergic reactions. Coverage of the link between high levels of hygiene and the rising occurrence of allergies and autoimmune diseases in Westernized countries is expanded.

Chapter 49 An Introduction to Ecology The introduction is revised to clarify the relationship between traditional ecology and the study of human impacts. The niche concept is introduced as a tool to relate organisms to environmental conditions. The theory of plate tectonics and a figure showing continental drift are added to the section on biogeography. The Coriolis effect, prevailing winds, ocean gyres, and El Niño are added to the climate section. Information from the Fifth Edition's biome boxes is integrated into the text and included in new photographic summary tables on terrestrial and aquatic biomes.

Chapter 50 Behavioral Ecology The introduction includes increased emphasis on fitness trade-offs and variation among organisms in a population (population thinking). Section case studies are updated, including a new opportunity for students to practice with optimal foraging in bees, a new data graphic on sexual selection in *Anolis* lizards, and a new photo of monkeys engaged in reciprocal grooming. A new section addresses the misconception that individuals act for the good of the species.

Chapter 51 Population Ecology The mark–recapture Quantitative Methods box is expanded. The figure and discussion of the life-history continuum are expanded. The exponential growth section is revised for a clearer walk-through of the equations and more direct assistance with common misconceptions. A new photographic summary table of density-dependent factors is added. Human population content is updated. Applications to conservation are expanded.

Chapter 52 Community Ecology More plant examples are included. The case studies on species interactions are updated and clarified. The community structure section now begins with a discussion of how pairwise interactions combine to form webs of interactions, introducing the food web as an example. A discussion of bottom-up and top-down influences on community structure is now included.

Chapter 53 Ecosystems and Global Ecology Updates and clarifications are made throughout the chapter, particularly in the section on climate change, including updated data graphics. Nutrient-cycle figures are modified to distinguish natural and human-caused processes. A section on phosphorus cycling is added. The concept of tipping points is added, and the interaction of multiple variables is emphasized.

Chapter 54 Biodiversity and Conservation Biology Updates and clarifications are made throughout the chapter. A new figure contrasts resistance and resilience. A new data graphic emphasizes the resource intensity of beef. Overall, more emphasis is placed on the positive effects of conservation action, including a new full-page photographic summary table of conservation strategies.

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The peer review system is the key to quality and clarity in science publishing. In addition to providing a filter, the investment that respected individuals make in vetting the material—catching errors or inconsistencies and making suggestions to improve the presentation—gives authors, editors, and readers confidence that the text meets rigorous professional standards.

Peer review plays the same role in textbook publishing. The time and care that this book's reviewers have invested is a tribute to their professional integrity, their scholarship, and their concern for the quality of teaching. Virtually every page in this edition has been revised and improved based on insights from the following individuals.

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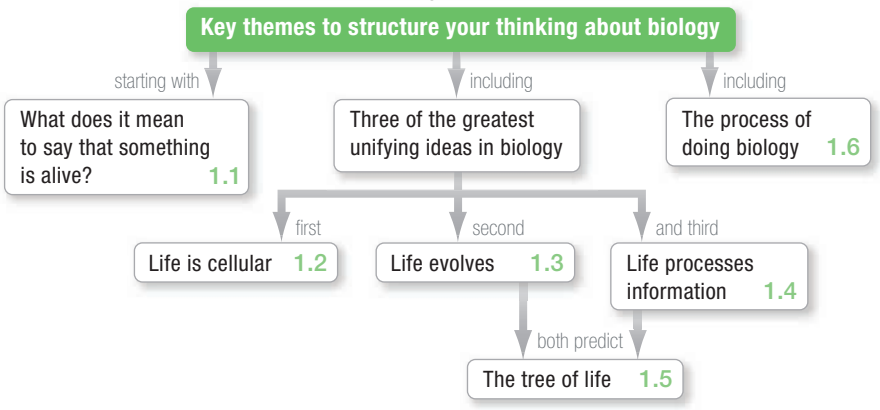
1 Biology and the Tree of Life

This vervet monkey baby is exploring its new world and learning how to find food and stay alive. It represents one of the key characteristics of life introduced in this chapter—replication.

In this chapter you will learn about



In essence, biological science is the study of life. It searches for ideas and observations that unify our understanding of the diversity of life—from bacteria living in hot springs to humans and majestic sequoia trees.

The goals of this chapter are to introduce the nature of life and explore how biologists go about studying it. The chapter also introduces themes that will resonate throughout this book:

- Analyzing how organisms work at the molecular level.
- Understanding organisms in terms of their evolutionary history.
- Helping you learn to think like a biologist.

Let's begin with what may be the most fundamental question of all: What is life?

BIG PICTURE

This chapter is part of the Big Picture. See how on pages 16–17.

1.1 What Does It Mean to Say That Something Is Alive?

An **organism** is a life-form—a living entity made up of one or more cells. Although there is no simple definition of life that is endorsed by all biologists, most agree that organisms share a suite of five fundamental characteristics. You can think of this text as one long exploration of these five traits.

- ***Cells*** Organisms are made up of membrane-bound units called **cells.** The membrane of a cell regulates the passage of materials between exterior and interior spaces.
- ***Replication*** One of the great biologists of the twentieth century, François Jacob, said that the "dream of a bacterium is to become two bacteria." Almost everything an organism does contributes to one goal: replicating itself.
- ***Evolution*** Organisms are the products of evolution, and their populations continue to evolve today.
- ***Information*** Organisms process hereditary, or genetic, information encoded in units called **genes.** Organisms also respond to information from the environment and adjust to maintain stable internal conditions. Right now, cells throughout your body are using information to make the molecules that keep you alive; your eyes and brain are decoding information on this page that will help you learn some biology, and if your room is too hot you might be sweating to cool off.
- ***Energy*** To stay alive and reproduce, organisms have to acquire and use energy. To give just two examples: plants absorb sunlight; animals ingest food.

Three of the greatest unifying ideas in all of science, which depend on the five characteristics just listed, laid the groundwork for modern biology: the cell theory, the theory of evolution, and the chromosome theory of inheritance. Formally, scientists define a **theory** as an explanation for a very general class of phenomena or observations that are supported by a wide body of evidence. Note that this definition contrasts sharply with the everyday usage of the word "theory," which often carries meanings such as "speculation" or "guess."

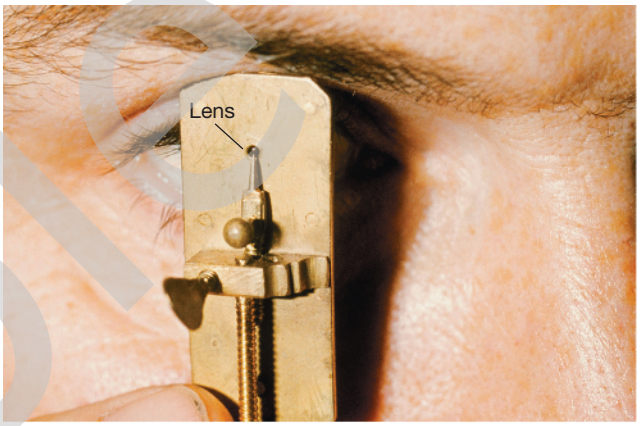
The cell theory, the theory of evolution, and the chromosome theory of inheritance address fundamental questions: What are organisms made of? Where do they come from? How is hereditary information transmitted from one generation to the next?

When these theories emerged in the mid-1800s, they revolutionized the way biologists think about the world. None of these insights came easily, however. The cell theory, for example, emerged after some 200 years of work. Let's examine some of the pivotal discoveries made along the way.

1.2 Life Is Cellular

In 1665 the Englishman Robert Hooke devised a crude microscope to examine the structure of cork (a bark tissue) from an oak tree. The instrument magnified objects to just 30× (30 times) their normal size, but it allowed Hooke to see something extraordinary. In the cork he observed small, pore-like compartments that were invisible to the naked eye. Hooke coined the term "cells" for these structures because he thought they resembled the cells inhabited by monks in a monastery.

(a) van Leeuwenhoek built his own microscopes—which, while small, were powerful. They allowed him to see, for example ...



(b) ... human blood cells (this modern photo was shot through one of van Leeuwenhoek's original microscopes).

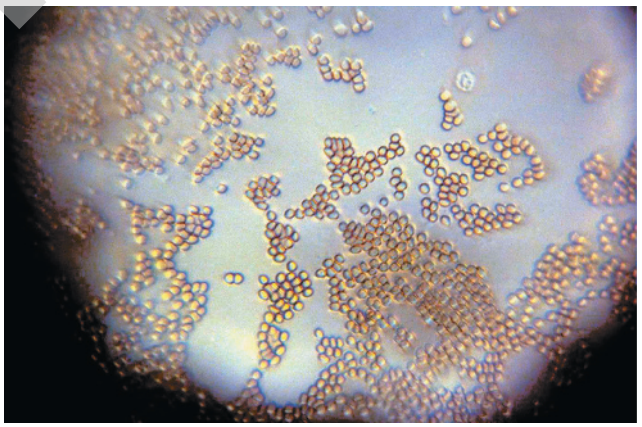


Figure 1.1 Van Leeuwenhoek's Microscope Made Cells Visible.

Soon after Hooke published his results, the Dutch scientist Anton van Leeuwenhoek developed much more powerful microscopes, some capable of magnifications up to 300× (**Figure 1.1**). With these instruments, van Leeuwenhoek inspected samples of pond water and made the first observations of a dazzling collection of single-celled organisms that he called "animalcules."

In the 1670s an Italian researcher who was studying the leaves and stems of plants with a microscope concluded that plant tissues were composed of many individual cells. By the early 1800s, enough data had accumulated for a German biologist to claim that *all* organisms consist of cells. Did this claim hold up?

All Organisms Are Made of Cells

Advances in microscopy have made it possible to examine the amazing diversity and complexity of cells at higher and higher magnifications. Microscopes tens of thousands of times more powerful than van Leeuwenhoek's have revealed that cells are

highly organized compartments separated from their environment by a membrane barrier. With these instruments, biologists have described over a million new species. The basic conclusion made in the 1800s remains intact: All organisms are made of cells.

The smallest organisms known today are bacteria that are barely 200 nanometers wide, or 200 *billionths* of a meter. (See BioSkills 1 to review the metric system.[1]) It would take 5000 of these organisms lined up side by side to span a millimeter. This is the distance between the smallest hash marks on a metric ruler. In contrast, sequoia trees can be over 100 meters tall, the equivalent of a 20-story building. Bacteria and sequoias are composed of the same fundamental building block, however—the cell. Bacteria consist of a single cell; sequoias are made up of trillions of cells.

The realization that all organisms are made of cells was fundamentally important, but it formed only the first part of the cell theory. In addition to understanding what organisms are made of, scientists wanted to understand how cells come to be.

Where Do Cells Come From?

In 1858, a German scientist named Rudolph Virchow proposed that all cells arise from cells already in existence. The complete **cell theory** builds on this concept: All organisms are made of cells, and all cells come from preexisting cells.

Two Hypotheses

The cell theory was a direct challenge to the prevailing explanation of where cells come from, called spontaneous generation. In the mid-1800s, most biologists believed that organisms could arise spontaneously under certain conditions. The bacteria and fungi that spoil foods such as milk and wine were thought to appear in these nutrient-rich media of their own accord—springing to life from nonliving materials. In contrast, the cell theory maintained that cells do not arise spontaneously but are produced only when preexisting cells grow and divide. The all-cells-from-cells explanation was a **hypothesis:** a testable statement to explain a phenomenon or a set of observations.

Biologists usually use the word "theory" to refer to proposed explanations for broad patterns in nature and prefer hypothesis to refer to explanations for more tightly focused questions. A theory serves as a framework for developing new hypotheses.

An Experiment to Settle the Question

Soon after Virchow's all-cells-from-cells hypothesis appeared in print, a French scientist named Louis Pasteur set out to test its predictions in an **experiment.** Experiments are a powerful scientific tool because they allow researchers to test the effect of a single, well-defined factor on a particular phenomenon. An experimental **prediction** describes a measurable or observable result that must be correct if a hypothesis is valid.

Pasteur wanted to determine whether organisms could arise spontaneously in a nutrient broth or whether they appear only when a broth is exposed to a source of preexisting cells. To address the question, he created two treatment groups that were identical in every respect but one: the factor being tested—in this case, a broth's exposure to preexisting cells.

Both treatments used glass flasks filled with the same amount of the same nutrient broth (Figure 1.2). Both flasks were boiled for the same amount of time to kill any existing organisms. After sterilization by boiling, however, any bacteria and fungi that cling to dust particles in the air could drop into the broth in the flask shown in Figure 1.2a because the neck of this flask was straight.

[1] BioSkills are located after Chapter 1. They focus on general skills that you'll use throughout this course. More than a few students have found them to be a lifesaver. Please use them!

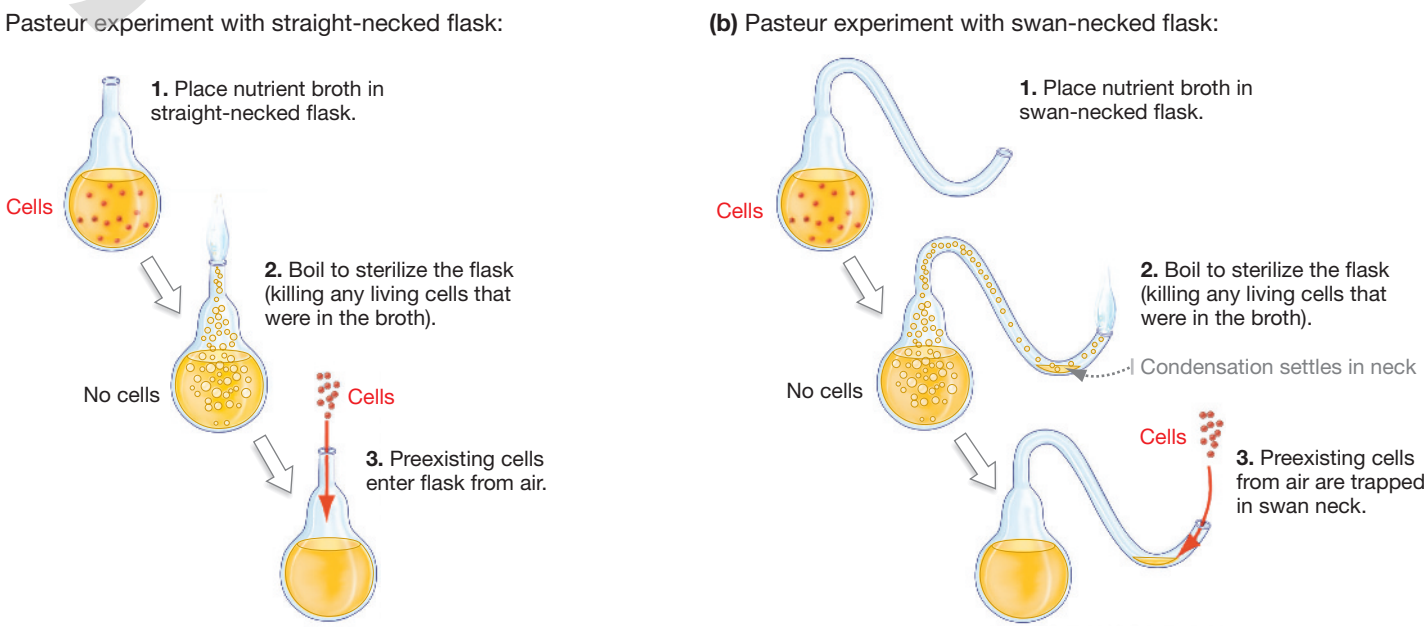


Figure 1.2 The Spontaneous Generation and All-Cells-from-Cells Hypotheses Were Tested Experimentally.

✓ **PROCESS OF SCIENCE** What problem would arise in interpreting these results if Pasteur had (1) put different types of broth in the two treatments, or (2) used a ceramic flask for one treatment and a glass flask for the other?

In contrast, in the flask with a long swan neck (Figure 1.2b), water would condense in the crook of the swan neck after boiling and this pool of water would trap any bacteria or fungi that entered on dust particles. Thus, the contents of the swan-necked flask were isolated from any source of preexisting cells even though they were still open to the air.

The spontaneous generation hypothesis predicted that cells would appear in both treatment groups. The all-cells-from-cells hypothesis predicted that cells would appear only in the treatment exposed to a source of preexisting cells.

And Pasteur's results? The broth in the straight-necked flask exposed to preexisting cells quickly filled with bacteria and fungi. This observation was important because it showed that the sterilization step had not altered the nutrient broth's capacity to support growth. The broth in the swan-necked flask remained sterile, however. Even when the flask was left standing for months, no organisms appeared in it. This result was inconsistent with the hypothesis of spontaneous generation.

Because Pasteur's data were so conclusive—meaning that there was no other reasonable explanation for them—the results persuaded most biologists that the all-cells-from-cells hypothesis was correct.

If all cells come from existing cells, where did the first cells come from? Biologists now have evidence that life arose from nonlife early in Earth's history, through a process called **chemical evolution.**

Life Replicates Through Cell Division

For life on Earth to continue to exist, cells must replicate. Most cells are capable of reproducing by dividing—in effect, by making a copy of themselves. As predicted by the cell theory, all the cells present in your body and in most other multicellular individuals are descended from preexisting cells, tracing back to a fertilized egg. A fertilized egg is a cell created by the fusion of sperm and egg—cells that formed in individuals of the previous generation.

New cells arise when preexisting cells split. In multicellular organisms they become specialized for particular functions by intricate processes. In this way, all the cells in a multicellular organism are connected by a common lineage. Is the tremendous diversity among organisms also related to common ancestry?

The second great founding idea in biology, published the same year as the all-cells-from-cells hypothesis, provided an answer. This was the realization, made independently by the English scientists Charles Darwin and Alfred Russel Wallace, that all the diverse **species**—all distinct, identifiable types of organisms—are connected by common ancestry.

1.3 Life Evolves

In 1858 short papers written separately by Darwin and Wallace were read to a small group of scientists attending a meeting of the Linnean Society of London. A year later, Darwin published a book that expanded on the idea summarized in those brief papers. The book was called *On the Origin of Species*. The first edition sold out in a day.

Figure 1.3 Sketch from Darwin's Notebook Dated 1837. Darwin wrote this in the notes that follow: "Thus genera would be formed. Bearing relation to ancient types with several extinct forms."

What Is Evolution?

Darwin and Wallace's theory made two important claims concerning patterns that exist in the natural world.

1. Species are related by common ancestry (**Figure 1.3**). This idea contrasted with the prevailing view in science at the time, which was that species represent independent entities created separately by a divine being.
2. The characteristics of species can be modified from generation to generation. Darwin called this process descent with modification. This claim argued against the popular view at the time that species do not change.

Evolution is a change in the characteristics of a population over time. A **population** is defined as a group of individuals of the same species living in the same area at the same time. To put it another way, species are related to one another and can change through time.

What Is Natural Selection?

Several other scientists had already come to the same conclusions as Darwin and Wallace about the relationships between species. The great insight by Darwin and Wallace was in proposing a process, called **natural selection,** that explains *how* evolution occurs.

Two Conditions of Natural Selection

Natural selection occurs whenever two conditions are met.

1. Individuals within a population vary in characteristics that are **heritable**—meaning, traits that can be passed on to offspring.

2. In a particular environment, certain versions of these heritable traits help individuals survive better or reproduce more than do other versions.

If certain heritable traits lead to increased success in producing offspring, then those traits become more common in the population over time. In this way, the population's characteristics change as a result of natural selection acting on individuals. This is a key insight: Natural selection acts on individuals, but evolutionary change occurs in populations.

Evolution occurs when heritable variation leads to differential success in reproduction. Individual populations change through time in response to natural selection. But over the past several decades, biologists have also documented dozens of cases in which natural selection has caused populations of one species to diverge and form new species. This divergence process is called **speciation.**

Research on speciation has two important implications: All species come from preexisting species, and all species, past and present, trace their ancestry back to a single common ancestor.

Fitness and Adaptation Darwin also introduced some new terminology to identify what happens during natural selection.

- In everyday English, "fitness" means "health and well-being." But in biology, **fitness** means "an individual's ability to produce viable offspring." Individuals with high fitness produce many surviving offspring.
- In everyday English, "adaptation" means that an individual is adjusting and changing to function in new circumstances. But in biology, an **adaptation** is a trait that increases the fitness of an individual in a particular environment.

Darwin and Wallace's ideas arose from their observations of nature. For example, in finches from the Galápagos Islands Darwin noted the remarkable variation in beak size and shape in species that otherwise appeared similar. He proposed that the birds on different islands in the chain were similar because they descended from a common ancestor—the finch populations that colonized different islands had changed through time and formed new species with distinct beaks.

Long-term studies by biologists over the past several decades have documented dramatic changes in a population of finches on one of the Galápagos Islands (you will learn more about this study in Chapter 22). When small, soft seeds were abundant there due to increased rainfall, finches with small, pointed beaks produced more offspring and had higher fitness than individuals with large, deep beaks. In this population and with this food source, a small, pointed beak was an adaptation that allowed certain individuals to thrive, and the incidence of finches with such beaks increased in the population.

Note that during this process, the beak shape of any individual finch did not change within its lifetime—the change occurred in the characteristics of the population over time. Darwin's finches continue to evolve today in response to changes in the environment.

Together, the cell theory and the theory of evolution provided the young science of biology with two central, unifying ideas:

1. The cell is the fundamental structural unit in all organisms.
2. All species are related by common ancestry and have changed over time in response to natural selection.

But what was the source of the heritable variation in traits? And how was information stored and transmitted from one generation to the next? The third unifying idea—the chromosome theory of inheritance—provided the foundation for biologists to answer these questions.

CHECK YOUR UNDERSTANDING

If you understand that …

- Natural selection occurs when heritable variation in certain traits leads to improved success in reproduction. Because individuals with these traits produce many offspring with the same traits, the traits increase in frequency and evolution occurs.
- Evolution is a change in the characteristics of a population over time.

✔ You should be able to …

Discuss the following statement: "Various species of Galápagos finches are adapted to their particular habitats."

Answers are available in Appendix A.

1.4 Life Processes Information

After Walter Sutton and Theodor Boveri proposed the **chromosome theory of inheritance** in 1902, the pieces of the genetic puzzle began to fall into place. The key point? Inside cells, hereditary or genetic information is encoded in genes, the units located on chromosomes.

But it wasn't until experiments were carried out in the 1950s that biologists figured out the molecular nature of the genetic material—a **chromosome** consists of a molecule of **deoxyribonucleic acid,** or **DNA.** To sum up, DNA is the heredity material. Genes consist of specific segments of DNA that code for products in the cell.

The Central Dogma

In what is considered one of the greatest scientific breakthroughs of biology, James Watson and Francis Crick proposed that DNA is a double-stranded helix (**Figure 1.4**). Crucial insights that led to this model came from structural analyses performed by Rosalind Franklin in Maurice Wilkins' laboratory.

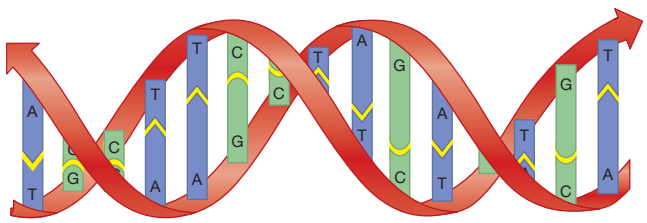


Figure 1.4 DNA Is a Double Helix.

Each strand of the **double helix** is made up of varying sequences of four different kinds of building blocks. In terms of structure, on each strand of the helix the building blocks of DNA are connected one to another linearly. In terms of function, they are like letters of the alphabet—the four different kinds of molecular building blocks are symbolized by the letters A, T, C, and G. A sequence of this letter code is like the sequence of letters in a word—it has meaning. In this way, DNA carries, or encodes, the information required for an organism's growth and reproduction.

The two strands of the double helix are joined by connections between the building blocks that occur only between certain letters: A always pairs with T, and C always pairs with G (see Figure 1.4). This pairing is key: DNA can be copied, and the information encoded in the DNA is faithfully preserved. The pairs are arranged much like the rungs on a ladder, with the strands acting as the sides of the ladder.

How is this information transmitted? The **central dogma**—first articulated by Crick—describes the flow of information in cells. In this context, the term "dogma" means a framework for understanding. Put simply, DNA codes for RNA, which codes for proteins (Figure 1.5).

Molecular machinery in cells makes a copy of a particular gene's information in the form of a closely related molecule called **ribonucleic acid,** or **RNA.** RNA molecules carry out a number of specialized functions in cells. For example, molecular machinery reads a messenger RNA molecule to determine what building blocks to use to make a **protein.** Proteins are crucial to most tasks required for a cell to exist, from forming structural components to promoting the chemical reactions that sustain life.

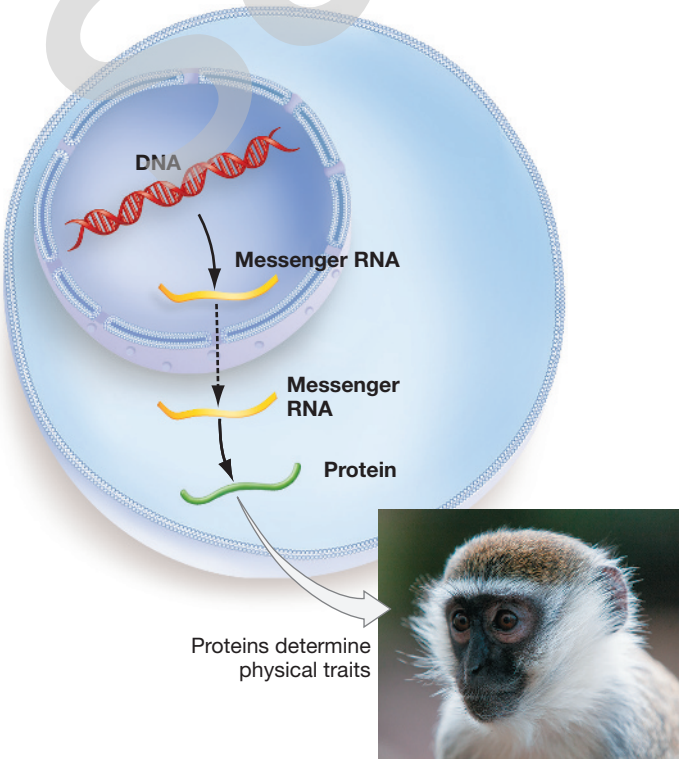


Figure 1.5 The Central Dogma Describes the Flow of Genetic Information. Genetic information flows from DNA to RNA to proteins. Differences in DNA sequences may lead to different physical traits.

Understanding the structure of DNA provided insight into how genetic information is passed from cell to cell or from one organism to its offspring. Making a copy of DNA in a cell is a highly accurate process, but mistakes can occur. What happens when a mistake is made? Differences in DNA sequences may lead to differences in the sequence of building blocks of proteins.

The implications are profound: The outward appearance of an organism is a product of the proteins produced by its molecular machinery, so differences in DNA sequences might lead to a difference, for example, in finch beak size and shape, or in the length of a giraffe's neck. At the level of individuals, such changes might increase or decrease fitness. At the population level, changes in sequence lead to the heritable variations that underlie the diversity of life and make evolution possible.

Life Requires Energy

The chemical reactions that sustain the diversity of life take place inside cells. Transmitting genetic information, and the other work carried out by cells, requires energy. Organisms—whether single-celled or multicellular—are capable of living in a wide array of environments because they vary in cell structure and how they acquire and use energy.

Organisms have two fundamental nutritional needs—acquiring chemical energy in the form of a molecule called **ATP** (or **adenosine triphosphate**) and obtaining molecules that can be used as building blocks for the synthesis of DNA, RNA, proteins, the cell membrane, and other large, complex compounds required by the cell. How organisms do this—whether acquiring energy from the sun or through chemical compounds—is central to the tremendous diversification of life after it first arose on Earth.

1.5 The Tree of Life

The theory of evolution by natural selection predicts that biologists should be able to construct a **tree of life**—a family tree of organisms. If life on Earth arose just once, then such a diagram would describe the genealogical relationships between species with a single, ancestral species at its base. Has this task been accomplished? If the tree of life exists, what does it look like?

Using Molecules to Understand the Tree of Life

One of the great breakthroughs in research on the tree of life occurred when American biologist Carl Woese (pronounced *woze*) and colleagues began analyzing the molecular components of organisms as a way to understand their evolutionary relationships. Their goal was to understand the **phylogeny** of all organisms—their actual genealogical relationships. Translated literally, "phylogeny" means "tribe-source."

To understand which organisms are closely versus distantly related, Woese and co-workers needed to study a molecule found in all organisms. They selected an RNA molecule, an essential

part of the machinery that all cells use to grow and reproduce. The researchers based their initial work on the sequence of building blocks observed in this RNA molecule. At the time it was not yet possible to easily analyze DNA sequences. With advances in technology, biologists now use DNA sequences to investigate phylogenetic relationships.

Analyzing Genetic Variation Why might DNA (or RNA) be useful for understanding the relationships between organisms? The answer is that the sequence of building blocks in DNA is a trait that can change during the course of evolution. Although a gene may code for an RNA or protein molecule that performs the same function in all organisms, the corresponding DNA sequence is not identical among species.

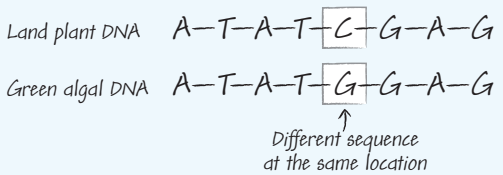
How is such genetic variation analyzed? Recall that the building blocks in DNA are symbolized by the letters A, T, C, and G. Biologists use this letter code to depict DNA sequences (**Making Models 1.1**). In land plants, for example, a section of DNA might start with the sequence A-T-A-T-**C**-G-A-G. In green algae, which are closely related to land plants, the same section of the molecule might contain A-T-A-T-**G**-G-A-G. But in brown algae, which are not closely related to green algae or to land plants, the same part of the molecule might consist of A-**A**-A-T-**G**-G-A-**C**.

The next step in analyzing genetic variation is to consider what the similarities and differences in the sequences imply about relationships between species. The goal is to produce a diagram that describes the phylogeny of the organisms being compared.

A diagram that depicts evolutionary history in this way is called a **phylogenetic tree.** (For help in learning how to read a phylogenetic tree, see **BioSkills 13**.) Just as a family tree shows relationships between individuals, a phylogenetic tree shows relationships between species. On a phylogenetic tree, branches that share a recent common ancestor—that is, an ancestral population—represent species that are closely related; branches that don't share recent common ancestors represent species that are more distantly related.

Making Models 1.1 Tips on Drawing DNA Sequences

In models focused on the information content in DNA, structural details can be left out and the double-stranded DNA double helix simplified to show the letter code on one strand only. Sequences can then be compared for similarities and differences.



MODEL Suppose that in the same section of DNA, molds and other fungi have the sequence A-T-A-T-G-G-A-C. Draw a model that compares the sequences. Do fungi appear to be more closely related to green algae or to land plants? Explain your logic.

To see this model in action, go to https://goo.gl/rXkXrM

The Tree of Life Estimated from Genetic Data To construct a phylogenetic tree, such as the one shown in **Figure 1.6**, researchers use sophisticated computer programs to find the arrangement of branches that is most consistent with the similarities and differences observed in the genetic data.

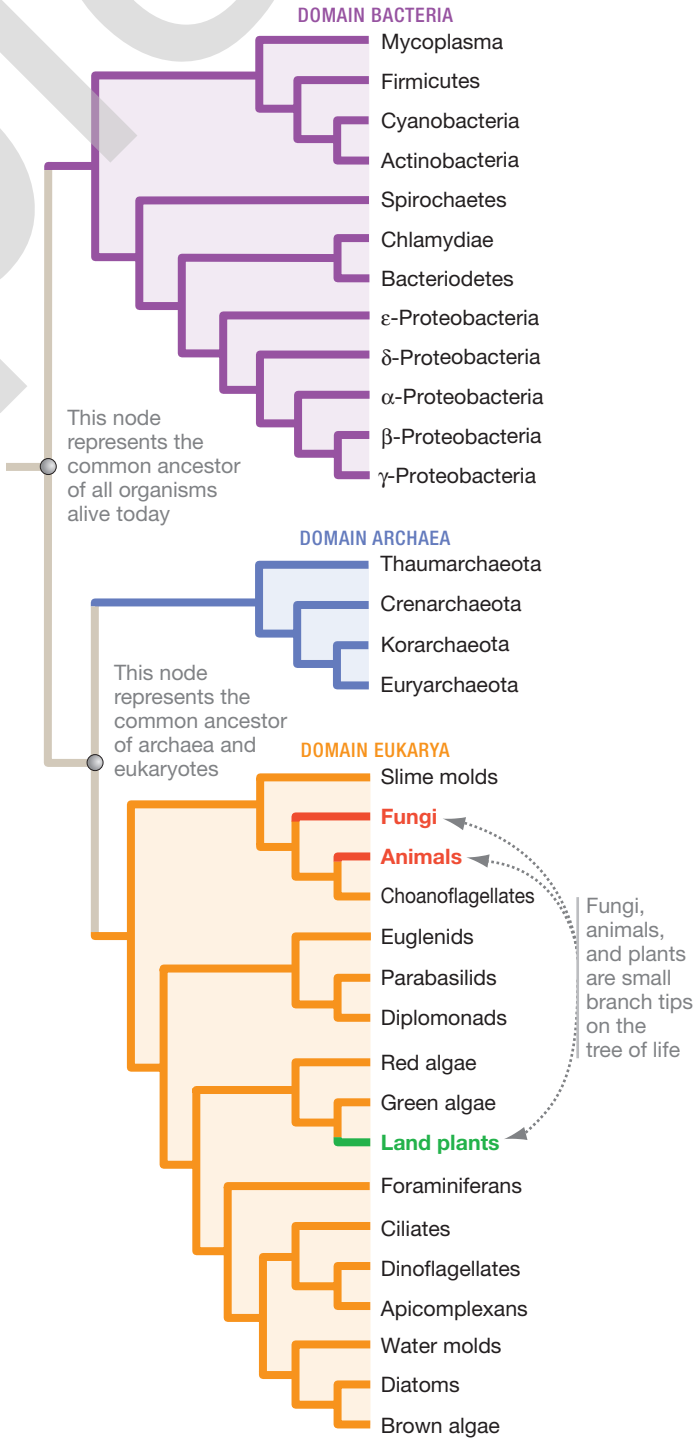


Figure 1.6 The Tree of Life Was Produced by Comparing Genetic Sequence Data. The three domains of life revealed by the analysis are labeled. Common names are given for lineages in the domains Bacteria and Eukarya. Phyla names are given for lineages in the domain Archaea, because most of them have no common names.

Because this tree includes such a diverse array of species, it is often called the universal tree, or the tree of life. Notice that the tree's main node is the common ancestor (ancestral population) of all living organisms. Researchers who study the origin of life propose that the tree's root extends even further back to the "*l*ast *u*niversal *c*ommon *a*ncestor" of cells, or **LUCA.**

The tree of life implied by genetic sequence data established that there are three fundamental groups or lineages of organisms: **(1)** the Bacteria, **(2)** the Archaea, and **(3)** the Eukarya. In all **eukaryotes** (literally, "true kernel"), cells have a prominent component called the nucleus (**Figure 1.7a**). Because the vast majority of bacterial and archaeal cells lack a nucleus, they are referred to as **prokaryotes** (literally, "before-kernel"; see **Figure 1.7b**). The vast majority of bacteria and archaea are unicellular ("one-celled"); many eukaryotes are multicellular ("many-celled").

When results based on genetic data were first published, biologists were astonished. For example:

- Prior to Woese's work and follow-up studies, biologists thought that the most fundamental division among organisms was between prokaryotes and eukaryotes. The Archaea were virtually unknown—much less recognized as a major and highly distinctive branch on the tree of life.
- Fungi were thought to be closely related to plants. Instead, they are actually much more closely related to animals.

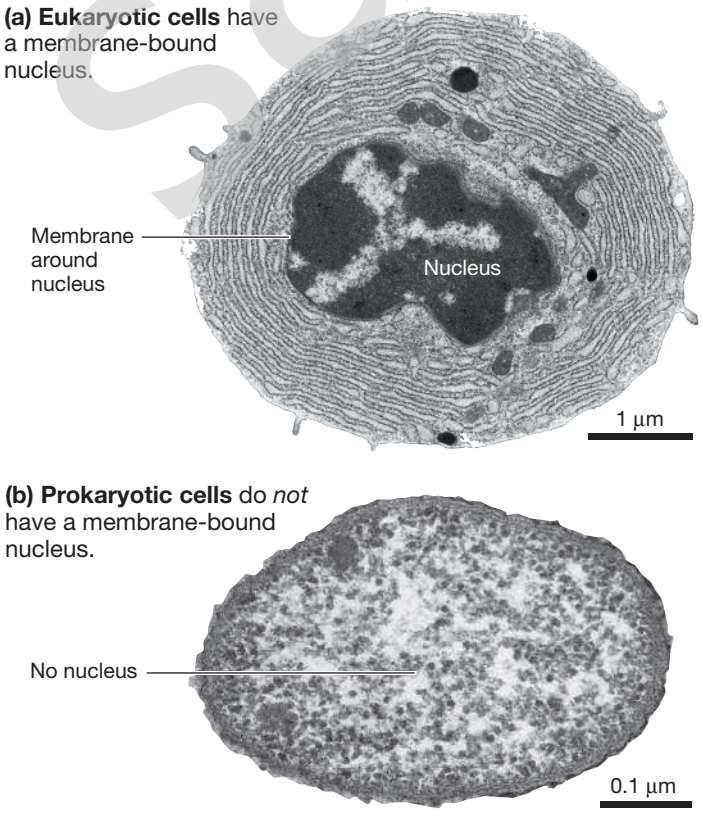


Figure 1.7 Eukaryotic and Prokaryotic Cells Differ in Structure.

✓ **QUANTITATIVE** How many times larger is the eukaryotic cell in this figure than the prokaryotic cell? (Hint: Study the scale bars.)

- Traditional approaches for classifying organisms—including the system of five kingdoms divided into various classes, orders, and families that you may have learned in high school—are inaccurate in many cases, because they do not reflect the actual evolutionary history of the organisms involved.

The Tree of Life Is a Work in Progress Just as researching your family tree can help you understand who you are and where you came from, so the tree of life helps biologists understand the relationships between organisms and the history of species. The discovery of the Archaea and the accurate placement of lineages such as the fungi qualify as exciting breakthroughs in our understanding of evolutionary history and life's diversity.

Work on the tree of life continues at a furious pace, however, and the location of certain branches on the tree is hotly debated. As databases expand and as techniques for analyzing data improve, the shape of the tree of life will undoubtedly change. Our understanding of the tree of life, like our understanding of every other topic in biological science, is dynamic.

How Should We Name Branches on the Tree of Life?

In science, the effort to name and classify organisms is called **taxonomy.** Any named group is called a **taxon** (plural: **taxa**). Currently, biologists are working to create a taxonomy, or naming system, that accurately reflects the phylogeny of organisms. Based on the tree of life, Woese proposed a new taxonomic category called the **domain.** He designated the Bacteria, Archaea, and Eukarya as the three domains of life.

Biologists often use the term **phylum** (plural: **phyla**) to refer to major lineages within each domain. Although the designation is somewhat arbitrary, each phylum is considered a major branch on the tree of life. Within the lineage called animals, biologists currently name 30–35 phyla—each of which is distinguished by distinctive aspects of its body structure as well as by distinctive gene sequences. For example, the mollusks (clams, squid, octopuses) constitute a phylum, as do chordates (the vertebrates and their close relatives).

Because the tree of life is so new, though, naming systems are still being worked out. For example, recent genetic data have fueled an ongoing debate about whether there are only two domains of life: Bacteria as one domain, and the rest of life the other. One thing that hasn't changed for centuries, however, is the naming system for individual species.

Scientific (Latin) Names In 1735, a Swedish botanist named Carolus Linnaeus established a system for naming species that is still in use today. Linnaeus created a two-part name unique to each type of organism.

- ***Genus*** The first part indicates the organism's **genus** (plural: **genera**). A genus is made up of a closely related group of species. For example, Linnaeus put humans in the genus *Homo*. Although humans are the only living species in this genus, at least six extinct organisms, all of which walked upright and made extensive use of tools, were later also assigned to *Homo*.

- ***Species*** The second term in the two-part name identifies the organism's species. Linnaeus gave humans the species name *sapiens*. A species name is always preceded by its genus.

An organism's genus and species designation is called its **scientific name** or Latin name. Scientific names are always italicized. Genus names are always capitalized, but species names are not—as in *Homo sapiens*.

Linnaeus maintained that different types of organisms should not be given the same genus and species names. Other species may be assigned to the genus *Homo* (from the Latin for "man"), and members of other genera may be named *sapiens* (from the Latin for "wise" or "knowing"), but only humans are named *Homo sapiens*. Each scientific name is unique.

Scientific Names Are Often Descriptive Scientific names and terms are often based on Latin or Greek word roots that are descriptive. For example, consider the yeast *Saccharomyces cerevisiae*. *Saccharomyces* is aptly named—the domesticated strains of yeast used in commercial baking and brewing are often fed sugar (Greek root *saccharo*), and yeast is a fungus (Greek root *myces*). The species name of this organism, *cerevisiae*, is Latin for "beer." Loosely translated, then, the scientific name of brewer's yeast means "sugar-fungus for beer."

Scientific names and terms often seem daunting at first glance. So, most biologists find it extremely helpful to memorize some of the common Latin and Greek roots. To aid you in this process, new terms in this text are often accompanied by a translation of their Latin or Greek word roots in parentheses. (A glossary of common root words with translations and examples is also provided in BioSkills 15.)

CHECK YOUR UNDERSTANDING

If you understand that …

- A phylogenetic tree shows the evolutionary relationships between species.
- To infer where species belong on a phylogenetic tree, biologists examine their genetic and other characteristics. Closely related species should have similar characteristics, while less closely related species should be less similar.

✔ You should be able to …

Examine the following DNA sequences and determine which two species would be closest on a phylogenetic tree.

Species A: A A C T A G C G C G A T
Species B: A A C T A G C G C C A T
Species C: T T C T A G C G G T A T

Answers are available in Appendix A.

1.6 Doing Biology

This chapter has introduced some of the great ideas in biology. The development of the cell theory, the theory of evolution, and the chromosome theory of inheritance provided cornerstones when the science was young. The central dogma explained the flow of information from DNA to physical traits of an organism, and the more recent insights of the tree of life have revolutionized our understanding of life's diversity.

These three unifying ideas are considered great because they explain fundamental aspects of nature, and because they have consistently been shown to be correct. They are considered correct because they have withstood extensive testing.

How do biologists go about testing their ideas? Before answering this question, let's step back a bit and consider the types of questions that researchers can and cannot ask.

The Nature of Science

Biologists ask questions about organisms, just as physicists and chemists ask questions about the physical world or geologists ask questions about Earth's history and the processes that shape landforms. No matter what their field, all scientists ask questions that can be answered by observing or measuring things—by collecting data. Conversely, scientists cannot address questions that can't be answered by observing or measuring things.

This distinction is important. It is at the root of continuing controversies about teaching evolution in publicly funded schools. In the United States and in Turkey, in particular, some Christian and Islamic leaders have been particularly successful in pushing their claim that evolution and religious faith are in conflict. Even though the theory of evolution is considered one of the most successful and best-substantiated ideas in the history of science, they object to teaching it.

The vast majority of biologists and many religious leaders reject this claim; they see no conflict between evolution and religious faith. Their view is that science and religion are compatible because they address different types of questions.

- Science is about formulating hypotheses and finding evidence that supports or conflicts with those hypotheses.
- Religious faith addresses questions that cannot be answered by data. The questions addressed by the world's great religions focus on why we exist and how we should live.

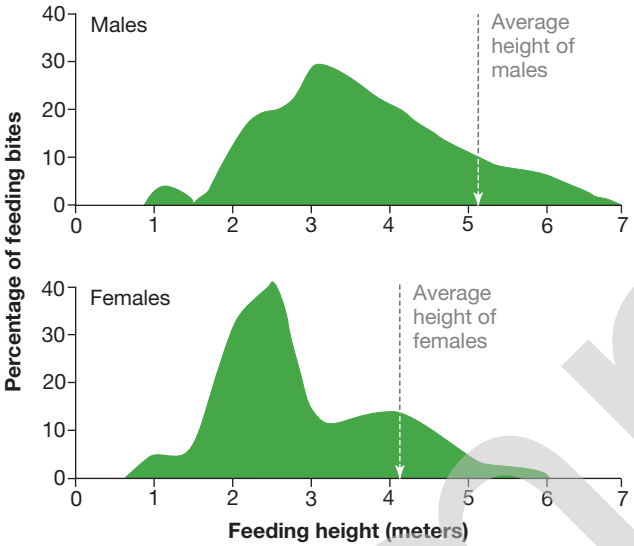
So how do biologists go about answering questions? After formulating hypotheses, biologists perform studies that yield experimental data or descriptive data, such as observing a behavior, characterizing a structure within a cell by microscopy, or sequencing DNA. Let's consider two examples of this process.

Why Do Giraffes Have Long Necks? An Introduction to Hypothesis Testing

If you were asked why giraffes have long necks, you might say based on your observations that long necks enable giraffes to reach food that is unavailable to other mammals. This hypothesis is expressed in African folktales and has traditionally been accepted by many biologists. The food competition hypothesis is so plausible, in fact, that for decades no one thought to test it.

In the mid-1990s, however, Robert Simmons and Lue Scheepers assembled data suggesting that the food competition hypothesis is only part of the story. Their analysis supports an alternative hypothesis: Long necks allow giraffes to use their heads as effective weapons for battering their opponents, and longer-necked giraffes have a competitive advantage in fights.

(a) Most feeding is done at about shoulder height.



(b) Typical feeding posture in giraffes

Figure 1.8 Giraffes Do Not Usually Extend Their Necks Upward to Feed.
DATA: Young, T. P., and L. A. Isbell. 1991. *Ethology* 87: 79–89.

✔ **QUANTITATIVE** At what height of vegetation do male and female giraffes spend most of their time feeding?

Before exploring these alternative explanations, it's important to recognize that hypothesis testing is a two-step process:

Step 1 State the hypothesis as precisely as possible and list the predictions it makes.

Step 2 Design an observational or experimental study that is capable of testing those predictions.

If the predictions are accurate, the hypothesis is supported. If the predictions are not met, then researchers do further tests, modify the original hypothesis, or search for alternative explanations. But the process does not end here. Biologists communicate their results to the scientific community and beyond; for example, via informal conversations, scientific meetings, or publications. (You can see the Big Picture of Doing Biology on pages 16–17.)

Now that you understand more about hypothesis testing, let's return to the giraffes. How did biologists test the food competition hypothesis? What data support their alternative explanation?

The Food Competition Hypothesis: Predictions and Tests The food competition hypothesis claims that giraffes compete for food with other species of mammals. When food is scarce, as it is during the dry season, giraffes with longer necks can reach food that is unavailable to other species and to giraffes with shorter necks. As a result, the longest-necked individuals in a giraffe population survive better and produce more young than do shorter-necked individuals, and average neck length of the population increases with each generation.

To use the terms introduced earlier, long necks are adaptations that increase the fitness of individual giraffes during competition for food. This type of natural selection has gone on so long that the population has become extremely long necked.

The food competition hypothesis makes several explicit predictions. For example, it predicts that

- neck length is variable among giraffes;
- neck length in giraffes is heritable; and
- giraffes feed high in trees, especially during the dry season, when food is scarce and the threat of starvation is high.

The first prediction is correct. Studies in zoos and natural populations confirm that neck length is variable among individuals. The researchers were unable to test the second prediction, however, because they studied giraffes in a natural population and could not do breeding experiments. As a result, they simply had to accept this prediction as an assumption. In general, though, biologists prefer to test every assumption behind a hypothesis.

What about the prediction regarding feeding high in trees? According to Simmons and Scheepers, this is where the food competition hypothesis breaks down.

Consider, for example, data collected by a different research team on the amount of time that giraffes spend feeding in vegetation of different heights (**Figure 1.8a**). Note that this graph plots the height of vegetation on the *x*-axis, starting from ground level (0 meters on the graph) and continuing up to 7 meters. The percentage of bites taken by a giraffe is plotted on the *y*-axis, for males and for females from the same population in Kenya. The dashed line on each graph indicates the average height of a male or female in this population. (For more help on reading graphs, see **BioSkills 2**.)

Note that the average height of a giraffe in this population is much greater than the height where most feeding takes place. In this population, both male and female giraffes spend most of their feeding time eating vegetation that averages just 60 percent of their full height. Studies on other populations of giraffes,

during both the wet and dry seasons, are consistent with these data. Giraffes usually feed with their necks bent (Figure 1.8b).

These data cast doubt on the food competition hypothesis, because one of its predictions does not appear to hold. Biologists have not abandoned this hypothesis completely, though, because feeding high in trees may be particularly valuable during extreme droughts, when a giraffe's ability to reach leaves far above the ground could mean the difference between life and death. Still, Simmons and Scheepers have offered an alternative explanation for why giraffes have long necks. The new hypothesis is based on the mating system of giraffes.

The Sexual Competition Hypothesis: Predictions and Tests

Giraffes have an unusual mating system. Breeding occurs year-round rather than seasonally. To determine when females are coming into estrus or "heat" and are thus receptive to mating, the males nuzzle the rumps of females. In response, the females urinate into the males' mouths. The males then tip their heads back and pull their lips to and fro, as if tasting the liquid. Biologists who have witnessed this behavior have proposed that the males taste the females' urine to detect whether estrus has begun.

Once a female giraffe enters estrus, males may fight among themselves for the opportunity to mate, though confrontation often is resolved by the males standing very tall and staring hard at each other until one male turns and runs away. When combat does occur, it is spectacular. The bulls stand next to one another, swing their necks, and strike thunderous blows with their heads. Researchers have seen males knocked unconscious for 20 minutes after being hit and have cataloged numerous instances in which the loser died.

These observations inspired a new explanation for why giraffes have long necks. The sexual competition hypothesis is based on the idea that longer-necked giraffes are able to strike harder blows during combat than can shorter-necked giraffes. In engineering terms, longer necks provide a longer "moment arm." A long moment arm increases the force of an impact. (Think about the type of sledgehammer you'd use to bash down a concrete wall—one with a short handle or one with a long handle?)

The idea here is that longer-necked males should win more fights and, as a result, father more offspring than shorter-necked males do. If neck length in giraffes is inherited, then the average neck length in the population should increase over time. Under the sexual competition hypothesis, long necks are adaptations that increase the fitness of males during competition for females.

Although several studies have shown that long-necked males are more successful in fighting and that the winners of fights gain access to estrous females, the question of why giraffes have long necks is not closed. With the data collected to date, many biologists would probably conclude that both the food competition hypothesis and the sexual competition hypothesis need further testing and refinement. It could be that both hypotheses are correct. For our purposes, the important take-home message is that all hypotheses must be tested rigorously.

In many cases in biological science, testing hypotheses rigorously involves experimentation. Experimenting on giraffes is difficult. But in the case study considered next, biologists were able to test an interesting hypothesis experimentally.

How Do Ants Navigate? An Introduction to Experimental Design

Let's consider a question that is easier to test than the one about factors that determine giraffe neck length: When ants leave their nest to search for food, how do they find their way back?

The Saharan desert ant lives in colonies and makes a living by scavenging the dead carcasses of insects. Individuals leave the burrow and wander about searching for food at midday, when temperatures at the surface can reach 60°C (140°F) and predators are hiding from the heat. Foraging trips can take the ants hundreds of meters—an impressive distance when you consider that the ants are only about a centimeter long. But when an ant returns, it doesn't follow the same wandering route it took away from the nest. Instead, its return path is a straight line (Figure 1.9).

Once individuals are close to the nest, they engage in a characteristic set of back-and-forth U-turns until they find their nest hole. How do they do know how far they are from the nest?

The Pedometer Hypothesis

Early work on navigation in desert ants showed that they use the Sun's position as a compass—meaning that they always know the approximate direction of the nest relative to the Sun. But how do they know how far to go?

Experiments had shown that the ants do not use landmarks to navigate, so Matthias Wittlinger and co-workers set out to test a novel idea. The biologists proposed that these ants know how far they are from the nest by using information from leg movements.

According to this pedometer hypothesis, the ants always know how far they are from the nest because they track the number of steps they have taken and their stride length. The idea is that they can make a beeline back toward the burrow because they integrate information on the angles they have traveled *and* the distance they have gone—based on step number and stride length.

If the pedometer hypothesis is wrong, however, then stride length and step number should have no effect on the ability of an ant to get back to its nest. This latter possibility is called a **null hypothesis.** A null hypothesis specifies what should be observed when the hypothesis being tested isn't correct.

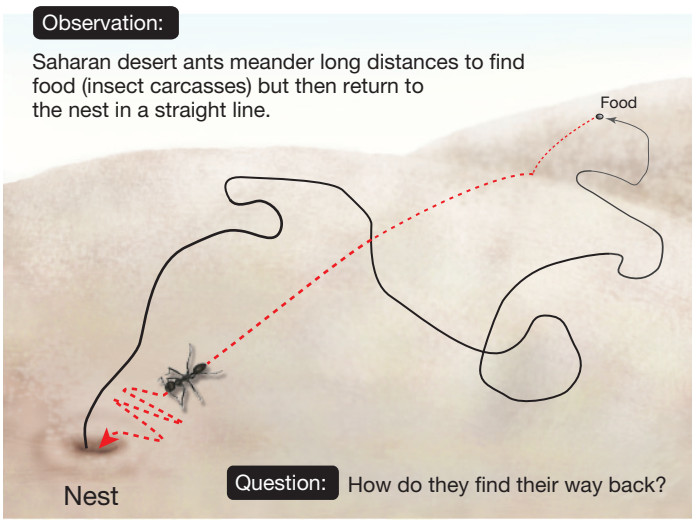


Figure 1.9 Foraging Desert Ants Can Navigate.

Testing the Hypothesis To test their idea, Wittlinger's group allowed ants to walk from a nest to a feeder through a channel—a distance of 10 m. Then they caught ants at the feeder and created three test groups, each with 25 individuals (**Figures 1.10** and **1.11**):

- ***Stumps*** By cutting the lower legs of some individuals off, the biologists created ants with shorter-than-normal legs.
- ***Normal*** Some individuals were left alone, meaning that they had normal leg length.
- ***Stilts*** By gluing pig bristles onto each leg, the biologists created ants with longer-than-normal legs.

Next they put the ants in a different channel and recorded how far they traveled in a direct line before starting their nest-searching behavior. To see the data they collected, look at the graph on the left side of the "Results" section in Figure 1.10.

- ***Stumps*** The ants with stumps stopped short, by about 5 m, before starting to search for the nest opening.

RESEARCH

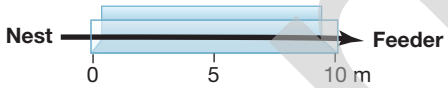
QUESTION: How do desert ants find their way back to their nest?

PEDOMETER HYPOTHESIS: Desert ants keep track of stride number and length to calculate how far they are from the nest.

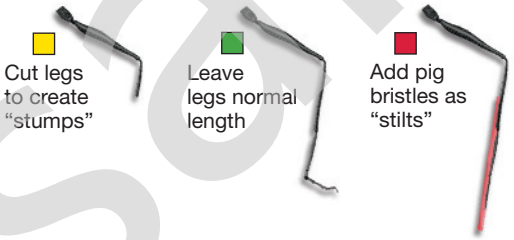
NULL HYPOTHESIS: Stride number and length have nothing to do with navigation (the ants use some other mechanism to navigate).

EXPERIMENTAL SETUP (TEST 1):

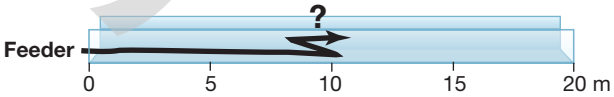
1. Ants walk from nest to feeder. 75 ants are collected.



2. Manipulation of legs. Three treatments, 25 ants each.



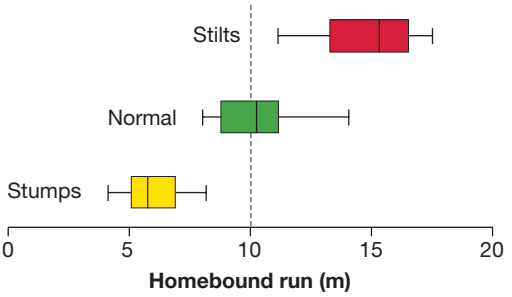
3. Ants return "home" from feeder and search for nest hole.



PREDICTION:
Ants with stilts will go too far; ants with stumps will stop short.

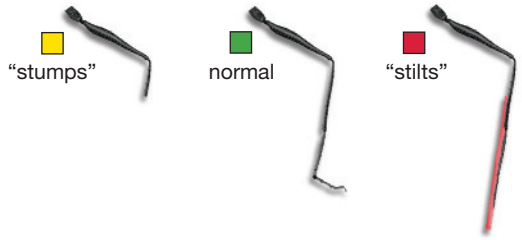
PREDICTION OF NULL HYPOTHESIS:
No differences among the three groups.

RESULTS:

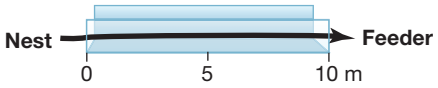


EXPERIMENTAL SETUP (TEST 2):

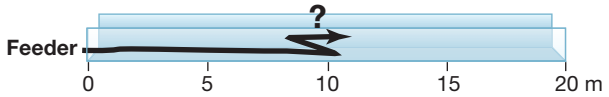
1. Recapture manipulated ants from Test 1.



2. The three treatments of ants walk from nest to feeder again.

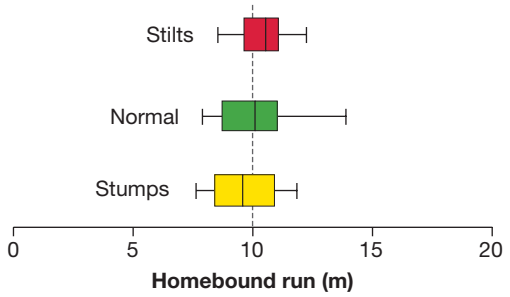


3. Ants walk back "home" from feeder again.



PREDICTION:
All three groups will start looking for nest after walking 10 m.

PREDICTION OF NULL HYPOTHESIS:
No difference from the observed results in Test 1.



CONCLUSION: Desert ants use information on stride length and number to calculate how far they are from the nest.

Figure 1.10 An Experimental Test: Do Desert Ants Use a "Pedometer"?

SOURCE: Wittlinger, M., R. Wehner, and H. Wolf. 2006. The ant odometer: Stepping on stilts and stumps. *Science* 312: 1965–1967.

✓ **PROCESS OF SCIENCE** What is the advantage of using 25 ants in each group instead of just one?

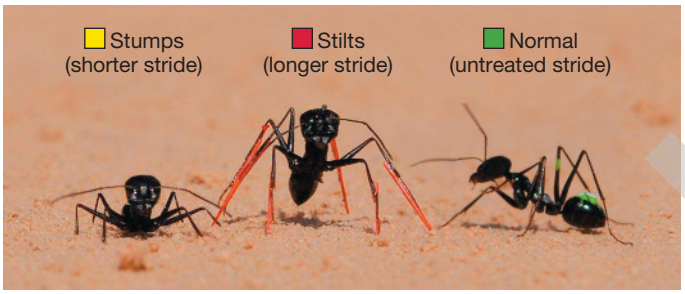


Figure 1.11 Manipulating Desert Ant Legs Changes Stride Length.

- ***Normal*** The normal ants walked the correct distance—about 10 m.
- ***Stilts*** The ants with stilts walked about 5 m too far before starting to search for the nest opening.

To check the validity of this result, the researchers put the test ants back in the nest and recaptured them one to several days later, when they had walked to the feeder on their stumps, normal legs, or stilts. Now when the ants were put into the other channel to "walk back," they all traveled the correct distance—10 m—before starting to search for the nest (see the graph on the right side of the "Results" section in Figure 1.10).

The graphs in the "Results" display "box-and-whisker" plots that allow you to easily see where most of the data fall. Each box indicates the range of distances where 50 percent of the ants stopped to search for the nest. The whiskers indicate the lower extreme (stopping short of the nest location) and the upper extreme (going too far) of where the ants stopped to search. The vertical line inside each box indicates the median—meaning that half the ants stopped above this distance and half below. (For more details on how biologists report medians and indicate the variability and uncertainty in data, see BioSkills 3.)

Interpreting the Results The pedometer hypothesis predicts that an ant's ability to walk home depends on the number and length of steps taken on its outbound trip. Recall that a prediction specifies what we should observe if a hypothesis is correct. Good scientific hypotheses make testable predictions—predictions that can be supported or rejected by collecting and analyzing data. In this case, the researchers tested the prediction by altering stride length and recording the distance traveled on the return trip. Under the null hypothesis in this experiment, all the ants—altered and unaltered—should have walked 10 m in the first test before they started looking for their nest.

Important Characteristics of Good Experimental Design This study illustrates several important points related to designing effective experiments:

- It is critical to include a **control.** A control checks for factors, other than the one being tested, that might influence the experiment's outcome. In this case, there were two controls. Including a normal, unmanipulated individual controlled for the possibility that switching the individuals to a new channel altered their behavior. In addition, the researchers had to control for the possibility that the manipulation itself—and not the change in leg length—affected the behavior of the stilts and stumps ants. This is why they did the second test, where the outbound and return runs were done with the same legs.
- The experimental conditions must be as constant or equivalent as possible. The investigators used ants of the same species, from the same nest, at the same time of day, under the same humidity and temperature conditions, at the same feeders, in the same channels. Controlling all the variables except one—leg length in this case—is crucial because it eliminates alternative explanations for the results.
- Repeating the test is essential. It is almost universally true that larger sample sizes in experiments are better. By testing many individuals, researchers can reduce the amount of distortion or "noise" in the data caused by unusual individuals or circumstances.

From the outcomes of these experiments, the researchers concluded that desert ants use stride length and number to measure how far they are from the nest. They interpreted their results as strong support for the pedometer hypothesis.

The giraffe and ant studies demonstrate a vital point: Biologists practice evidence-based decision making. They ask questions about how organisms work, pose hypotheses to answer those questions, and use experimental or observational evidence to decide which hypotheses are correct.

The data on giraffes and ants offer a taste of things to come. In this text you will encounter hypotheses and research on questions ranging from how water gets to the top of 100-meter-tall sequoia trees to how the bacterium that causes tuberculosis has become resistant to antibiotics. As you work through this book, you'll get lots of practice thinking about hypotheses and predictions, analyzing the nature of control treatments, and interpreting graphs.

A commitment to tough-minded hypothesis testing and sound experimental design is a hallmark of biological science. Understanding their value is an important first step in becoming a biologist.

CHECK YOUR UNDERSTANDING

If you understand that …

- Hypotheses are proposed explanations that make testable predictions.
- Predictions describe observable outcomes of particular conditions.
- Well-designed experiments alter just one condition—a condition relevant to the hypothesis being tested.

✓ You should be able to …

PROCESS OF SCIENCE Design an experiment to test the hypothesis that desert ants feed during the hottest part of the day because it allows them to avoid being eaten by lizards. Then answer the following question about your experimental design: How are experimental conditions controlled or standardized in a way that precludes alternative explanations of the data?

Answers are available in Appendix A.

1.1 What Does It Mean to Say That Something Is Alive?

- There is no single, well-accepted definition of life. Instead, biologists point to five characteristics that organisms share.
- Three of the greatest unifying ideas in biology are the cell theory, the theory of evolution, and the chromosome theory of inheritance.

1.2 Life Is Cellular

- The cell theory identified the fundamental structural unit common to all life.

1.3 Life Evolves

- The theory of evolution states that all organisms are related by common ancestry.
- Natural selection is a well-tested explanation for why species change through time and why they are so well adapted to their habitats.

1.4 Life Processes Information

- The chromosome theory of inheritance states that genes are located on chromosomes.
- A chromosome consists of a molecule of DNA—the hereditary material. Genes, located on chromosomes, consist of specific segments of DNA that code for products in the cell.
- The flow of information from DNA to RNA to protein is called the central dogma.
- Organisms are highly diverse in how they acquire and use energy.

1.5 The Tree of Life

- The theory of evolution predicts that all organisms are part of a genealogy of species, and that all species trace their ancestry back to a single common ancestor.
- To construct this phylogeny, biologists have analyzed the sequences in an array of genetic material found in all cells.
- A tree of life, based on similarities and differences in these molecules, has three fundamental lineages, or domains: the Bacteria, the Archaea, and the Eukarya.

1.6 Doing Biology

- Biology is a hypothesis-driven, experimental science.

Answers are available in Appendix A

✓ TEST YOUR KNOWLEDGE

1. Anton van Leeuwenhoek made an important contribution to the development of the cell theory. How?
   a. He articulated that all organisms are made of cells.
   b. He articulated that all cells come from preexisting cells.
   c. He invented the first microscope and saw the first cell.
   d. He invented more powerful microscopes and was the first to describe the diversity of cells.
2. **PROCESS OF SCIENCE** What does it mean to say that experimental conditions are controlled?
   a. The test groups consist of the same individuals.
   b. The null hypothesis is correct.
   c. There is no difference in outcome between the control and experimental treatment.
   d. All physical conditions except for one are identical for all groups tested.
3. What does it mean to say that a characteristic is heritable?
   a. The characteristic evolves.
   b. The characteristic can be passed on to offspring.
   c. The characteristic is advantageous to the organism.
   d. The characteristic does not vary in the population.
4. Could *both* the food competition hypothesis and the sexual competition hypothesis explain why giraffes have long necks? Why or why not?

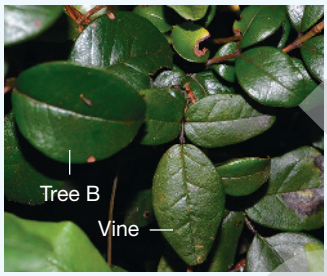
✓ TEST YOUR UNDERSTANDING

5. What would researchers have to demonstrate to convince you that they had discovered life on another planet?
6. What did Linnaeus' system of naming organisms ensure?
   a. Two different organisms never end up with the same genus and species name.
   b. Two different organisms have the same genus and species name if they are closely related.
   c. The genus name is different for closely related species.
   d. The species name is the same for each organism in a genus.
7. What is "selected" during natural selection? Explain your answer.
8. **PROCESS OF SCIENCE** Explain why researchers formulate a null hypothesis in addition to a hypothesis when designing an experimental study.

✓ TEST YOUR PROBLEM-SOLVING SKILLS

9. **CAUTION** A friend tells you that the theory of evolution is just an educated guess by biologists about how things work. Evaluate this statement.
10. Some humans have genes that make them resistant to infection by HIV. Would human populations likely evolve differently in areas of the world where HIV infection rates are high? Explain your logic.

✓ PUT IT ALL TOGETHER: Case Study



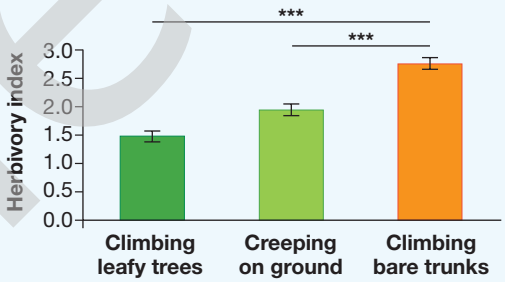
Can a plant act like a chameleon?

You may be familiar with chameleons turning different colors to blend in with their environment. Now biologists have observed that *Boquila trifoliolata*, a climbing vine found in the rain forest of southern Chile, can mimic the leaves of a dozen host species. When the vine climbs up a leafy tree, it adjusts the size, shape, and color of its own leaves to match that tree's leaves. But when a vine climbs up a bare tree trunk, it looks exactly the same as one that creeps along the rain forest floor.

11. Outline the flow of information from the genetic material to the physical appearance of the vine pictured earlier.

12. What does the species name of *Boquila trifoliolata* mean? Why is this name appropriate? (Hint: See BioSkills 15.)

13. **QUANTITATIVE** Researchers hypothesized that leaf mimicry by *B. trifoliolata* provides protection from plant-eating animals (herbivores). The results of a study of 45 individual vines are shown in the following graph. Light conditions were very similar in all cases. Researchers compared the level of leaf damage by plant eaters (herbivory index) in vines climbing leafy host trees, vines creeping on the ground with no support, and vines climbing on bare tree trunks. Use the *P* values provided to determine if the differences are significant or not (*** means $P < 0.001$, see BioSkills 3). What conclusion, if any, can be drawn about leaf mimicry from this study? What might the researchers do next to further explore the role of leaf mimicry?



Source: Gianoli, E., and F. Carrasco-Urra. 2014. *Current Biology* 24: 984–987.

14. **PROCESS OF SCIENCE** If the researchers had compared vines growing under variable light conditions, how might this have changed their interpretation of the data?

15. **PROCESS OF SCIENCE** What was the purpose of including bare tree trunks in the study?

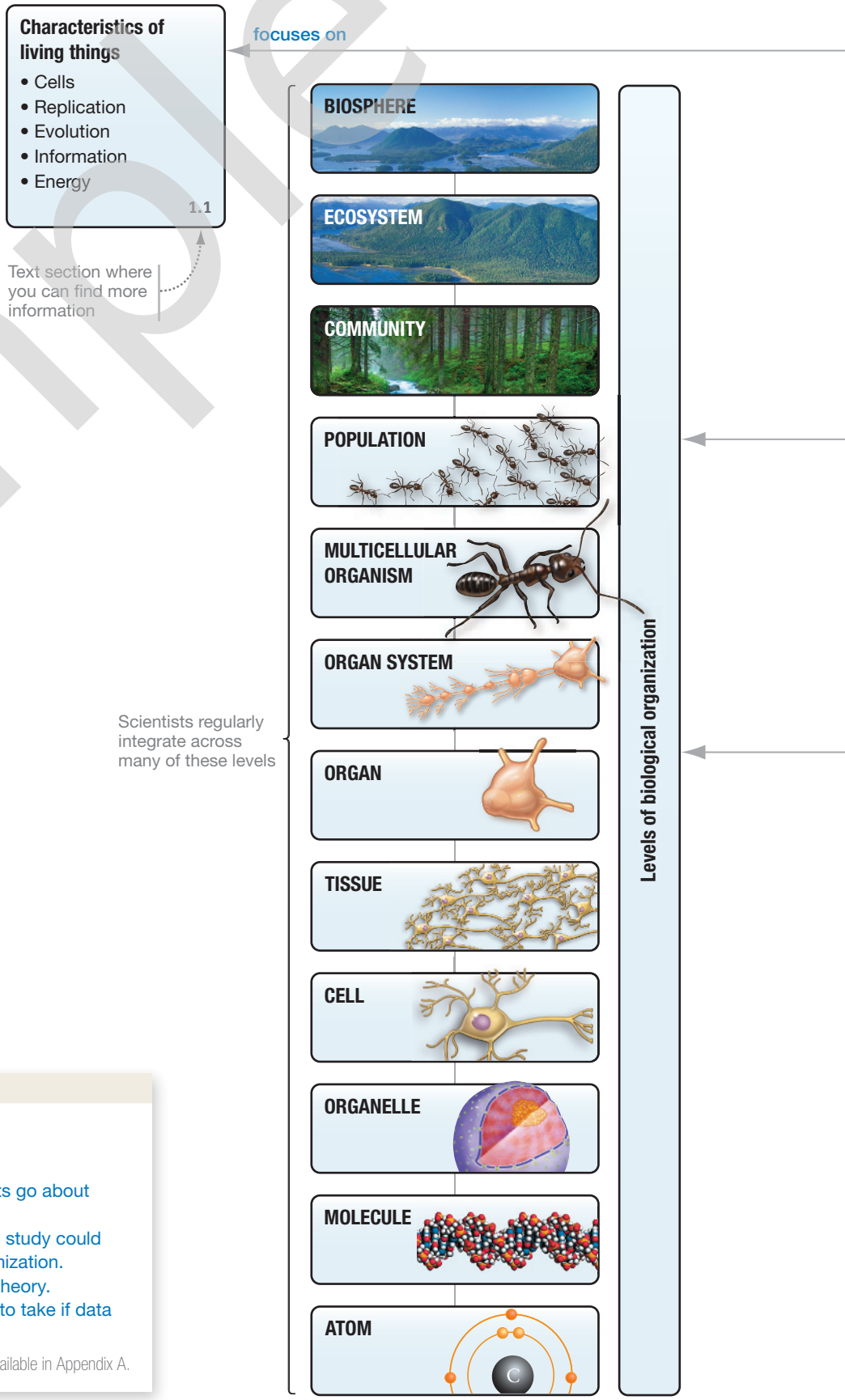
16. By avoiding being eaten, *B. trifoliolata* individuals would have increased fitness. In biology, what does the term "fitness" mean?

THE BIG PICTURE

Biologists study the characteristics of life. The cell theory, the theory of evolution by natural selection, the chromosome theory of inheritance, and the tree of life are some of the great ideas in biology that came about by biologists asking questions that can be answered by observing or measuring things—that is, by collecting data.

Notice that the study of life is not a series of linear steps with a beginning and an end. Instead, the process of doing biology is dynamic and ongoing. The answer to one question may lay the foundation for 20 more questions. Working together, biologists from different disciplines integrate data across many levels, from atoms to the biosphere.

Note that the gray numbers in boxes tell you where to go in the book for more information. Also, be sure to do the blue exercises in the Check Your Understanding box below.



CHECK YOUR UNDERSTANDING

If you understand the big picture …

✔ You should be able to …

1. **PROCESS OF SCIENCE** Describe how biologists go about testing their ideas.
2. Provide an example of how an experimental study could span more than one level of biological organization.
3. Compare and contrast a hypothesis with a theory.
4. **PROCESS OF SCIENCE** Propose the next step to take if data support the hypothesis you are testing.

Answers are available in Appendix A.

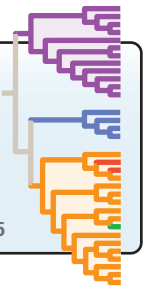
DOING BIOLOGY

involves the

Evidence-based study of life 1.1

reveals

The Tree of Life
Describes the evolutionary relationships among species
1.5, Ch 25, Unit 5



begins with

Observations (planned or chance)

Exploring the literature
BioSkills 16

Sharing ideas with other scientists

Inspiration

provides

enables

leads to

lead to

Defining a problem and forming a question

leads to

may involve one or many

Society

to

Scientific community
- Discussions with colleagues
- Scientific meetings
- Peer-reviewed publications

BioSkills 16

leads to

Stating a hypothesis and a null hypothesis, and making predictions

reject hypothesis; propose alternative hypothesis

leads to

may involve one or many

Designing a study (either observational or experimental) to test predictions

revisit assumptions of study

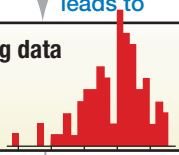
to

Characteristics of good experimental design
- Controls
- Consistent experimental conditions
- Large sample sizes 1.6

usually involves

leads to

Gathering data



then

Communicate findings

then

Theory
- Explains fundamental aspects of nature
- Consistently shown to be correct after extensive testing
- Serves as a framework for developing new hypotheses 1.2

relate to larger

such as

Cell theory 1.2

and

Theory of evolution 1.3, Ch 22

and

Chromosome theory of inheritance 1.4, Ch 14

leads to

Analyzing and interpreting data
BioSkills 1–3, 6–11, 13

evaluated by asking

Do the data support the hypothesis?

if NO

if YES

BioSkills

In this book you will learn that

BioSkills
are essential for doing biology

starting with

Asking Questions and Designing Studies

Chapter 1: Introduces core principles and best practices

Big Picture 1: Provides a visual summary of how to think like a biologist

The narrative throughout the text models how to think like a biologist, including end-of-chapter case studies.

Experiment boxes, graphs, and other visual models in each chapter help you to visualize scientific ideas.

then using this BioSkills section to review and practice with

Quantifying Biology

1: Using the Metric System and Significant Figures

2: Reading and Making Graphs

3: Interpreting Standard Error Bars and Using Statistical Tests

4: Working with Probabilities

5: Using Logarithms

Using Common Lab Tools

6: Separating and Visualizing Molecules

7: Separating Cell Components by Centrifugation

8: Using Spectrophotometry

9: Using Microscopy

10: Using Molecular Biology Tools and Techniques

11: Using Cell Culture and Model Organisms as Tools

Visualizing Biology

12: Reading and Making Visual Models

13: Reading and Making Phylogenetic Trees

14: Reading Chemical Structures

See 2: Reading and Making Graphs

Reading Biology

15: Translating Greek and Latin Roots in Biology

16: Reading and Citing the Primary Literature

where success requires

where success requires

where success requires

Monitoring Your Own Learning

17: Recognizing and Correcting Misconceptions

18: Using Bloom's Taxonomy for Study Success

BioSkill 1 Using the Metric System and Significant Figures

Scientists ask questions that can be answered by observing or measuring things—by collecting data. What units are used to make measurements? When measurements are reported, how can you tell how reliable the data are?

Metric System Units and Conversions

The metric system is the system of units of measure used in every country of the world but three (Liberia, Myanmar, and the United States). It is also the basis of the SI system—the International System of Units (abbreviated from the French, *Système international d'unités*)—used in scientific publications.

The popularity of the metric system is based on its consistency and ease of use. These attributes arise from the system's use of the base 10. For example, each unit of length in the system is related to all other measures of length in the system by a multiple of 10. There are 10 millimeters in a centimeter, 100 centimeters in a meter, and 1000 meters in a kilometer.

Measures of length in the English system, in contrast, do not relate to each other in a regular way. Inches are routinely divided into 16ths; there are 12 inches in a foot, 3 feet in a yard, and 5280 feet (or 1760 yards) in a mile.

If you have grown up in the United States and are accustomed to using the English system, it is extremely important that you begin developing a working familiarity with metric units and values. **Tables B1.1** and **B1.2** should help you get started with this process.

As an example, consider the following question: An American football field is 120 yards long, while rugby fields are 144 meters long. In meters, how much longer is a rugby field than an American football field? To solve this problem, first convert yards to meters:

$$120 \text{ yards} \times \text{m}/1.09 \text{ yards} = 110 \text{ m}$$

Note that the unit "yards" cancels out. The difference in meters is thus $144 - 110 = 34$ m. If you did the unit conversion calculation on a calculator, you might have come up with 110.09 m. Why was the number of meters rounded off? The answer lies in significant figures. Let's take a closer look.

Table B1.1 Metric System Units and Conversions

Measurement	Unit of Measurement and Abbreviation	Metric System Equivalent	Converting Metric Units to English Units
Length	kilometer (km)	1 km = 1000 m = 10^3 m	1 km = 0.62 mile
	meter (m)	1 m = 100 cm	1 m = 1.09 yards = 3.28 feet = 39.37 inches
	centimeter (cm)	1 cm = 0.01 m = 10^{-2} m	1 cm = 0.3937 inch
	millimeter (mm)	1 mm = 0.001 m = 10^{-3} m	1 mm = 0.039 inch
	micrometer (μm)	1 μm = 10^{-6} m = 10^{-3} mm	
	nanometer (nm)	1 nm = 10^{-9} m = 10^{-3} μm	
Area	hectare (ha)	1 ha = 10,000 m^2	1 ha = 2.47 acres
	square meter (m^2)	1 m^2 = 10,000 cm^2	1 m^2 = 1.196 square yards
	square centimeter (cm^2)	1 cm^2 = 100 mm^2 = 10^{-4} m^2	1 cm^2 = 0.155 square inch
Volume	liter (L)	1 L = 1000 mL	1 L = 1.06 quarts
	milliliter (mL)	1 mL = 1000 μL = 10^{-3} L	1 mL = 0.034 fluid ounce
	microliter (μL)	1 μL = 10^{-6} L	
Mass	kilogram (kg)	1 kg = 1000 g	1 kg = 2.20 pounds
	gram (g)	1 g = 1000 mg	1 g = 0.035 ounce
	milligram (mg)	1 mg = 1000 μg = 10^{-3} g	
	microgram (μg)	1 μg = 10^{-6} g	
Temperature	Kelvin (K)*		K = °C + 273.15
	degrees Celsius (°C)		$°C = \frac{5}{9}(°F - 32)$
	degrees Fahrenheit (°F)		$°F = \frac{9}{5}(°C + 32)$

*Absolute zero is −273.15°C = 0 K.

Table B1.2 Prefixes Used in the Metric System

Prefix	Abbreviation	10^n	Decimal	English expression
pico–	p	10^{-12}	0.000000000001	one trillionth
nano–	n	10^{-9}	0.000000001	one billionth
micro–	μ	10^{-6}	0.000001	one millionth
milli–	m	10^{-3}	0.001	one thousandth
centi–	c	10^{-2}	0.01	one hundredth
deci–	d	10^{-1}	0.1	one tenth
–	–	10^{0}	1	one
deca–	da	10^{1}	10	ten
hecto–	h	10^{2}	100	one hundred
kilo–	k	10^{3}	1000	one thousand
mega–	M	10^{6}	1,000,000	one million
giga–	G	10^{9}	1,000,000,000	one billion
tera–	T	10^{12}	1,000,000,000,000	one trillion

Significant Figures

Significant figures, or "sig figs," are critical when reporting scientific data. The number of significant figures in a measurement, such as 3.524, is the number of digits that are known with some degree of confidence (3, 5, and 2) plus the last digit (4), which is an estimate or approximation. How do scientists know how many digits to include when reporting a measurement?

Rules for Working with Significant Figures The rules for counting significant figures in a reported measurement are:

- All nonzero numerals are always significant.
- Leading zeros are never significant; these zeros do nothing but set the decimal point.
- Embedded zeros are always significant.
- Trailing zeros are significant *only* if the decimal point is specified (Hint: Change the number to scientific notation. It is easier to see the "trailing" zeros.)

Table B1.3 shows examples of how to apply these rules. The bottom line? Significant figures indicate the accuracy of measurements.

Using Scientific Notation Scientific notation is the way that biologists deal with very large or very small numbers. For example, instead of writing 0.00027, you could write this value as the product of two numbers: 2.7 (the digit term) and 10^{-4} (the exponential term), or 2.7×10^{-4}. The digit term shows the number of significant figures, and the exponential term places the decimal point. A negative exponent of 10 shows that to write the number in long form, you should shift the decimal point that number of places to the left. A positive exponent shows that the decimal point should be shifted that number of places to the right.

Precision versus Accuracy If biologists count the number of bird eggs in a nest, they report the data as an exact number—say, 3 eggs. But if the same biologists are measuring the diameter of the eggs, the numbers will be inexact. Just how inexact they are depends on the equipment used to make the measurements.

If you measure the width of your textbook with a ruler several times, you'll get essentially the same measurement again and again with some variation. See **Figure B1.1** for a graphical representation of this. Precision refers to how closely individual measurements agree with each other. You may have determined

Table B1.3 Rules for Working with Significant Figures

Example	Number of Significant Figures	Scientific Notation	Rule
35,214	5	3.5214×10^{4}	All nonzero numbers are always significant.
0.00352	3	3.52×10^{-3}	Leading zeros are not significant.
1.035	4	$1.035 (\times 10^{0})$	Imbedded zeros are always significant.
200	1	2×10^{2}	Trailing zeros are significant only if the decimal point is specified.
200.0	4	2.000×10^{2}	Trailing zeros are significant only if the decimal point is specified.

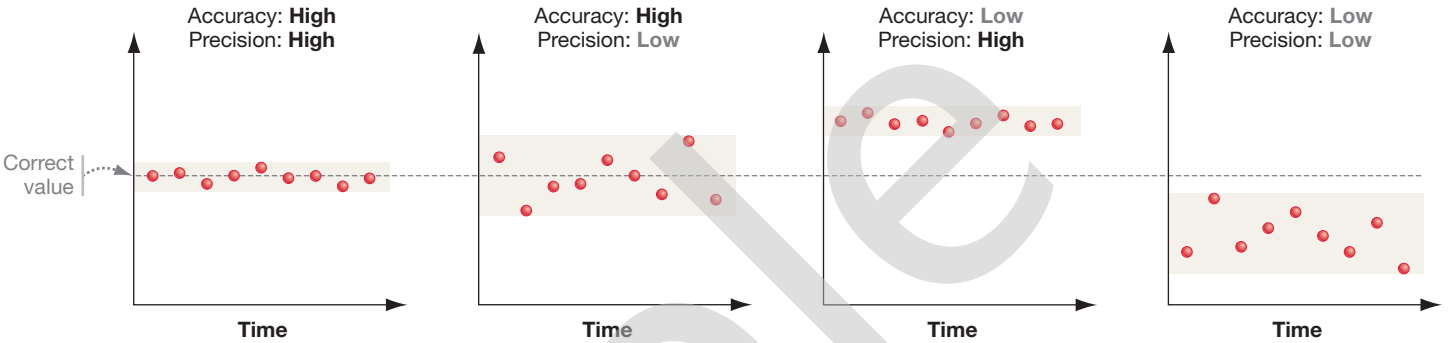


Figure B1.1 Accuracy Versus Precision in Measurement. The dashed line shows the correct value (in the example given, the actual width of your textbook), and the red dots indicate measurements made over time.

the book's width with precision, but how do you know if your ruler is accurate?

Accuracy refers to how closely a measured value agrees with the correct value. You don't know the accuracy of a measuring device unless you calibrate it. For instance, you could calibrate your ruler by comparing it against a ruler that is known to be accurate. As the sensitivity of equipment used to make a measurement increases, the number of significant figures increases. For example, if you used a kitchen scale to weigh some sodium chloride, you might obtain a weight of 3 ± 1 g (an accuracy of 1 significant figure); but an analytical balance in the lab might give a value of 3.524 ± 0.001 g (an accuracy of 4 significant figures).

It is important to follow the "sig fig rules" when reporting a measurement, so that data do not appear to be more accurate than the equipment allows.

Combining Measurements How do you deal with combining measurements with different degrees of accuracy and precision? A simple rule to follow when combining measurements is that the accuracy of the final answer can be no greater than the least accurate measurement. When you multiply or divide measurements, the answer can have no more significant figures than the least accurate measurement. When you add or subtract measurements, the answer can have no more decimal places than the least accurate measurement.

As an example, consider adding the following measurements: 5.9522, 2.065, and 1.06. If you add these numbers with your calculator, the answer your calculator will give you is 9.0772. However, this is incorrect—you must round your answer off to 9.08, which has two decimal places, the least number of decimal places in your data.

It is important to practice working with metric units and to nail down the concept of significant figures. The Check Your Understanding questions in this BioSkill should help you get started with this process.

CHECK YOUR UNDERSTANDING

If you understand BioSkill 1

✔ **You should be able to . . .**

1. **QUANTITATIVE** Calculate how many miles a runner completes in a 5.0-kilometer run.
2. **QUANTITATIVE** Calculate your normal body temperature in degrees Celsius. (Normal body temperature is 98.6 °F.)
3. **QUANTITATIVE** Calculate your current weight in kilograms.
4. **QUANTITATIVE** Calculate how many liters of milk you would need to buy to get approximately the same volume as a gallon of milk.
5. **QUANTITATIVE** Multiply the measurements 2.8723 and 1.6. How many significant figures does your answer have? Why?

Answers are available in Appendix A.

BioSkill 2 Reading and Making Graphs

Graphs are the most common way to report data, for a simple reason. Compared to reading raw numerical values in a table or list, a graph makes it much easier to understand what the data mean.

Learning how to read and interpret graphs is one of the most basic skills you'll need to acquire as a biology student. As when learning piano or soccer or anything else, you need to understand a few key ideas to get started and then have a chance to practice—a lot—with some guidance and feedback. At the same time, you'll also be developing the skills you need to make your own graphs.

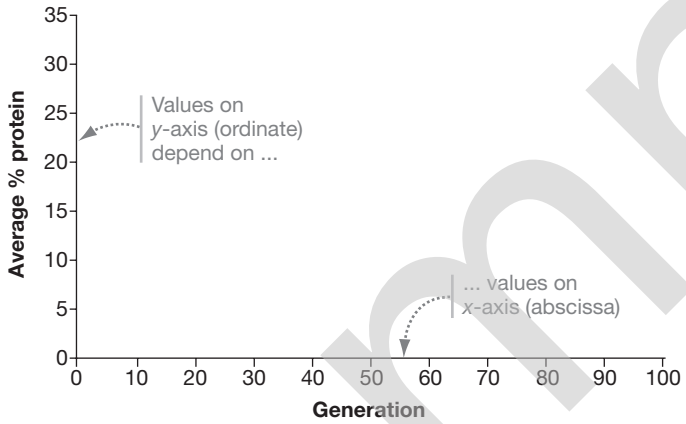
Getting Started

To start reading a graph, you need to do three things: read the axes, figure out what the data points represent—that is, where they came from—and think about the overall message of the data. Let's consider each step in turn.

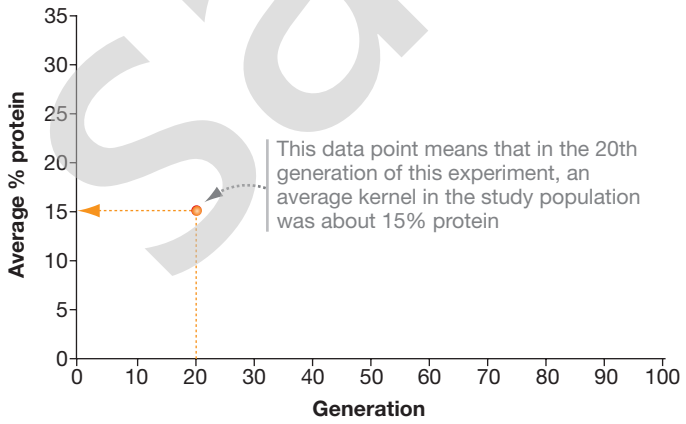
What Do the Axes Represent? Most graphs have two axes: one horizontal and one vertical. The horizontal axis of a graph is also called the *x*-axis or the abscissa. The vertical axis of a graph is also called the *y*-axis or the ordinate. Each axis represents a variable that takes on a range of values. These values are indicated by the tick marks and labels on the axis. Note that each axis should *always* be clearly labeled with the unit or treatment it represents.

Figure B2.1 shows the steps in reading a *scatterplot*—a type of graph where continuous data are graphed on each axis and individual data points are plotted. Continuous data can take an array of values over a range. In contrast, discrete data can take only a restricted set of values. In a graph of the average height of men and women in your class, height is a continuous variable, but sex is a discrete variable.

(a) Read the axes—what is being plotted?



(b) Look at the data points (or bars)—what do they represent?



(c) What's the punch line?

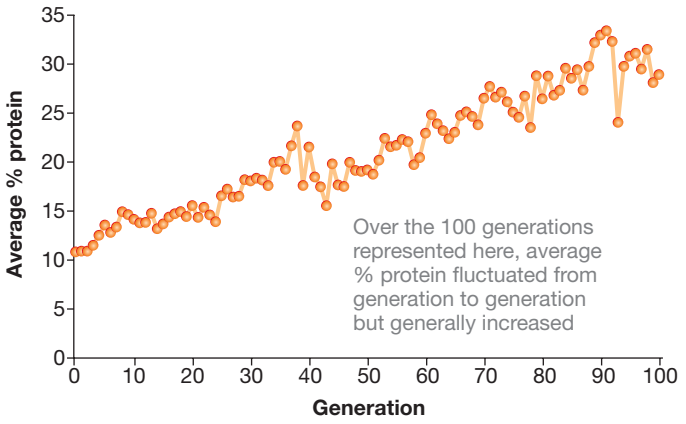


Figure B2.1 Scatterplots Are Used to Graph Continuous Data.

To create a graph, researchers plot the independent variable on the *x*-axis and the dependent variable on the *y*-axis (Figure B2.1a). The terms "independent" and "dependent" are used because the values on the *y*-axis *depend* on the *x*-axis values. For the example in this figure, the researchers wanted to show how the protein content of maize (corn) kernels in a study population changed over time. Thus, the protein concentration plotted on the *y*-axis depended on the generation plotted on the *x*-axis. The value on the *y*-axis always depends on the value on the *x*-axis, but not vice versa.

In many graphs in biology, the independent variable is either time or the various treatments used in an experiment. In these cases, the *y*-axis records how some quantity changes as a function of time or as the outcome of the treatments applied to the experimental cells or organisms.

What Do the Data Points Represent? Once you've read the axes, you need to figure out what each data point is. In our maize kernel example, the data point in Figure B2.1b represents the average percentage of protein found in a sample of kernels from a study population in a particular generation.

If it's difficult to figure out what the data points are, ask yourself where they came from—meaning how the researchers got them. You can do this by understanding how the study was done and by understanding what is being plotted on each axis. The *y*-axis will tell you what the researchers measured; the *x*-axis will usually tell you when they measured it or what group they measured. In some cases—for example, in a plot of average body size versus average brain size in primates—the *x*-axis will report a second variable that was measured.

In other cases, a data point on a graph may represent a relative or arbitrary unit of measurement, such as the amount of gene expression relative to a control, with the control set at an arbitrary value of 1.0 (for an example, see Figure 19.5). The data point shows the ratio of the amount of a substance, intensity, or other quantity, relative to a predetermined reference measurement. For example, the *y*-axis might show the percentage of relative activity of an enzyme—the rate of the enzyme-catalyzed reaction, scaled to the highest rate of activity observed (100 percent)—in experiments conducted under conditions that are identical except for one variable, such as pH or temperature (see Figure 8.15).

What Is the Overall Trend or Message? Look at the data as a whole, and figure out what they mean. Figure B2.1c suggests an interpretation of the maize kernel example. If the graph shows how some quantity changes over time, ask yourself if that quantity is increasing, decreasing, fluctuating up and down, or staying the same. Then ask whether the pattern is the same over time or whether it changes over time.

When you're interpreting a graph, it's extremely important to limit your conclusions to the data presented. Don't extrapolate beyond the data, unless you are explicitly making a prediction based on the assumption that present trends will continue. For example, you can't say that the average percentage of protein content was increasing in the population before the experiment started, or that it will continue to increase in the future. You can say only what the data tell you.

Types of Graphs

Many of the graphs in this text are scatterplots like the one shown in Figure B2.1c. But you will also come across other types of graphs in this text. When creating your own graphs, you'll want to think carefully about which type of graph is the most appropriate to use for a particular data set.

Scatterplots, Lines, and Curves Some scatterplots, like the one in Figure B2.1c, have data points that are connected by dot-to-dot lines to help make the overall trend clearer. In other scatterplots, the data points are unconnected or have a smooth line drawn through them.

A *smooth line* through data points—sometimes straight, sometimes curved—is a "line of best fit." It represents a mathematical function that summarizes the relationship between the *x* and *y* variables. It is "best" in the sense of fitting the data points most accurately. The line may intersect with some of the points, none of the points, or all of the points.

Curved lines often take on characteristic shapes depending on the relationships between the *x* and *y* variable. For example, bell-shaped curves typically fit data from studies on enzyme kinetics (see Chapter 8), while sigmoid, or S-shaped, curves fit data from many studies on oxygen–hemoglobin dissociation (see Chapter 42) and population growth (see Chapter 51).

Bar Charts, Histograms, and Box-and-Whisker Plots Scatterplots, or line-of-best-fit graphs, are the most appropriate type of graph when the data have a continuous range of values and you want to show individual data points. But other types of graphs are used to represent different types of data distributions:

- *Bar charts* plot data that have discrete or categorical values instead of a continuous range of values. In many cases the bars might represent different treatment groups in an experiment, as in **Figure B2.2a**. In this graph, the height of the bar indicates the mean value. To interpret the graph, ask yourself how different the values are. If the bar chart reports means over discrete ranges of values, ask what trend is implied—as you would for a scatterplot.
- *Histograms* illustrate frequency data and can be plotted as numbers or percentages. **Figure B2.2b** shows an example where height (in inches) is plotted on the *x*-axis, and the number of students in a population in the United States is plotted on the *y*-axis. Each bar indicates the number of individuals in each interval of height, which reflects the relative frequency, in this population, of people whose heights are in that interval. The measurements could also be recalculated so that the *y*-axis reported the percentage of the population in each interval. Then the total percentage for all the bars would equal 100 percent. Note that if you were to draw a smooth curve connecting the tops of the bars in this histogram, the curve would be roughly bell shaped. To interpret a histogram, ask whether there is a "hump" in the data—indicating a group of values on the *x*-axis that are more frequent than others. If so, what does it mean? Is the hump in the center of the distribution of values, toward the left, or toward the right?

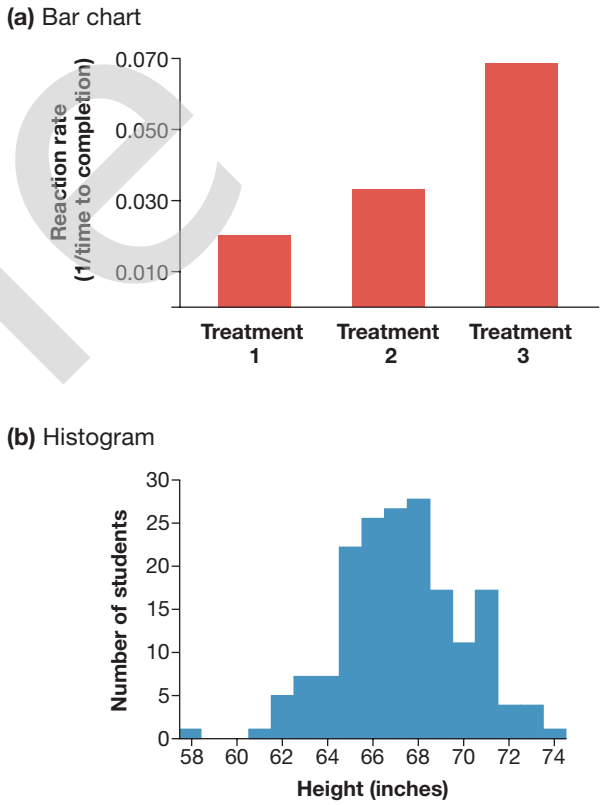


Figure B2.2 Bar Charts and Histograms. (a) Bar charts are used to graph data that are discontinuous or categorical. **(b)** Histograms show the distribution of frequencies or values in a population.

- *Box-and-whisker plots* allow you to easily see where most of the data fall (see Figure 1.10 for an example). Each box indicates where half of the data numbers are. The whiskers indicate the lower extreme and the upper extreme of the data. The vertical line inside each box indicates the median—meaning that half of the data are greater than this value and half are less. To interpret a box-and-whisker plot, ask yourself what information the graph gives you. What is the range of values for the data? Where are half the data points? Below what value is three quarters of the data?

In all types of graphs, statistical tests can be used to determine whether a difference between treatment groups, or a difference in the relationship between two continuous ranges of values, is significant. If differences are statistically significant, it means that they are not likely to have occurred by chance, but rather are likely to be attributable to a specific variable (see **BioSkill 3**).

Getting Practice

Working with this text will give you lots of practice with reading and interpreting graphs—they appear in almost every chapter. In many graphs, arrows and labels have been added to suggest an interpretation or draw your attention to an important point on the graph. In other graphs, you should be able to figure out what the data mean on your own or with the help of other students or your instructor.

CHECK YOUR UNDERSTANDING

If you understand BioSkill 2

✔ You should be able to …

1. **QUANTITATIVE** Refer to Figure B2.1 and determine the total change in average percentage of protein in maize kernels, from the start of the experiment until the end.
2. **QUANTITATIVE** Determine the trend in average percentage of protein in maize kernels between generation 37 and generation 42 in Figure B2.1.
3. Explain whether the conclusions from the bar chart in Figure B2.2a would be different if the data and label for Treatment 3 were put on the far left and the data and label for Treatment 1 on the far right.
4. **QUANTITATIVE** Determine the most common height in the class graphed in Figure B2.2b. Convert your answer to centimeters (cm).
5. **MODEL** Make a bar graph from the data in this table that shows how 16 children with central nervous system leukemia responded to treatment with the anti-tumor drug topotecan. Being free of leukemia was considered a complete response. Which is the dependent variable? Which is the independent variable?

Response	Percentage of Children
Complete response	37.5
Stable disease	50
Progressive disease	12.5

DATA: Potter, S. L., et al. 2012. *Pediatric Blood Cancer* 58: 362–365.

Answers are available in Appendix A.

BioSkill 3 Interpreting Standard Error Bars and Using Statistical Tests

When biologists do an experiment, they collect data on individuals or samples in a treatment group and a control group, or several such comparison groups. Then they typically test whether the mean (average) values of the dependent variable are different in two (or more) of the groups.

Standard Error Bars

For example, in one experiment student researchers measured how fast a product formed when they set up a reaction with three concentrations of reactants (see Figure 8.4). Each treatment—meaning each combination of reactant concentrations—was replicated many times.

Figure B3.1 graphs the mean reaction rate for each of the three treatments in the students' experiment. Note that Treatments 1, 2, and 3 represent increasing concentrations of reactants. The thin "I-beams" at the top of each bar indicate the standard error of each mean. The standard error of the mean is a quantity that indicates the uncertainty in a calculated mean. In effect, it quantifies how confident you are that the mean you've calculated is the mean you'd observe if you did the experiment under the same conditions an extremely large number of times. It is a measure of precision (see BioSkill 1).

Note that sometimes the error bars represent the confidence interval of the mean. A *confidence interval* gives an estimated range of values that is likely to include the population parameter being studied, such as the survival rate of animals after exposure to a pathogen. The estimated range is calculated from a given set of sample data. A 95 percent confidence level means that 95 percent of the confidence intervals would include the population parameter. You might also have heard the term "standard deviation." How are standard error and standard deviation related? When biologists calculate the standard deviation of a sample, they are using it as an estimate of the variability of the population that the sample was taken from. For data with a normal distribution, about 95 percent of individuals will have values within two standard deviations of the mean. The standard deviation will not tend to change as sample size increases.

In contrast, the standard error of the mean (SEM) depends on both the standard deviation (SD) and the sample size:

$$\text{SEM} = \frac{\text{SD}}{\sqrt{\text{sample size}}}$$

The standard error decreases as the sample size increases, because the extent of chance variation is reduced.

Let's consider again the experiment carried out by the student researchers (see Figure B3.1). Suppose two trials with the same concentration of reactants had a reaction rate of 0.075, and two other trials had a reaction rate of 0.025. The mean reaction rate of all four trials would be 0.050. In this case, the standard error would be large. But what if two trials had a reaction rate of 0.051 and two had a reaction rate of 0.049? The mean would still be 0.050, but the standard error would be small.

Once they had calculated these means and standard errors, the student researchers wanted to answer a question: Does reaction rate increase when reactant concentration increases? After looking at the data, you might conclude that the answer is yes. But how could you come to a conclusion like this objectively, instead of subjectively? The answer is to use a statistical test to determine, for example, whether the difference between the rate

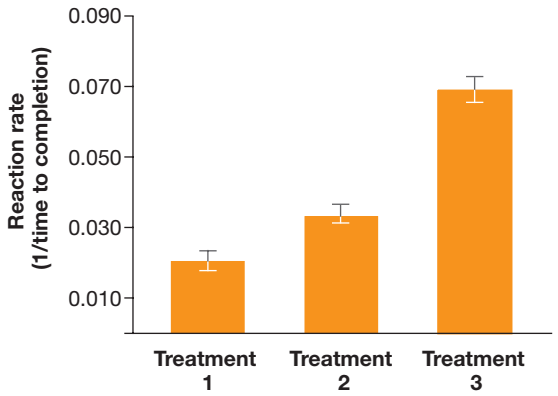


Figure B3.1 Standard Error Bars Indicate the Uncertainty in a Mean.

at the highest reactant concentration and the rate at the lowest reactant concentration is significant. If the difference is found to be statistically significant, then it is not likely to have occurred by chance—it's likely to be attributable to the change in reactant concentration. Let's take a closer look at using and interpreting statistical tests.

Using Statistical Tests

If you take a statistics course, you'll learn which statistical tests are most appropriate for analyzing different types of data. Three commonly used statistical tests are the chi-square test, t-test, and analysis of variance. Other tests examine regression and correlation:

- *Chi-square* tests are used to compare observed data with data you would expect to obtain according to a specific hypothesis. For example, if, according to Mendel's laws (see Chapter 14), you expected equal numbers of male and female offspring from a cross but you observed 9 males and 23 females, you might want to know whether the difference between the observed and expected numbers was due to chance or to other factors. How much of a difference can occur before you must conclude that something other than chance is at work? The chi-square test always tests the null hypothesis (see Chapter 1), which states that there is no significant difference between the observed and expected results.
- *T-tests* are used to determine if there is a significant difference between the mean values of two groups, such as the mean body sizes of mainland and island tortoises (see Chapter 39). In this case, the null hypothesis would be that there is no significant difference between the means of the two data sets.
- *Analysis of variance (ANOVA)* compares the means of two or more sets of data by calculating how widely individual values in each data set vary. If they vary greatly from the mean, the variance is large, and vice versa. When applied to only two data sets, ANOVA will give the same result as a t-test. ANOVA is a powerful statistical test because it allows you to test for each factor while controlling for others and to detect whether one variable affects another. As an example, if you were comparing the activity of a particular enzyme in mainland and island tortoises, you might want to determine whether sex affects enzyme activity, so you could also separate the data sets by sex.
- *Regression and correlation* analyses are done when a researcher wants to know whether there is a relationship or correlation between two variables and, if so, is it positive (positive slope) or negative (negative slope). For example, when patients are given increasing amounts of a drug, does their blood pressure increase or decrease proportionally? Correlation is a way to express the relationship between two variables, whereas linear regression is about the best fit line in a graph (see BioSkill 2).

You'll likely do statistical tests early in your undergraduate career. To use this textbook, though, you need to know only what statistical testing does and how to interpret a test statistic—a number that characterizes the size of the difference among the data sets.

Interpreting *P* Values and Statistical Significance

How do you use a statistical test to determine if differences are significant? Let's return to the experiment shown in Figure B3.1 and work through a three-step process:

1. Specify the null hypothesis, which is that reactant concentration has no effect on reaction rate.
2. Calculate a test statistic. In this experiment, the test statistic compares the actual differences in reaction rates at the three reactant concentrations to the difference predicted by the null hypothesis. The null hypothesis predicts that there should be no difference.
3. Determine the probability (see BioSkill 4) of getting by chance a test statistic at least as large as the one calculated. This probability, called the ***P* value,** comes from a reference distribution—a mathematical function that specifies the probability of getting various values of the test statistic if the null hypothesis is correct. The *P* value is the estimated probability of rejecting the null hypothesis when that hypothesis is correct. For example, a *P* value of 0.01 means that there is a 1 percent chance that the null hypothesis has been rejected when it is actually correct. One percent is considered a very small chance of making such an error; thus, very small *P* values indicate that researchers have high confidence in the significance of differences in their data.

By convention, most researchers consider a difference among treatment groups to be *statistically significant* if there is less than a 5 percent probability (*P*) of observing it by chance, or $P < 0.05$. When presenting *P* values in the scientific literature, researchers often use an asterisk rating system as well as quoting the *P* values (Table B3.1).

Table B3.1 Asterisk Rating System for *P* Values and Statistical Significance

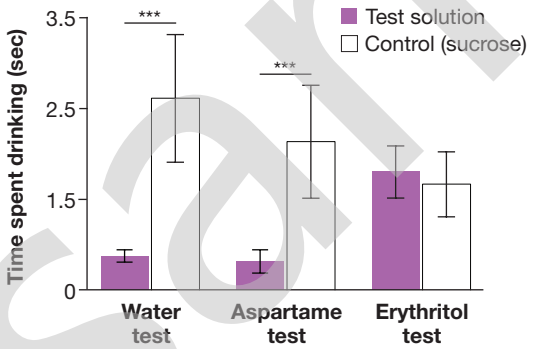
P Value	Asterisk Rating	Statistical Significance Level	Meaning
$P > 0.05$	None	Not significant	Greater than a 1 in 20 chance of being wrong (i.e., incorrect rejection of the null hypothesis)
$P < 0.05$	*	Statistically significant	Less than a 1 in 20 chance of being wrong
$P < 0.01$	**	Statistically significant	Less than a 1 in 100 chance of being wrong
$P < 0.001$	***	Statistically significant	Less than a 1 in 1000 chance of being wrong

CHECK YOUR UNDERSTANDING

If you understand BioSkill 3

✔ You should be able to …

1. **QUANTITATIVE** Determine which of the following tests used to estimate the average height of individuals in a class is likely to have the smaller standard error, and why.
   - Test 1: Measuring the height of two individuals chosen at random
   - Test 2: Measuring the height of every student who showed up for class on a particular day
2. Interpret data from a recent study in which researchers investigated the evolution of sweet taste perception in hummingbirds. Captive hummingbirds were presented with a control solution (sucrose) and a test solution (either water, the artificial sweetener aspartame, or erythritol, a substance that stimulates the sweet taste receptor). The length of time the birds spent drinking each solution was recorded. What can you conclude from the data shown in the graph below? (Hint: Consult Table B3.1 on the asterisk rating system for *P* values).



DATA: Baldwin, M. W., et al. 2014. *Science* 345: 929–933.

Answers are available in Appendix A.

You are very likely to see small differences among treatment groups just by chance. If you flipped a coin ten times, for example, you are unlikely to get exactly five heads and five tails, even if the coin is fair. A reference distribution tells you how likely you are to get, by chance, each of the possible outcomes, such as six heads and four tails.

In the case of the student researchers' experiment (see Figure B3.1), the reference distribution indicated that if the null hypothesis of no difference in reaction rates is correct, you would see differences at least as large as those observed only 0.01 percent of the time by chance ($P < 0.0001$). Because 0.0001 is less than 0.05, the students were able to conclude that the null hypothesis—that reactant concentration has no effect on reaction rate—is not correct. According to their data, the reaction they studied really does happen more rapidly when reactant concentration increases.

What does a result that is not statistically significant mean ($P > 0.05$)? You can conclude that no effect of the treatment was detected in the experiment. However, this doesn't necessarily mean there was no underlying effect. If the sample size in a study is small—particularly in a population with lots of natural variability—researchers may not detect an effect of a particular treatment, even when an effect is actually there.

When reading graphs in this book, you should take care to inspect the standard error bars. As a *very* rough rule of thumb, means often turn out to be significantly different, according to an appropriate statistical test, if there is no overlap between two times the standard errors. When you are asked to make conclusions about the significance of data shown in a graph, however, you will be provided with *P* values to interpret.

BioSkill 4 Working with Probabilities

What is probability? Probability is the chance or likelihood that an event will occur or that a hypothesis or scientific prediction is correct. In biology, probability is used to evaluate the significance of experimental results and to predict the outcome of genetic crosses.

To answer certain questions, biologists sometimes need to combine the probabilities of different events. You'll encounter examples of this when you solve genetics problems (see Chapter 14) and analyze changes in allele frequencies using the Hardy–Weinberg principle (see Chapter 23).

Two fundamental rules apply when probabilities are combined. Each rule pertains to a distinct situation.

The Both-And Rule

The both-and rule—also known as the product rule or multiplication rule—applies when you want to know the probability of two or more independent events occurring together. Let's use the rolling of two dice as an example. What is the probability of rolling two sixes? These two events are independent, because the probability of rolling a six on one die has no effect on the probability of rolling a six on the other die.

The probability of rolling a six on the first die is ⅙. The probability of rolling a six on the second die is also ⅙. The probability of rolling a six on *both* dice, then, is $⅙ \times ⅙ = 1/36$. In other words, if you rolled two dice 36 times, on average you would expect to roll two sixes once.

In the case of a cross between two parents heterozygous for the *R* gene (genotype *Rr*), the probability of getting a gamete (egg or sperm) with allele *R* from one parent has no effect on the probability of getting a gamete with allele *R* from the other parent. Gametes fuse randomly. The probability of a child getting allele *R* from the father is ½, and the probability of the child getting allele *R* from the mother is ½. Thus, the probability of getting both *R* alleles and having the genotype *RR* is $½ \times ½ = ¼$.

The Either-Or Rule

The either-or rule—also known as the sum rule or addition rule—applies when you want to know the probability of an event happening when there are two or more alternative ways for that event to occur. In this case, the probability that the event will occur is the sum of the probabilities of each way that it can occur.

For example, suppose you wanted to know the probability of rolling either a one or a six when you toss a die. The probability of rolling each number is ⅙, so the probability of rolling one or the other is ⅙ + ⅙ = ⅓. If you rolled a die three times, on average you'd expect to roll a one or a six once.

In the case of a cross between two parents heterozygous for the *R* gene, the probability of getting an *R* allele from the father and an *r* allele from the mother is ½ × ½ = ¼. Similarly, the probability of getting an *r* allele from the father and an *R* allele from the mother is ½ × ½ = ¼. Thus, the combined probability of getting the *Rr* genotype in either of the two ways is ¼ + ¼ = ½.

CHECK YOUR UNDERSTANDING

If you understand BioSkill 4

✔ You should be able to …

1. **QUANTITATIVE** Calculate the probability of getting four "tails" if four students each toss a coin.
2. **QUANTITATIVE** Calculate the probability of getting a two, a three, or a six after a single roll of a die.

Answers are available in Appendix A.

BioSkill 5 Using Logarithms

You will encounter logarithms at several points in this text. Logarithms are a way of working with powers—numbers that are multiplied by themselves one or more times.

Logarithms are useful when you are studying something that can have a large range of values, like the concentration of hydrogen ions in a solution or the intensity of sound that the human ear can detect. In cases like these, it's convenient to express the numbers involved as exponents. Using exponents makes a large range of numbers more manageable. For example, instead of saying that the hydrogen ion concentration in a solution can range from 10^0 to 10^{-14}, the logarithmic pH scale allows you to simply say that it ranges from 0 to 14. Instead of giving the actual value, the pH scale expresses concentration as an exponent.

Scientists use exponential notation to represent powers. For example,

$$a^x = y$$

means that if you multiply a by itself x times, you get y. In exponential notation, a is called the base and x is called the exponent. The entire expression is called an exponential function.

What if you know y and a, and you want to know x? This is where logarithms come in. You can solve for exponents by using logarithms:

$$x = \log_a y$$

This equation reads that x is equal to the logarithm of y to the base a. Logarithms are a way of working with exponential functions. They are important because so many processes in biology (and in chemistry and physics, for that matter) are exponential. To understand what's going on, you have to describe the process with an exponential function and then use logarithms to work with that function.

Although a base can be any number, most scientists use just two bases when they employ logarithmic notation: 10 and e (sometimes called Euler's number after Swiss mathematician Leonhard Euler). What is e? It is the limit of $(1 + 1/n)^n$ as n tends to infinity. Mathematicians have shown that the base e is an irrational number (like π) that is approximately equal to 2.718. Like 10, e is just a number; $10^0 = 1$ and, likewise, $e^0 = 1$. Both 10 and e have qualities that make them convenient to use in science.

Logarithms to the base 10 are so common that they are usually symbolized in the form $\log y$ instead of $\log_{10} y$. A logarithm to the base e is called a natural logarithm and is symbolized as ln (pronounced *EL-EN*) instead of log. You write "the natural logarithm of y" as $\ln y$.

Most scientific calculators have keys that allow you to solve problems involving base 10 and base e. For example, if you know y, they'll tell you what $\log y$ or $\ln y$ are—meaning that they'll solve for x in our first example equation. They'll also allow you to find a number when you know its logarithm to base 10 or base e. Stated another way, they'll tell you what y is if you know x, and y is equal to e^x or 10^x. This process is called finding an antilog. In most cases, you'll use the inverse or second function button on your calculator to find an antilog (above the log or ln key).

To get some practice with your calculator, consider this equation:

$$10^2 = 100$$

If you enter 100 in your calculator and then press the log key, the screen should say 2. The logarithm tells you what the exponent is. Now press the antilog key while 2 is on the screen. The screen should return to 100. The antilog solves the exponential function, given the base and the exponent.

If your background in algebra isn't strong, you'll want to get more practice working with logarithms because you'll see them frequently during your undergraduate career. Remember that once you understand the basic notation, there's nothing mysterious about logarithms. They are simply a way of working with exponential functions, which describe what happens when something is multiplied by itself a number of times—like cells that replicate and then replicate again and then again.

CHECK YOUR UNDERSTANDING

If you understand BioSkill 5

✔ You should be able to …

For questions 1 and 2, use the equation $N_t = N_0 e^{rt}$ (see Chapter 51).

1. Identify the type of function this equation describes.
2. **QUANTITATIVE** Rewrite this equation after taking the natural logarithm of both sides.

For questions 3 and 4, use the equation $pH = -\log [H^+]$ (see Chapter 2).

3. **QUANTITATIVE** Calculate the pH of a solution whose $[H^+]$ is 2.75×10^{-4}.
4. **QUANTITATIVE** Determine the $[H^+]$ of a solution whose pH is 5.43.

Answers are available in Appendix A.

BioSkill 6 Separating and Visualizing Molecules

To study a molecule, you have to be able to isolate it. Isolating a molecule is a two-step process: The molecule has to be separated from other molecules in a mixture and then physically picked out or located in a purified form. Let's explore the techniques that biologists use to separate proteins and nucleic acids and then find the particular one they are interested in.

Using Electrophoresis to Separate Molecules

In molecular biology, the standard technique for separating proteins and nucleic acids is called gel electrophoresis or, simply, electrophoresis (literally, "electricity-moving"). You may be using electrophoresis in a lab for this course, and you will be analyzing data derived from electrophoresis in this text.

The principle behind electrophoresis is simple. Nucleic acids carry a negative charge, as do proteins when they are denatured and coated with a charged (ionic) detergent. As a result, these molecules move when placed in an electric field. Negatively charged molecules move toward the positive electrode; positively charged molecules move toward the negative electrode.

An Example "Run" Figure B6.1 shows an electrophoresis setup. To separate a mixture (sample) of macromolecules so that each one can be isolated and analyzed, researchers add the sample to a gelatinous substance ("gel") consisting of long molecules that form a matrix of fibers. The matrix has pores that act like a sieve through which the molecules in the sample can pass.

As shown in step 1, each sample is placed in a slot ("well") in a sheet or slab of the gel. In many cases, researchers also fill a well with a sample containing proteins or DNA molecules of known size, called a size standard or "ladder."

In step 2, the gel is immersed in a solution that conducts electricity. When an electric field is applied across the gel, the molecules in each well move through the gel toward the positive electrode, forming a lane. Molecules that are smaller or more highly charged for their size move faster through the sieve than do larger or less highly charged molecules. As they move, then, the molecules separate by size or by charge. Small or highly charged molecules end up near the bottom of the gel; large or less-charged molecules remain near the top.

Once molecules of different size or charge have separated from one another, the electric field is removed by turning off the power supply (step 3).

Is charge or size more important in separating molecules by electrophoresis? When it comes to nucleic acids, the answer is size. The same is true for proteins that are treated with a charged detergent before they are run on a gel. In these cases, there is a fixed amount of charge for a given length of the molecule. This means that the size of the molecule determines how fast it runs on the gel. For proteins that are run without treatment with a charged detergent, size and charge work together to determine how fast they separate on a gel.

Why Do Separated Molecules Form Bands? When researchers visualize a particular molecule on a gel, using techniques described later in this BioSkill, the image that results consists of bands: lines of varying thickness that are as wide as a lane in the gel. Why?

PROCESS: GEL ELECTROPHORESIS

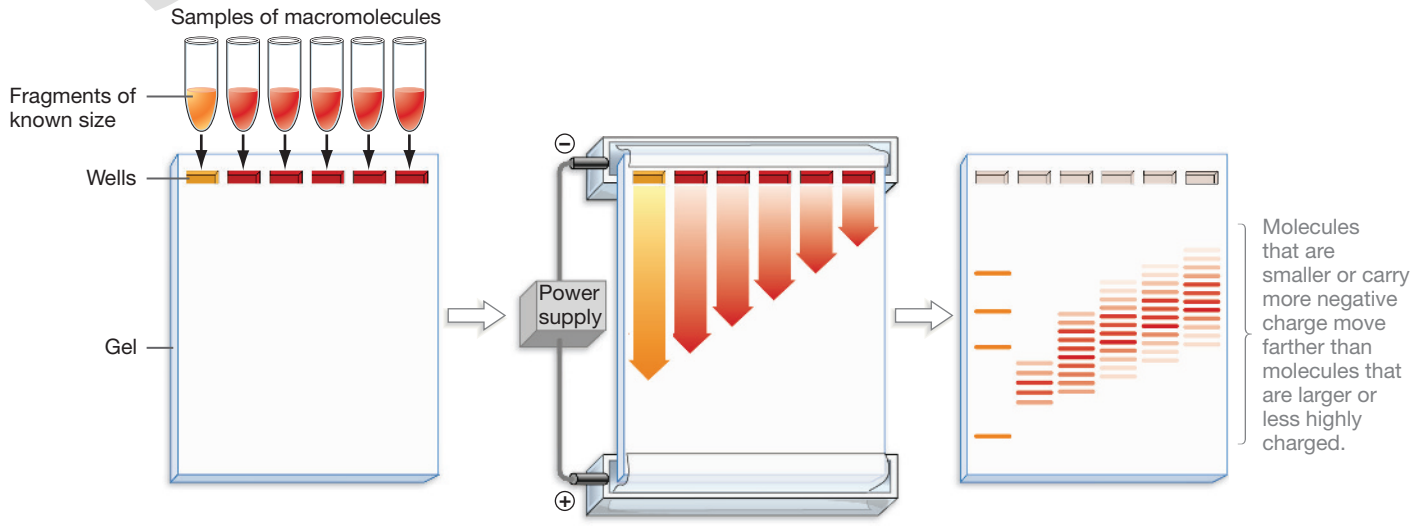


Figure B6.1 Macromolecules Can Be Separated via Gel Electrophoresis.

✔ DNA and RNA move toward the positive electrode. What makes these molecules negatively charged?

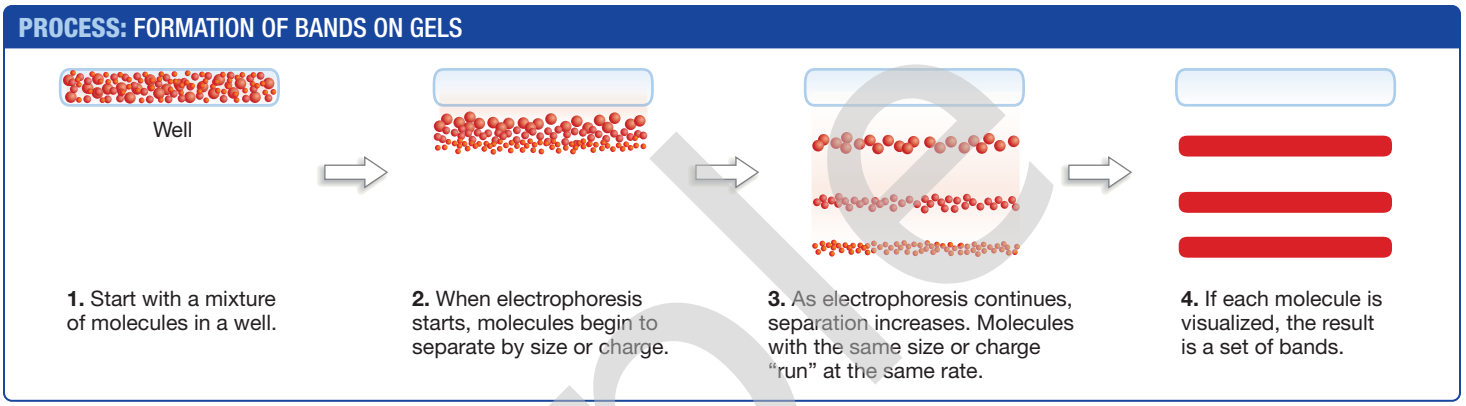


Figure B6.2 On a Gel, Molecules That Are Alike Form Bands.

To understand the answer, study **Figure B6.2**. The left panel shows the original mixture of molecules. In this diagram, the size of each dot represents the size of each molecule. The key is to realize that the original sample contains many copies of each specific molecule, and that these copies run down the length of the gel together—meaning, at the same rate—because they have the same size or charge.

It's that simple: Molecules that are alike form a band because they stay together.

Using Thin Layer Chromatography to Separate Molecules

Gel electrophoresis is one of many ways to separate molecules. Another common method is called thin layer chromatography. This method was developed in the early 1900s by botanists who were analyzing the different-colored pigments from leaves of a plant (see Chapter 10). The name chromatography comes from the Greek words *khroma* for "color" and *graphein*, "to write."

In this method, rather than loading samples into wells in a gel, the samples are deposited or "spotted" near the bottom of a stiff support, either glass or plastic, that is coated with a thin layer of silica gel, cellulose, or a similar porous material. The coated support is then placed in a solvent. As the solvent moves up through the coating by capillary action, it carries the molecules in the samples with it. Molecules are carried at different rates, based on their size and solubility in the solvent.

Visualizing Molecules

Once molecules have been separated using electrophoresis or thin layer chromatography, they have to be detected. Although plant pigments are colored, nucleic acids and most proteins are invisible unless they are labeled in some way.

Using Radioactive Isotopes When molecular biology was getting under way, the first types of labels in common use were radioactive isotopes—forms of atoms that are unstable and release energy in the form of radiation. Radioactive isotopes can be incorporated into proteins or nucleic acids, and the radiation then can be used to detect the labeled macromolecules.

Once electrophoresis is complete, the labeled proteins or nucleic acids can be visualized by laying X-ray film over the gel. Because radiation exposes film, a black dot appears wherever a radioactive atom is located in the gel. So many black dots occur so close together that they form a dark band. This technique for visualizing macromolecules is called autoradiography.

Advances in technology have led to the development of another technique for visualizing labeled proteins or nucleic acids. In this technique, called phosphorimaging, the gel is placed on a specially coated plate in a laser scanner that then produces a digital image of the gel.

Using Fluorescent Tags Starting in the late 1990s and early 2000s, it became much more common to label macromolecules with fluorescent tags. Once electrophoresis is complete, fluorescence can be detected by exposing the gel to an appropriate wavelength of light; the fluorescent tag fluoresces, or glows, in response. (Fluorescence is explained in Chapter 10.)

Fluorescent tags have important advantages over radioactive isotopes: **(1)** They are safer to handle. **(2)** They are faster—you don't have to wait hours or days for the radioactive isotope to expose a film. **(3)** They come in multiple colors, so you can label several different molecules in the same experiment and detect them independently.

Using Nucleic Acid and Protein Stains DNA and RNA can be stained with a fluorescent dye such as ethidium bromide (EtBr). Ethidium bromide fits in and binds between the bases, causing nucleic acids to fluoresce orange when illuminated by ultraviolet light. Proteins can be detected by using silver stain or dyes such as Coomassie blue that bind to proteins in the gel.

An example of an EtBr-stained gel is shown in **Figure B6.3** on page 30. In this experiment, researchers wanted to determine the optimal temperature for primer annealing in a polymerase chain reaction (PCR; see **BioSkill 10**). The far-left lane contains DNA fragments of known size; this lane is used to estimate the size of the molecules in the other lanes, which are numbered: Lane 1 is a control sample containing no DNA template; lanes 2 through 8 are samples in which the primer annealing temperature was varied incrementally from 71°C to 51°C.

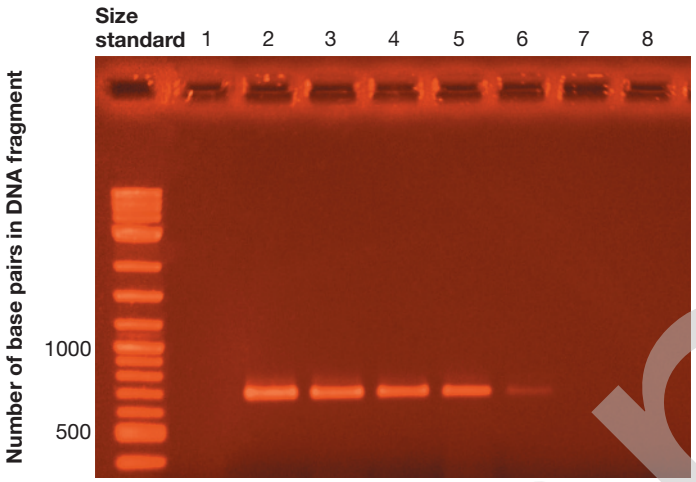


Figure B6.3 Ethidium Bromide Staining Is a Technique for Visualizing Nucleic Acids. The DNA molecules in this gel were stained with ethidium bromide and illuminated by ultraviolet light.

Reading a Gel One of the keys to interpreting, or "reading," a gel or an image of a gel is to realize that brighter or more intense bands contain more of the stain or label, indicating a greater amount of the stained or labeled molecule. Fainter bands contain less of the molecule.

To read a gel, then, you look for **(1)** the presence or absence of bands in some lanes—meaning some experimental samples—and **(2)** differences in the intensity of the bands—reflecting differences in the amount of DNA or protein present.

For example, several conclusions can be drawn from the data in Figure B6.3. A DNA fragment containing about 700 base pairs was amplified over a range of annealing temperatures (lanes 2–6). Lane 6 contained less of this fragment than lanes 2–5, and lanes 7 and 8 contained none at all. The fragment was not amplified in the absence of the DNA template (lane 1), indicating that it was specific for the DNA template used.

Using Nucleic Acid Probes In many cases, researchers want to find one specific molecule—a certain DNA sequence, for example—in a collection of molecules. How is this possible? The answer hinges on using a particular molecule as a probe. A probe is a labeled molecule that binds specifically to the molecule of interest. The label is often a radioactive atom, a fluorescent tag, or an enzyme that catalyzes a color-forming or light-emitting reaction.

For example, a nucleic acid probe is a single-stranded fragment of DNA or RNA that will bind to a particular single-stranded complementary sequence in a mixture of DNA or RNA molecules. By binding to the target sequence, the probe marks the fragment containing that sequence, distinguishing it from all the other nucleic acid fragments in the mixture. As **Figure B6.4** shows, a nucleic acid probe—in this case a labeled DNA probe—can be found after it has bound to the complementary sequence in the large mixture of fragments.

If you are looking for a particular DNA or RNA sequence on a gel, you will first need to transfer the nucleic acids to a nylon membrane by a technique called blotting.

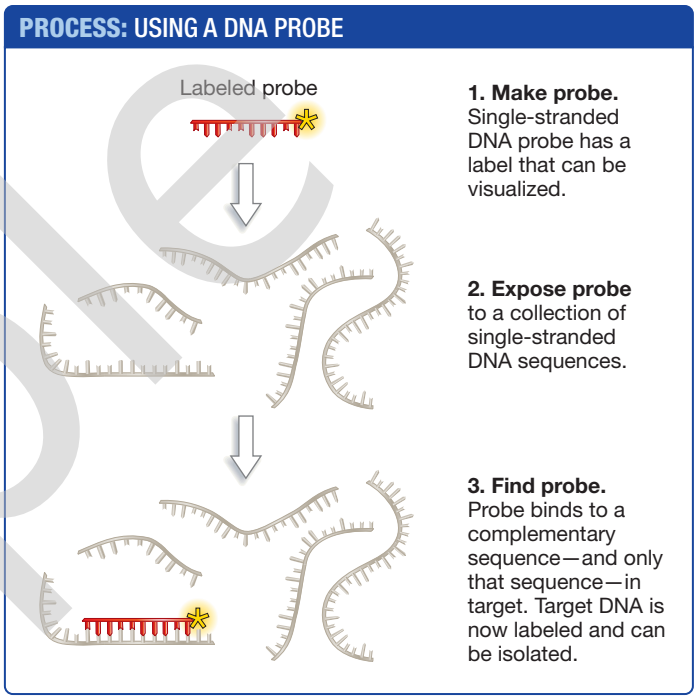


Figure B6.4 DNA Probes Bind to and Identify Specific Target Sequences.

✔ If you understand the concept of a DNA probe, you should be able to explain why the probe must be single stranded and labeled in order to work, and why it binds to just one specific fragment. You should also be able to indicate where a probe with the sequence AATCG will bind to a target DNA strand with the sequence TTTTACCCATTTACGATTGGCCT. (Recall that sequences are always written 5′ → 3′.)

- *Southern blotting*, invented by Edwin Southern, is a technique for identifying DNA segments of interest in a mixed sample. This involves making DNA fragments that have been run on a gel single stranded, transferring them from the gel to a nylon membrane, and then exposing the membrane to a single-stranded probe that binds to the target sequence by complementary base pairing. Once the probe has bound, you can detect the band that contains it through autoradiography, fluorescence, or a color change.
- *Northern blotting* is a technique for detecting target RNA segments. It involves transferring RNA fragments from a gel to a nylon membrane and then probing them to detect the segment of interest. The name is a play on Southern blotting, the protocol that it was derived from.

Using Antibody Probes How can researchers find a particular protein out of a large collection of different proteins? The answer is to use an antibody. An antibody is a protein that binds specifically to a section of a different protein (see Chapter 48 for more details on antibodies).

To use an antibody as a probe, investigators attach a tag molecule—often an enzyme that catalyzes a color-forming or light-emitting reaction—to the antibody and then add the tagged antibody to the collection of proteins. The antibody will bind to its target protein and can be visualized thanks to the tag it carries.

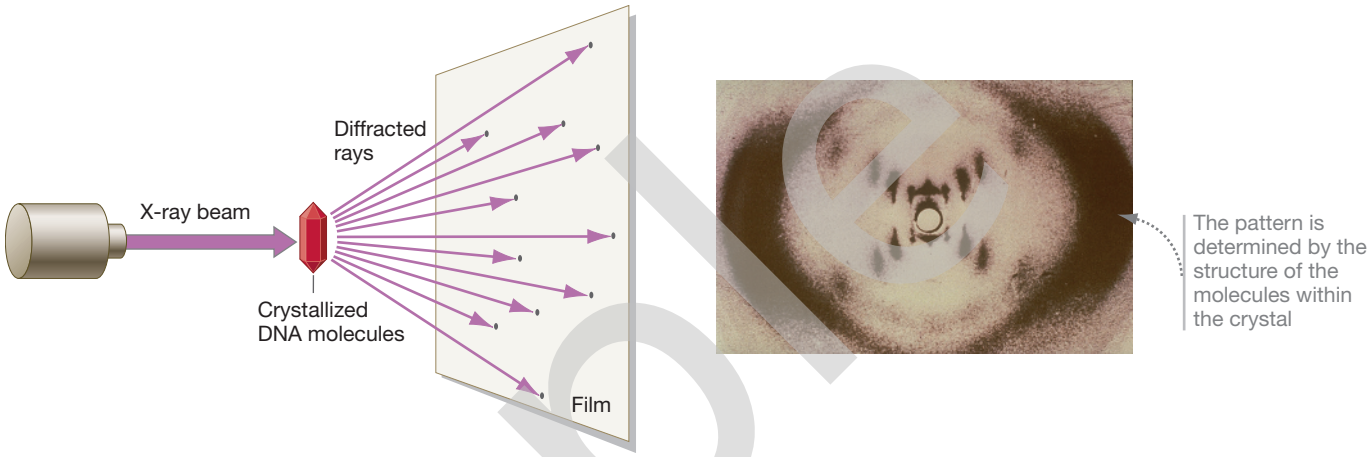


Figure B6.5 X-Ray Crystallography. When crystallized molecules are bombarded with X-rays, the radiation is scattered in distinctive patterns. The photograph at the right, obtained by Rosalind Franklin in 1953, shows an X-ray film that recorded the pattern of scattered radiation from DNA molecules.

If the proteins in question have been separated by gel electrophoresis and transferred to a membrane, the result is called a western blot. The name is an extension of the naming pattern for Southern and northern blots.

Using Radioimmunoassay and ELISA to Measure Amounts of Molecules Another important method that makes use of antibodies is called a radioimmunoassay. This method is used when investigators want to measure tiny amounts of a molecule, such as a hormone in the blood. In this case, a known quantity of a hormone is labeled with a radioactive isotope. This labeled hormone is then mixed with a known amount of antibody, and the two bind to one another. Next, a sample of blood, containing an unknown quantity of the same hormone, is added. The hormone from the blood and the radiolabeled hormone compete for antibody binding sites.

As the concentration of unlabeled hormone increases, more of it binds to the antibody, displacing more of the radiolabeled hormone. The amount of unbound radiolabeled hormone is then measured. Using known standards as a reference, the amount of hormone in the blood can be determined.

A commonly used technique based on similar principles is called enzyme-linked immunosorbent assay (ELISA). In ELISA, the amount of a particular molecule is measured using colorimetric signals instead of a radioactive signal.

Using X-ray Crystallography to Visualize Macromolecules To understand what the 3-D structure of individual macromolecules or macromolecular machines look like, researchers use a technique called X-ray crystallography, or X-ray diffraction analysis. The procedure is based on bombarding crystals of a molecule with X-rays. X-rays are scattered in precise ways when they interact with the atoms in a crystal, producing a diffraction pattern that can be recorded on X-ray film or other types of detectors (**Figure B6.5**).

By varying the orientation of the X-ray beam as it strikes a crystal and documenting the diffraction patterns that result, researchers can construct a map representing the density of electrons in the crystal. Relating these electron-density maps to information about the primary structure of the nucleic acid or protein allows researchers to build a 3-D model of the molecule. Virtually all of the molecular models used in this book were built from X-ray crystallographic data.

CHECK YOUR UNDERSTANDING

If you understand BioSkill 6

✔ **You should be able to …**

1. Consider a gel that has been stained for DNA products from a polymerase chain reaction using ethidium bromide. One lane contains no bands. Two lanes have a band in the same location, but one of the bands is very faint and the other is extremely bright. Interpret these results.
2. Explain why the effort to understand the structure of biological molecules is worthwhile even though X-ray crystallography is time-consuming and technically difficult. What's the payoff?

Answers are available in Appendix A.

BioSkill 7 Separating Cell Components by Centrifugation

Biologists use a technique called differential centrifugation to isolate specific cell components. A centrifuge accomplishes this task by spinning a cell sample in a solution that allows cell components to separate according to their density or size and shape. The individual parts of the cell can then be purified and studied in detail, in isolation from other parts of the cell.

The first step in preparing a cell sample for centrifugation is to release the cell components by breaking the cells apart. This can be done by putting them in a dilute (hypotonic) solution, by exposing them to high-frequency vibration, by treating them with a detergent, or by grinding them up. Each of these methods breaks apart plasma membranes and releases the contents of the cells.

The resulting pieces of plasma membrane quickly reseal to form small vesicles, often trapping cell components inside. The suspension that results from the homogenization step is a mixture of these vesicles, free-floating macromolecules released from the cells, and organelles. A suspension like this is called a cell extract or cell homogenate.

When a cell homogenate is placed in a centrifuge tube and spun at high speed, the suspended components move toward the bottom of the tube, along the red arrows in **Figure B7.1a**. The effect is similar to a merry-go-round, which seems to push you away from the spinning platform. At the same time, the solution in the tube exerts a centripetal (literally, "center-seeking") force that pushes the homogenate away from the bottom of the tube. Larger, denser components resist this inward force more readily than do smaller, less dense ones and so reach the bottom of the tube faster.

To separate the components of a cell extract, researchers often perform a series of centrifuge runs. Steps 1 and 2 of **Figure B7.1b** illustrate how an initial run at low speed causes larger, heavier parts of the homogenate to move below smaller, lighter parts. The material that collects at the bottom of the tube is called the pellet, and the solution and components left behind form the supernatant ("above-swimming"). The supernatant is placed in a fresh tube and centrifuged at increasingly higher speeds and longer durations. Each centrifuge run continues to separate cell components based on their size and density.

To separate macromolecules or organelles (for a list of eukaryotic cell components, see Summary Table 7.2), researchers carry out centrifugation at extremely high speeds. They also may fill the centrifuge tube with a series of sucrose solutions of decreasing density, starting with the highest density at the bottom of the tube (**Figure B7.1c**). The resulting density gradient allows cell components to separate on the basis of small differences in size, shape, and density. When the centrifuge run is complete, each cell component occupies a distinct band of material in the tube, based on where that component settled in the density gradient. A researcher can collect the material in each band for further study.

CHECK YOUR UNDERSTANDING

If you understand BioSkill 7

✔ **You should be able to …**

1. List the physical properties of molecules or cell components that allow their separation via centrifugation.
2. State which cell component—ribosomes or mitochondria—you would expect to form a pellet more quickly when you centrifuge a cell homogenate at medium speed using the method shown in Fig. B7.1b. Explain why.

Answers are available in Appendix A.

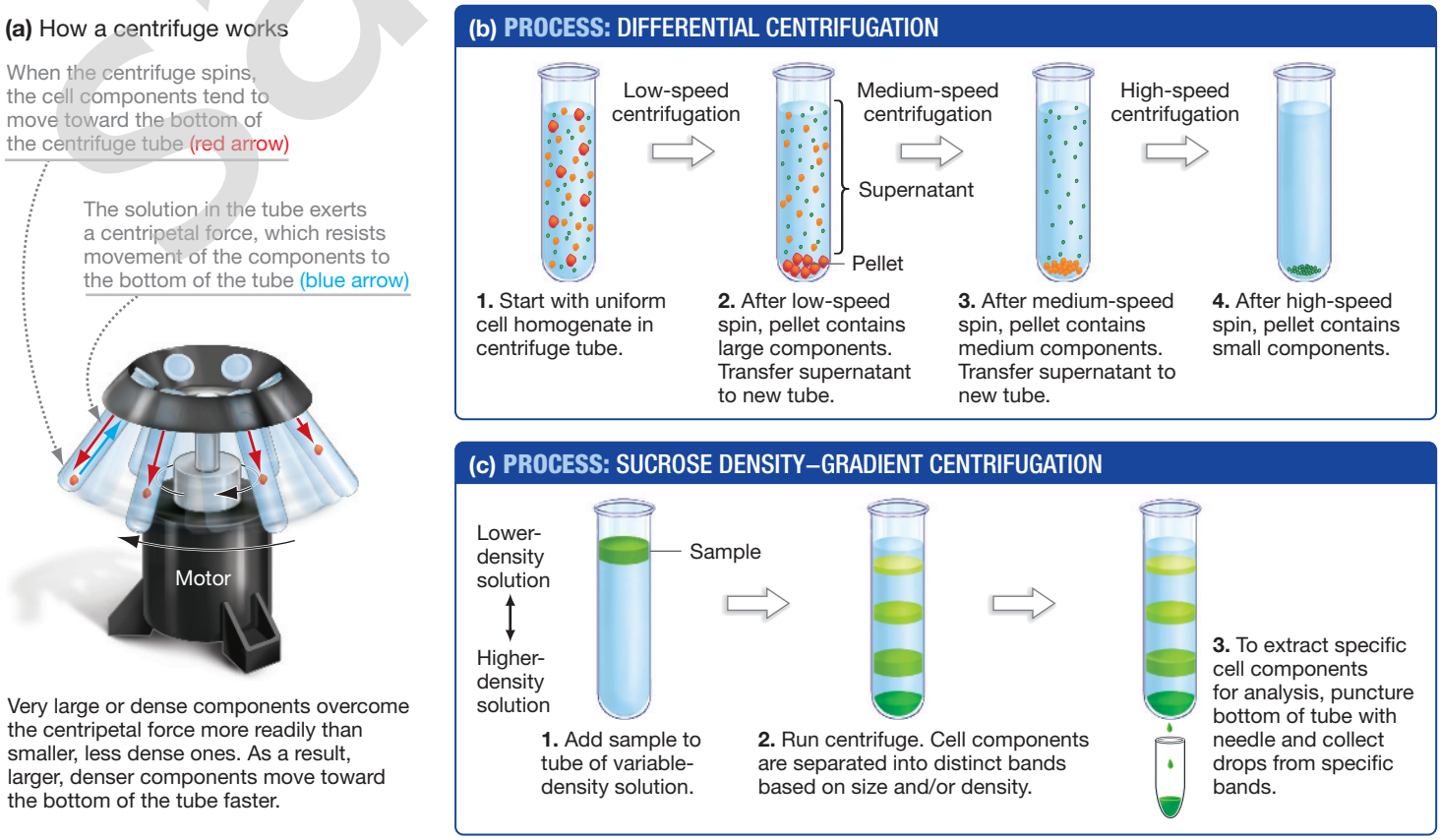


Figure B7.1 Cell Components Can Be Separated by Centrifugation. (a) The forces inside a centrifuge tube allow cell components to be separated. **(b)** Through a series of centrifuge runs made at increasingly higher speeds, an investigator can separate fractions of a cell homogenate by size via differential centrifugation. **(c)** A high-speed centrifuge run can achieve extremely fine separation among cell components by sucrose density–gradient centrifugation.

BioSkill 8 Using Spectrophotometry

Spectrophotometry is a versatile technique in which an instrument called a spectrophotometer measures light absorbance by a substance. This measurement can be used to determine the concentration of the substance. In the spectrophotometer, light is passed from a lamp through a prism or diffraction grating, which splits the light into individual wavelengths (**Figure B8.1**). A moveable slit is then positioned to allow only light of a single wavelength to reach the sample, which is placed in the light path in a transparent cuvette or test tube. On the other side of the sample holder is a detector that measures the amount of transmitted light that got through the sample. This value is then converted into the amount of light absorbance.

In the lab, you may use spectrophotometry in the following tasks: **(1)** calculating the concentration of DNA, RNA, or proteins in a solution; **(2)** following the growth of bacterial cells; **(3)** quantifying the amount of photosynthesis occurring in chloroplasts, or **(4)** determining the rate of an enzyme-catalyzed reaction. Let's examine the last example in more detail.

If an enzyme-catalyzed reaction produces a colored product or destroys a colored substrate, a spectrophotometer can measure how much of that product or substrate is present and thereby quantify the activity of the enzyme. How does this work?

Suppose an enzyme-catalyzed reaction produces a green product that absorbs light best at a wavelength of 475 nm (blue light). If a solution containing this product is placed in a spectrophotometer and illuminated with light of this wavelength, the solution will absorb most of the blue light and transmit only some of it (see Figure B8.1). A more concentrated solution will absorb more blue light than a less concentrated one, so the more colored product a solution contains, the darker it looks.

Even solutions that appear colorless may absorb specific wavelengths of light. For instance, a solution of DNA absorbs light best at a wavelength of 260 nm, which is in the ultraviolet range. There are no units for absorbance, but you should always state the wavelength used, for example, "absorbance at 260 nm."

CHECK YOUR UNDERSTANDING

If you understand BioSkill 8

✔ **You should be able to …**

Explain the relationship between absorbance and transmittance of light through the sample in the spectrophotometer shown in Figure B8.1.

Answers are available in Appendix A.

BioSkill 9 Using Microscopy

A lot of biology happens at levels that can't be detected with the naked eye. Biologists use an array of microscopes to study small multicellular organisms, individual cells, and the contents of cells.

You'll probably use dissecting microscopes and compound light microscopes to view specimens during your labs for this course, and throughout this text you'll see images generated from other types of microscopy. The key is to recognize that each approach for visualizing microscopic structures has strengths and weaknesses. As a result, each technique is appropriate for studying certain types or aspects of cells or molecules.

Light and Fluorescence Microscopy

If you use a dissecting microscope during labs, you'll recognize that it works by magnifying light that bounces off a whole specimen—often a live organism. You'll be able to view the specimen in three dimensions, which is why these instruments are sometimes called stereomicroscopes, but the maximum magnification possible is only about 20 to 40 times normal size (20× to 40×).

To view smaller objects, such as wet mounts or prepared slides of specimens, you'll probably use a compound microscope. Compound microscopes magnify light that passes *through* a specimen. The instruments used in introductory labs are usually capable of 400× magnification; the most sophisticated compound microscopes available can achieve magnifications of about 2000×. This is enough to view individual bacterial or eukaryotic cells and see large structures inside cells, like condensed chromosomes (see Chapter 12).

To prepare a specimen for viewing under a compound light microscope, researchers may need to slice the tissues or cells to create a section thin enough for light to pass through. The section is often stained to increase contrast and make structures visible. In many cases, different types of dyes are used to highlight different types of structures.

To visualize the location of specific proteins, such as structural or regulatory proteins, or to visualize organelles, such as mitochondria, researchers use a technique called immunostaining. After tissues or cells are prepared for viewing, the specimen is stained with fluorescently tagged antibodies. In this case, the cells are viewed under a fluorescence microscope. The fluorescing tag emits visible light when ultraviolet or other wavelengths of light are passed through the specimen. The result? Beautiful cells that glow green, red, or blue.

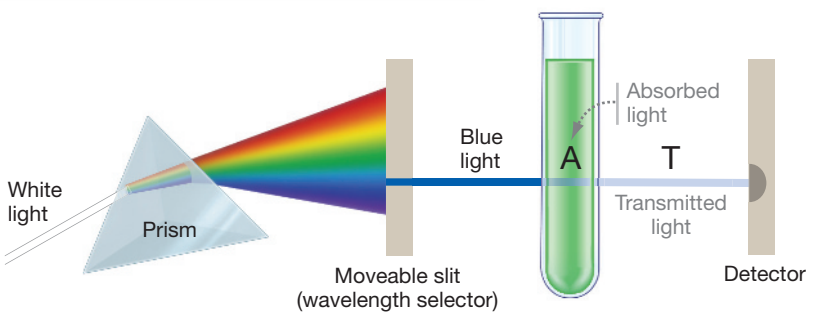


Figure B8.1 How a Spectrophotometer Works.

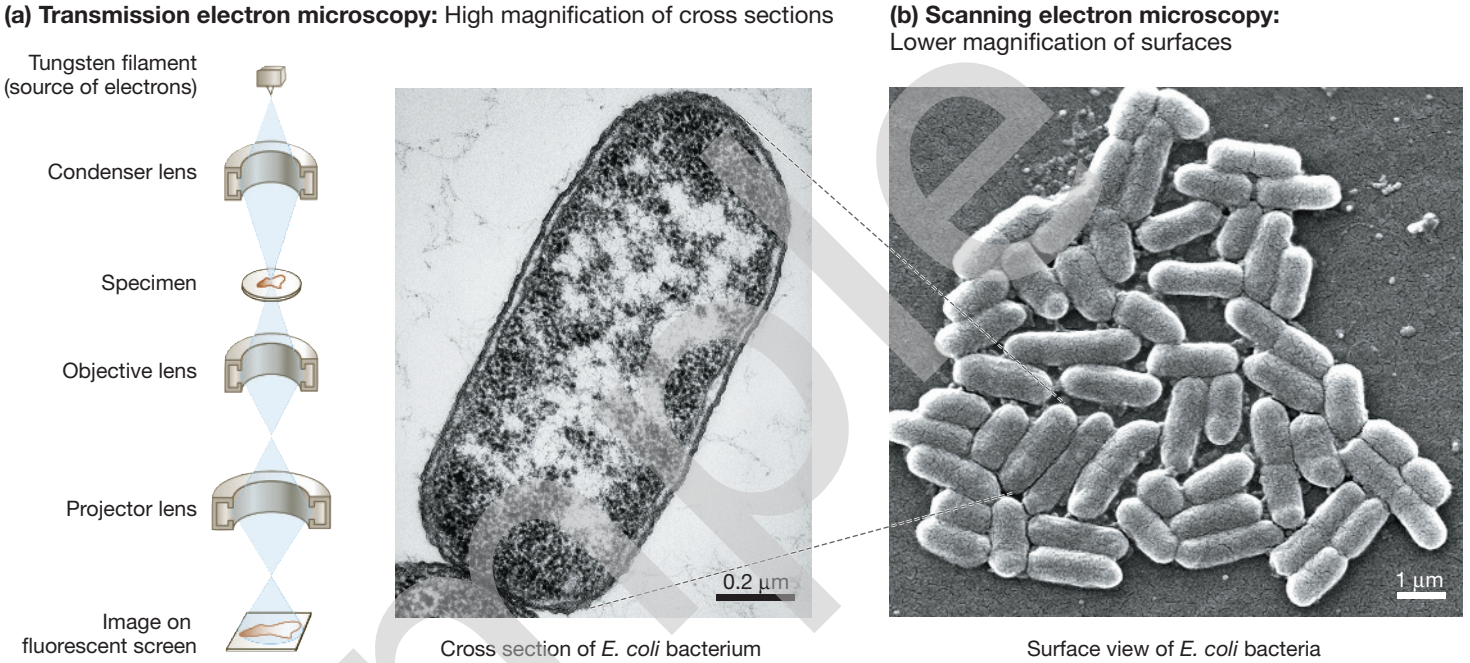


Figure B9.1 There Are Two Basic Types of Electron Microscopy.

Electron Microscopy

Until the 1950s, the compound microscope was the biologist's only tool for viewing cells directly. But the invention of the electron microscope provided a new way to view specimens. Two basic types of electron microscopy are now available: one that allows researchers to examine very thin cross sections of cells at extremely high magnification, and one that offers a view of surfaces at somewhat lower magnification.

Transmission Electron Microscopy The transmission electron microscope (TEM) is an extraordinarily effective tool for viewing cell structure at high magnification. TEM forms an image from electrons that pass through a specimen, just as a light microscope forms an image from light rays that pass through a specimen.

Biologists who want to view a cell under a transmission electron microscope begin by "fixing" the cell, meaning that they treat it with a chemical agent that stabilizes the cell's structure and contents while disturbing them as little as possible. Then the researcher permeates the cell with an epoxy plastic that stiffens the structure. Once this epoxy hardens, the cell can be cut into extremely thin sections with a glass or diamond knife. Finally, the sectioned specimens are saturated with a metal—often lead. (The reason for this last step is explained shortly.)

Figure B9.1a outlines how the transmission electron microscope works. A beam of electrons is produced by a tungsten filament at the top of a column and directed downward. (All of the air is pumped out of the column, so that the electron beam isn't scattered by collisions with air molecules.) The electron beam passes through a series of lenses and through the specimen. The lenses are actually electromagnets, which alter the path of the beam much like a glass lens in a dissecting or compound microscope bends light. The electromagnet lenses magnify and focus the image on a screen at the bottom of the column. There the electrons strike a coating of fluorescent crystals, which emit visible light in response. The light can be detected by a digital camera; the result is a micrograph—a photograph of an image produced by microscopy.

The image itself is created by electrons that pass through the specimen. If no specimen were in place, all the electrons would pass through and the screen (and micrograph) would be uniformly bright. However, cell materials by themselves would also appear fairly uniform and bright. This is because an atom's ability to deflect electrons depends on its mass, and the hydrogen, carbon, oxygen, and nitrogen atoms that dominate biological molecules have low masses. This is why cell biologists must saturate cell sections with solutions containing heavy metals such as lead. These metals have high atomic masses and scatter electrons effectively. Different macromolecules take up the metal atoms in different amounts, so the metals function as "stains" that produce contrast for different structures. With TEM, areas that take up the most metal atoms scatter the electron beam most, producing dark areas in micrographs.

The advantage of TEM is that it can magnify objects up to 250,000×, making intracellular structures clearly visible. The downsides are that researchers are restricted to observing dead, sectioned material, and that they must take care not to distort the specimen during the preparation process.

Scanning Electron Microscopy The scanning electron microscope (SEM) is the most useful tool biologists have for looking at the surfaces of structures. Materials are prepared for scanning electron microscopy by coating their surfaces with a layer of metal atoms. To create an image of this surface, the microscope scans the surface with a narrow beam of electrons. Electrons that are reflected back from the surface or that are emitted by

the metal atoms in response to the beam then strike a detector. The detector counts these electrons and sends the signals to an amplifier. The final image is built up from the number of electrons emitted from each spot on the sample and is displayed on a screen, magnified up to 50,000×. The image is captured directly in a computer.

Because SEM records shadows and highlights, it provides images with a three-dimensional appearance (**Figure B9.1b**). It cannot magnify objects nearly as much as TEM can, however.

Studying Live Cells and Real-Time Processes

Until the 1960s, biologists were unable to get clear, high-magnification images of living cells. But a series of innovations over the past 50 years has made it possible to observe organelles and subcellular structures in action.

The development of digital imaging proved revolutionary. It allowed specimens to be viewed at higher magnification, because digital cameras are more sensitive to small differences in contrast than are the human eye. It also made it easier to keep live specimens functioning normally, because the increased light sensitivity of digital cameras allows them to be used with low illumination, so specimens don't overheat. Digital imaging also made possible the use of computers to remove out-of-focus background material and increase image clarity.

A more recent innovation was the use of a fluorescent molecule called green fluorescent protein, or GFP, which allows researchers to tag specific molecules or structures and follow their movement in live cells over time. This was a major advance over immunostaining, in which cells have to be fixed. GFP is naturally synthesized in certain species of jellyfish. By affixing GFP to another protein (using genetic engineering techniques described in Chapter 20) and expressing that protein in a live cell, investigators can follow the protein's fate over time and record its movement. For example, researchers have made video recordings of GFP-tagged proteins being transported from the rough ER through the Golgi apparatus and out to the plasma membrane. This is cell biology: the movie.

GFP's influence has been so profound that the researchers who developed its use in microscopy were awarded the 2008 Nobel Prize in Chemistry. Many other fluorescent proteins have since been developed with colors ranging from cyan (greenish blue) to yellow to red.

Visualizing Cellular Structures in 3-D

The world is three-dimensional. To understand how microscopic structures work, it is essential to understand their shapes and spatial relationships. Consider two techniques currently being used to analyze the 3-D structure of cells and organelles.

- *Confocal microscopy* is carried out by mounting a specimen that has been treated with one or more fluorescent tags on a microscope slide and then focusing a beam of light at a certain wavelength through a pinhole at a specific depth within the specimen. The tag emits light at a different wavelength in response. A detector is set up at exactly the position where the emitted light comes into focus. The result is a sharp image of a precise plane in the tissue being studied (**Figure B9.2a**). Note that if you viewed the same specimen under a conventional fluorescence microscope, the image would be blurry because it results from light emitted by the entire specimen (**Figure B9.2b**). By altering the focal plane, a researcher can record images from an array of depths in the specimen; a computer can then be used to generate a 3-D image of a cell or tissue (**Figure B9.2c**).
- *Electron tomography* uses a transmission electron microscope to generate a 3-D image of an organelle or other subcellular structure. The specimen is rotated around a single axis while the researcher takes many "snapshots." The individual images are then pieced together with a computer. This technique has provided a much more accurate view of mitochondrial structure than was possible using traditional TEM (see Figure 9.8).

(a) Confocal fluorescence image of mouse intestine

(b) Conventional fluorescence image of same tissue as in (a)

(c) Confocal 3-D image of cells forming a blood vessel

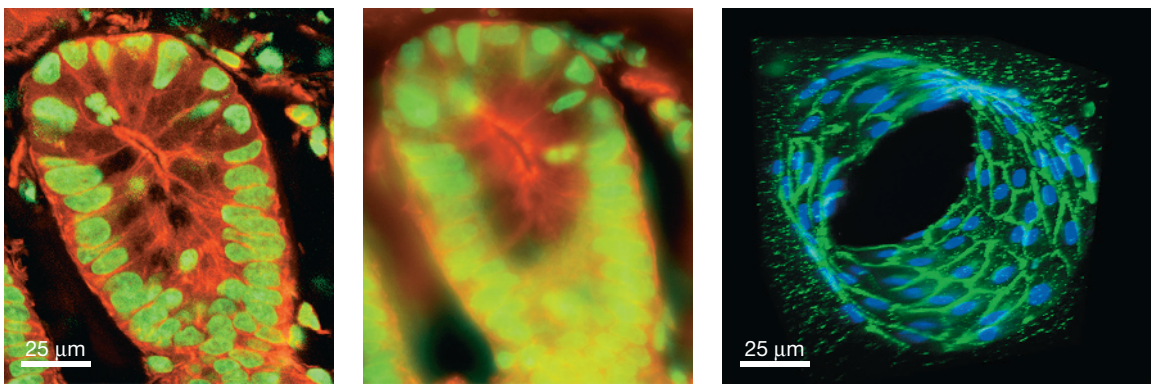


Figure B9.2 Confocal Microscopy Provides Sharp Images of Living Tissues. (a) The confocal image of this mouse intestine is sharp because it results from light emitted at a single plane within the tissue. **(b)** The conventional image of this same tissue is blurred because it results from light emitted by the entire tissue. **(c)** This 3-D confocal image was reconstructed from optical "sections" of cells forming a blood vessel.

CHECK YOUR UNDERSTANDING

If you understand BioSkill 9

✔ You should be able to …

Interpret whether the absence of mitochondria in a transmission electron micrograph of a cancerous human liver cell means that the cell lacks mitochondria.

Answers are available in Appendix A.

BioSkill 10 Using Molecular Biology Tools and Techniques

The basic tools and techniques of genetic engineering and genome analysis are revolutionary. (Some of the major breakthroughs achieved by molecular biologists are highlighted in Chapter 20). But unless you are doing research in the lab, it can be difficult to fully appreciate all the details underlying the methods employed by molecular biologists. The key is to understand some of the basic principles and steps in each technique, and then to recognize how biologists can use each technique to answer distinct questions.

Often in a molecular biology study, some of the first tools and techniques used are associated with cloning. Biologists clone a DNA region of interest to obtain millions of copies of that region for further analysis. One traditional approach is to make a collection of DNA sequences called a DNA library. Let's take a closer look.

Making and Using DNA Libraries

A collection of DNA sequences, each of which is inserted into a vector, is called a DNA library. DNA libraries are made up of cloned genes or portions of genes. Each gene can be produced in large quantity and isolated in pure form. If the sequences are fragments of DNA from the genome of an individual, the library is called a genomic library. If the sequences are complementary DNA (cDNA)—DNA copies of mRNAs made by a particular cell type or tissue—the library is called a cDNA library. How is a cDNA library made?

Creating a cDNA Library The enzyme reverse transcriptase catalyzes the synthesis of cDNA from an RNA template. This cDNA can then be used to make a cDNA library, as shown in **Figure B10.1**. The end result, shown in step 5, is a collection of transformed bacterial cells. Each of the cells contains a plasmid with one cDNA from the initial mRNAs isolated from a particular cell type or tissue.

DNA libraries are important because they give researchers a way to store DNA fragments from a particular cell type or genome in a form that is accessible for gene cloning. But like a college library, a DNA library isn't very useful unless there is a way to retrieve specific pieces of information. At your school's library, you use call numbers or computer searches to retrieve a particular book or article.

How do you go about retrieving a particular cDNA from a library?

Finding a Particular cDNA in a Library Molecular biologists are often faced with the task of finding one specific cDNA or gene in a large collection of DNA fragments. To do this requires a probe—a labeled molecule that binds to the molecule the biologist is looking for (see **BioSkill 6**).

PROCESS: CREATING A cDNA LIBRARY

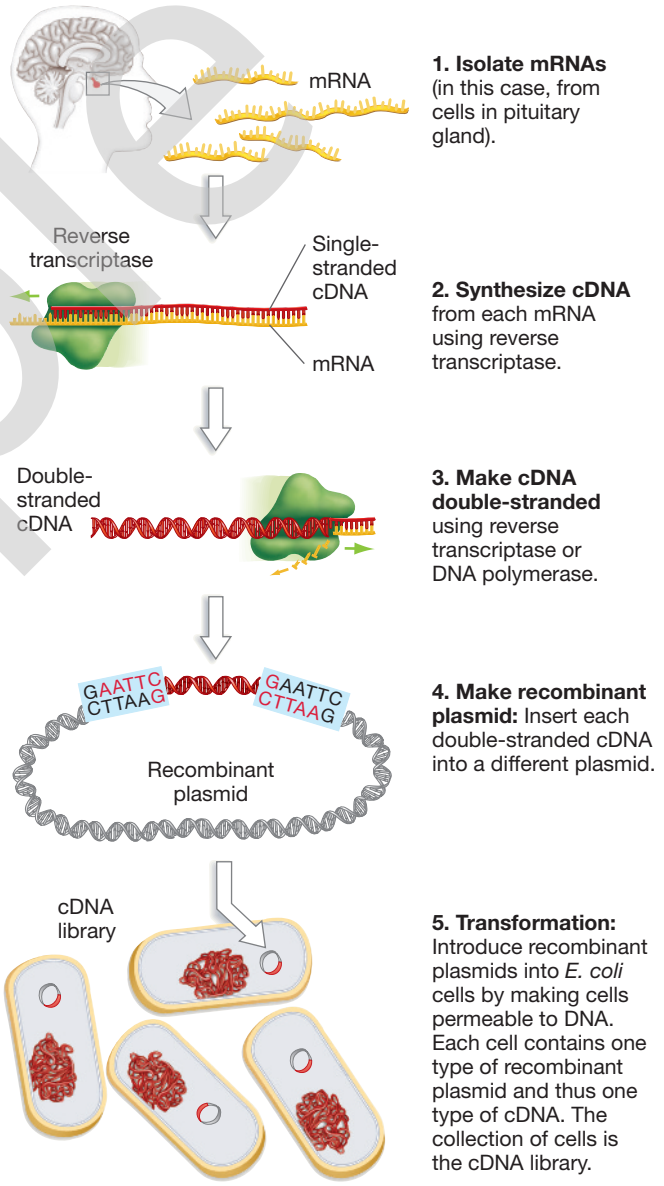


Figure B10.1 Complementary DNA (cDNA) Libraries Represent a Collection of the mRNAs in a Cell.

✔ Would each type of cDNA in the library be represented just once? Why or why not?

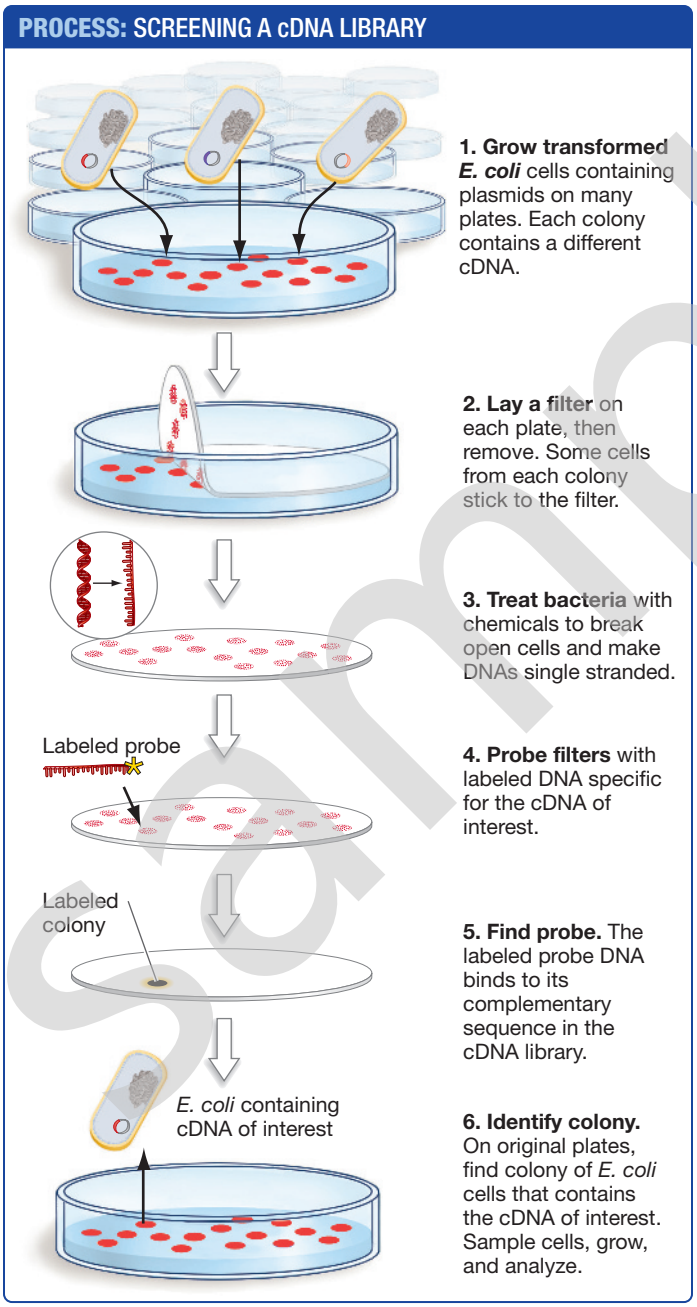


Figure B10.2 Finding a Specific cDNA by Probing a cDNA Library.

Figure B10.2 shows how researchers use a probe to find a particular plasmid in a cDNA library. The labeled probe will bind to its complementary sequence in the library. In this way, the recombinant cell that contains the specific cDNA of interest can be identified by the researchers.

Another powerful technique for making lots of identical copies of (amplifying) a particular region of DNA is the polymerase chain reaction. The amplified DNA can be used for cloning into a plasmid vector or for many other types of analyses (see Chapter 20).

Let's examine how PCR works.

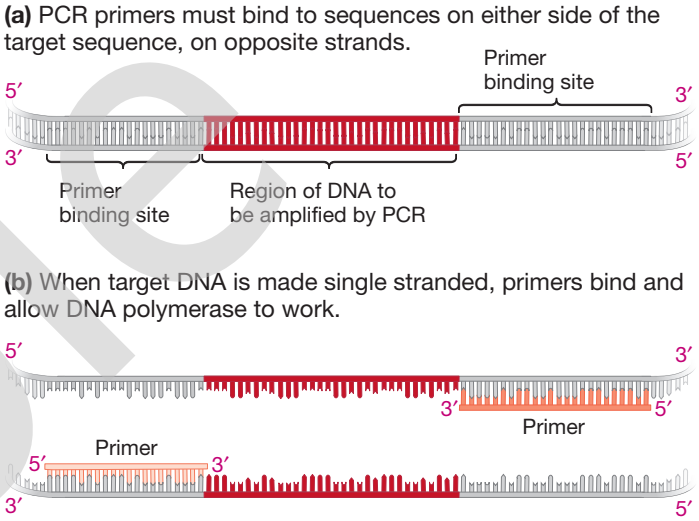


Figure B10.3 The Polymerase Chain Reaction Requires Appropriate Primers. (a) To design an appropriate primer, the base sequences at the primer binding sites must be known. **(b)** The primers bind by complementary base pairing to single-stranded target DNA.

✓ **MODEL** Indicate where DNA polymerase would begin to work on each strand; add an arrow indicating the direction of DNA synthesis.

Amplifying DNA Using the Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is an in vitro DNA synthesis reaction that uses DNA polymerase to replicate a specific section of DNA over and over.

DNA polymerase cannot work without a primer (see Chapter 15). As Figure B10.3a shows, the primer sequences used must be complementary to bases on either side of the target region—the DNA you want to copy. One primer is complementary to a sequence on one side of the target DNA; the other primer is complementary to a sequence on the opposite strand of DNA, on the other side of the target region. If the target DNA molecule is made single stranded, then the primers will bind to their complementary sequence, as shown in Figure B10.3b. Once the primers are bound, DNA polymerase can extend each new strand of DNA in the 5′ → 3′ direction.

Figure B10.4 on page 38 shows the steps involved in the polymerase chain reaction.

Step 1 The researcher creates a reaction mix containing an abundant supply of the four deoxyribonucleoside triphosphates (dNTPs; see Chapter 15), a DNA sample that includes the target DNA of interest, many copies of the two primers, and a heat-resistant DNA polymerase.

Step 2 The reaction mix is heated to 95°C. At this temperature, double-stranded DNA denatures. This means that the two DNA strands separate, forming single-stranded templates.

Step 3 The mixture is allowed to cool to 50°C–72°C, depending on the polymerase used and the primer sequences. In this temperature range, the primers bind, or anneal, to complementary portions of the single-stranded template DNA. This step is called primer annealing.

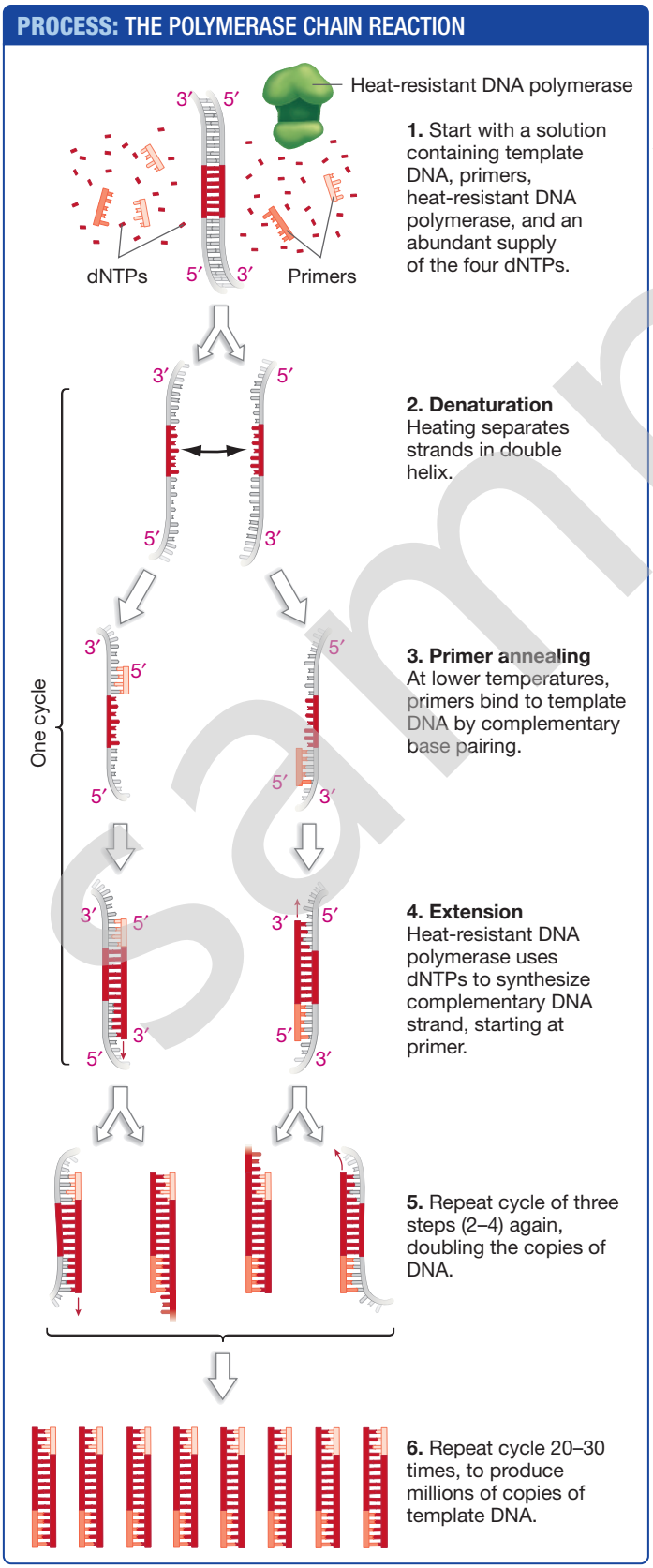


Figure B10.4 The Polymerase Chain Reaction Produces Many Copies of a Specific Sequence. Each PCR cycle (denaturation, primer annealing, and extension) results in a doubling of the number of target sequences.

Step 4 The reaction mix is heated to 72°C. At this temperature, the heat-resistant DNA polymerase efficiently synthesizes the complementary DNA strand from the dNTPs, starting at the primer. This step is called extension.

Step 5 Repeat steps 2 through 4.

Step 6 Continue repeating steps 2 through 4 until the necessary number of copies is obtained.

The temperature changes required in each step are controlled by automated PCR machines, and there is no need to add more components once the reaction starts.

The denaturation, primer annealing, and extension steps constitute a single PCR cycle. If one copy of the template sequence existed in the original sample, then two copies are present at the end of the first cycle (see step 4 in Figure B10.4). These two copies then act as templates for the second cycle—another round of denaturation, primer annealing, and extension—after which four copies of the target DNA are present (see step 5).

Each time the cycle repeats, the number of copies of template sequence in the reaction mixture doubles (step 6). Doubling occurs because each newly synthesized segment of DNA serves as a template in the subsequent cycle, along with the previously synthesized segments. Starting with a single copy, successive cycles result in the production of 2, 4, 8, 16, 32, 64, 128, 256 copies, and so on. A total of n cycles can generate 2^n copies. In just 20 cycles, one sequence can be amplified to over a million copies.

Dideoxy Sequencing

After cloning a gene or amplifying a region of DNA by PCR, molecular biologists often want to determine the DNA's base sequence. One way to do this, called dideoxy sequencing, is a clever variation on the basic in vitro DNA synthesis reaction (**Figure B10.5**).

The key is to use monomers for DNA synthesis called dideoxyribonucleoside triphosphates (ddNTPs) along with the normal deoxyribonucleoside triphosphates (dNTPs) in the reaction mix (see Chapter 15). The ddNTPs are identical to dNTPs, except they lack a hydroxyl group at their 3′ carbon. Four types of ddNTPs are used in dideoxy sequencing, each named according to whether it contains adenine (ddATP), thymine (ddTTP), cytosine (ddCTP), or guanine (ddGTP). The use of ddNTPs inspired the name dideoxy sequencing.

If a ddNTP is added to a growing DNA strand, it terminates synthesis. Why? After a ddNTP is added, no hydroxyl group is available on a 3′ carbon to link to the 5′ carbon on an incoming dNTP monomer. As a result, DNA polymerization stops once a ddNTP is added.

Every time a ddNTP is added to a growing strand, the result is a fragment with a length corresponding to the position in the template of a base complementary to the ddNTP. To produce these fragments, biologists create a reaction mix containing many copies of **(1)** the template DNA, **(2)** a primer, and **(3)** DNA polymerase, as well as **(4)** a large supply of the four dNTPs and **(5)** a small amount of the four ddNTPs (Figure B10.5, step 1). Each of the four ddNTPs carries a different fluorescent tag.

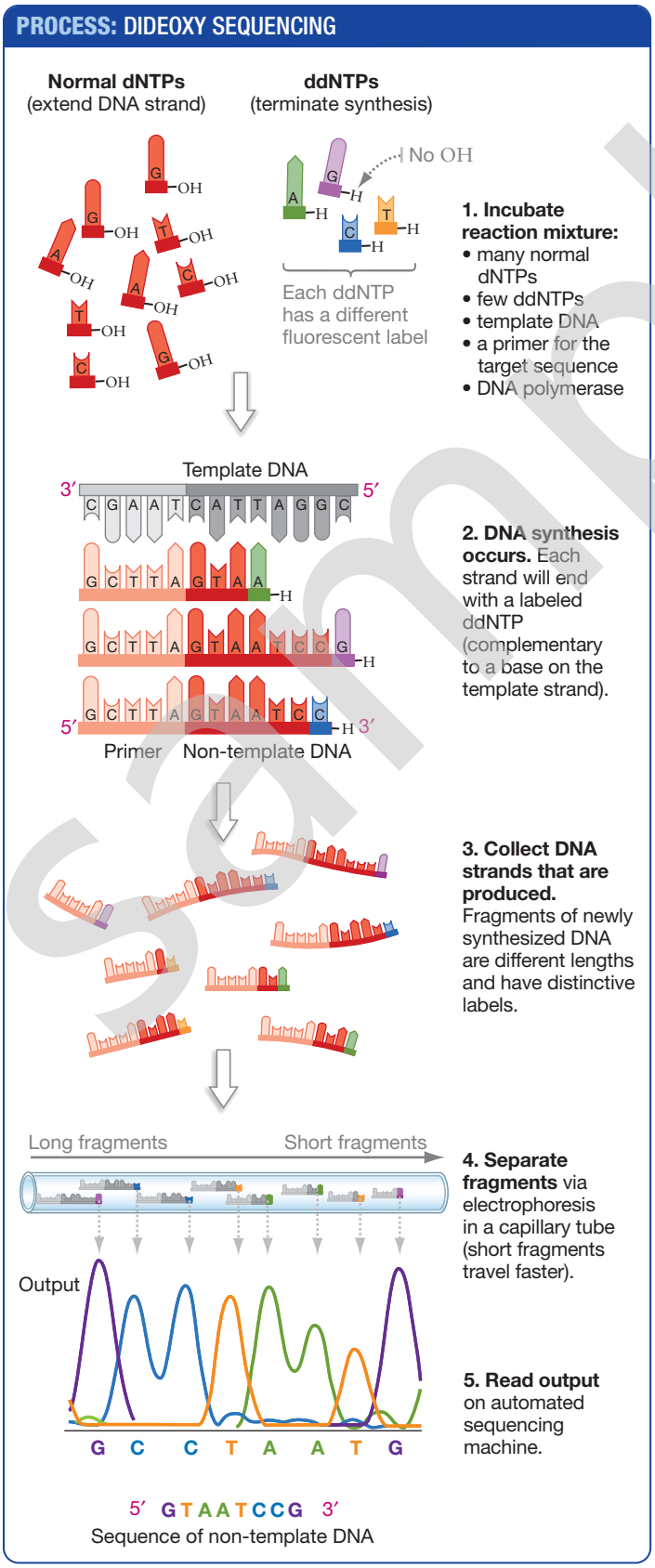


Figure B10.5 Dideoxy Sequencing Can Determine the Base Sequence of DNA.

Fluorescent molecules absorb light at one wavelength and reemit the light at a longer wavelength. As described in BioSkill 6, they provide a very sensitive way of detecting molecules.

Under these conditions, many daughter strands of different lengths are synthesized. All fragments that are the same length end in the same kind of ddNTP.

Step 2 in Figure B10.5 shows why:

- DNA polymerase synthesizes a complementary strand from each template in the reaction mix.
- The synthesis of each one of these complementary strands starts at the same point—the primer.
- Because there are many dNTPs and relatively few ddNTPs in the reaction mix, dNTPs are usually incorporated opposite each complementary base on the template strand as DNA polymerase works its way along the template strand. Incorporating a dNTP allows DNA synthesis to continue.
- Occasionally, one of the few ddNTPs is incorporated into the growing strand, opposite the corresponding base in the template. The complementary base in the template strand pairs randomly with either a ddNTP or a dNTP.
- The addition of the ddNTP stops further elongation.
- "Stops" of this kind happen for each base in the template strand. As a result, the overall reaction produces a collection of newly synthesized strands (fragments) whose various lengths correspond to the location of each base in the template strand (see step 3 in Figure B10.5). Each fragment will fluoresce in the color of its ddNTP.

Using gel electrophoresis in a capillary tube (step 4 in Figure B10.5), biologists can line up the fragments in order of size. When the fragments are lined up by size, the incorporated dideoxy nucleotides on the successive fragments reveal the sequence of bases in the template DNA. As step 5 shows, a machine can read the pattern of fluorescence, indicating the sequence of bases in the newly synthesized strand.

Sequencing a cDNA clone or PCR product is technically straightforward, but how can researchers sequence an entire genome?

Shotgun Sequencing

When researchers first set out to sequence the genome of a species, they usually relied on an approach known as shotgun sequencing. Molecular biologists still use shotgun sequencing, although newer sequencing technologies are streamlining the process. Let's take a look at key steps in the traditional method:

Step 1 Application of high-frequency sound waves, or sonication, is used to break a genome randomly into pieces about 160 kilobases (kb) long (1 kb = 1000 bases).

Step 2 Each 160-kb piece is inserted into an engineered version of a bacterial chromosome, called a bacterial artificial chromosome (BAC), that can be used as a cloning vector. BACs are able to replicate large segments of DNA. Each BAC is then inserted into a different bacterial cell. By allowing each cell to

grow into a colony, researchers can isolate large numbers of each 160-kb fragment.

Step 3 After many copies of each 160-kb fragment have been produced, each cloned DNA is again broken into fragments—but this time, the fragments are about 1 kb long.

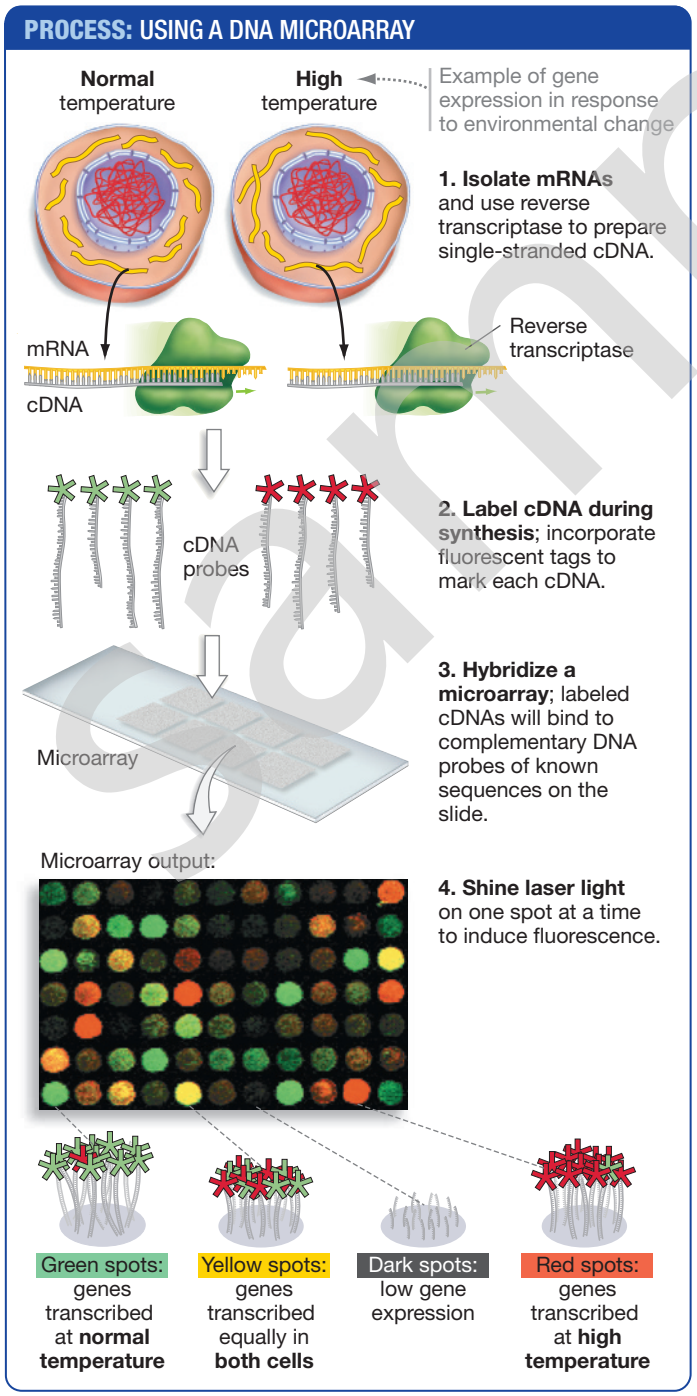


Figure B10.6 DNA Microarrays Can Be Used to Study Changes in Gene Expression. By hybridizing a microarray with labeled cDNAs synthesized from mRNAs, researchers can identify which sequences are being transcribed. Here cDNAs made from cells growing at normal temperature have a green tag, while cDNAs made from cells growing at high temperature have a red tag.

Step 4 These small fragments are then inserted into plasmids and placed inside bacterial cells. The plasmids are copied many times as each cell grows into a large population.

Step 5 Next, the cloned 1-kb fragments from each 160-kb BAC clone are sequenced, and computer programs analyze regions where the ends of different 1-kb fragments overlap. Overlaps occur because many copies of each 160-kb segment were made and then fragmented randomly by sonication.

Step 6 Based on the overlaps between 1-kb fragments from a single BAC clone, the computer stitches the sequences together until a continuous sequence across the BAC has been reconstructed.

Step 7 The ends of the reconstructed BACs are analyzed in a similar way. The goal is to link sequences from each 160-kb segment based on regions of overlap until the sequence of an entire genome is assembled.

In essence, the shotgun strategy consists of breaking a genome into many small fragments, sequencing each fragment, and then putting the sequence data back in the correct order. Whether the approach is traditional or modern, this principle holds.

Once genes are cloned and sequenced, researchers can then begin to address important questions about how genes function. (Chapter 20 covers some exciting recent discoveries.) Let's examine one method that allows researchers to find out how and when all the genes in an organism are expressed.

DNA Microarray

A DNA microarray lets researchers study the expression of thousands of genes at a time. The microarray consists of as many as 1 million different single-stranded DNA segments that are permanently attached at one end to a glass slide or silicon chip. The DNA sequence of each segment is known, as is its location on the slide or chip. Each segment serves as a probe for a specific transcript.

A typical experiment done with a DNA microarray follows the steps outlined in **Figure B10.6**. For example, suppose researchers wanted to learn how gene expression in a certain kind of cell is altered to meet the challenges of heat stress. They would begin by isolating mRNAs produced in control cells functioning at normal temperature and in cells of the same kind exposed to high temperatures (step 1).

Once they purified mRNAs from the two populations of cells, the researchers would use reverse transcriptase to make a single-stranded cDNA version of each RNA in the two samples. One of the nucleotides in the cDNA would carry a fluorescent tag (step 2). The tag used for the control cells would fluoresce one color (let's say green), while the tag for the heat-stressed cells would fluoresce another color (let's say red). The labeled cDNAs of both colors would then be added to the microarray, where they would bind to complementary DNA probes (step 3). This step is called hybridization because hybrids would form between probe DNAs and cDNAs.

Out of all the probes present on the microarray, then, only those that represent genes being expressed by the two populations of cells will be labeled on the microarray. In this example,

genes that are expressed by the control cells at the normal temperature will be labeled green, while those expressed by the cells during heat stress will be labeled red. If a gene is expressed under both sets of conditions, then both green- and red-labeled cDNAs will bind to the DNA in that spot on the microarray, and the spot will appear yellow (step 4).

CHECK YOUR UNDERSTANDING

If you understand BioSkill 10

✔ **You should be able to …**

1. Explain why no further nucleotides can be added after a ddNTP molecule is added to a growing chain of DNA.
2. Consider the following scenario: Suppose a friend of yours is doing a series of PCRs and comes to you for advice. She purchased two sets of primers, hoping that one set would amplify the template sequence shown here. (The dashed lines in the template sequence stand for a long sequence of unspecified bases in the target gene.) Neither of the primer pairs produced any product DNA, however.

	Primer a	Primer b
Primer Pair 1:	5′-CAAGTCC-3′	and 5′-GCTGGAC-3′
Primer Pair 2:	5′-GGACTTG -3′	and 5′-GTCCAGC-3′
Template:	5′-ATTCGGACTTG—GTCCAGCTAGAGG-3′ 3′-TAAGCCTGAAC—CAGGTCGATCTCC-5′	

   a. Explain why each primer pair didn't work. Indicate whether both primers are at fault, or just one of them.
   b. Your friend doesn't want to buy new primers. She asks you whether she can salvage this experiment. What should you tell her to do?
3. Explain how you would use a DNA microarray to compare the genes expressed in human brain cells with those expressed in human liver cells.

Answers are available in Appendix A.

BioSkill 11 Using Cell Culture and Model Organisms as Tools

Research in biological science starts with a question. In most cases, the question is inspired by an observation about a cell or an organism. To answer it, biologists often study cells or tissues in culture. At other times, they perform experiments on model organisms—aptly named because these organisms are intended to serve as models for what is going on in a wide array of species. Let's look at each approach in turn.

Cell and Tissue Culture Methods

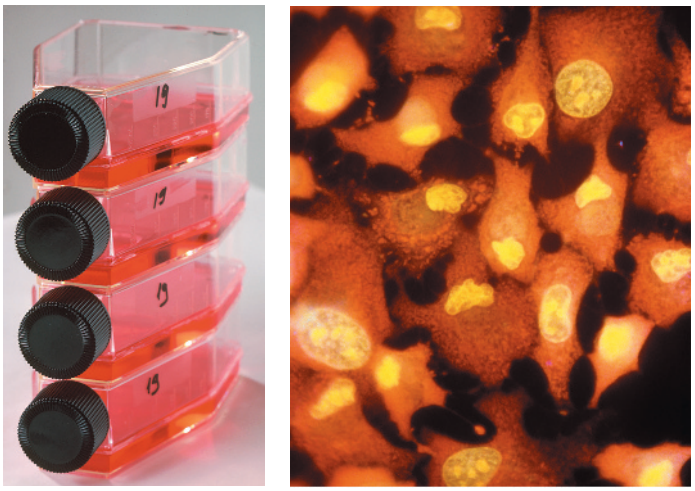
For researchers, there are important advantages to culturing plant and animal cells and tissues. Culturing involves growing a cell or tissue outside the organism itself. Cell and tissue cultures produce large populations of a single type of cell or tissue and enable biologists to control experimental conditions precisely.

Animal Cell Culture In 1907, a researcher successfully cultivated amphibian nerve cells in a drop of fluid from the spinal cord, but biologists weren't able to routinely culture animal cells in the laboratory until the 1950s and 1960s. It took years to figure out how to re-create the conditions that exist in the intact organism precisely enough for cells to grow normally.

To grow in culture, animal cells must be provided with a liquid mixture containing the nutrients, vitamins, and hormones that stimulate growth. Typically, this mixture is serum, the liquid portion of blood. Serum-free media that are much more precisely defined chemically are available for certain cell types.

Moreover, many types of animal cells will not grow in culture unless they are on a solid surface that mimics the types of surfaces they would adhere to in the intact animal. As a result, animal cells are typically cultured in flasks with special coatings (**Figure B11.1a**, left).

(a) Animal cell culture: immortal HeLa cancer cells



(b) Plant tissue culture: tobacco callus

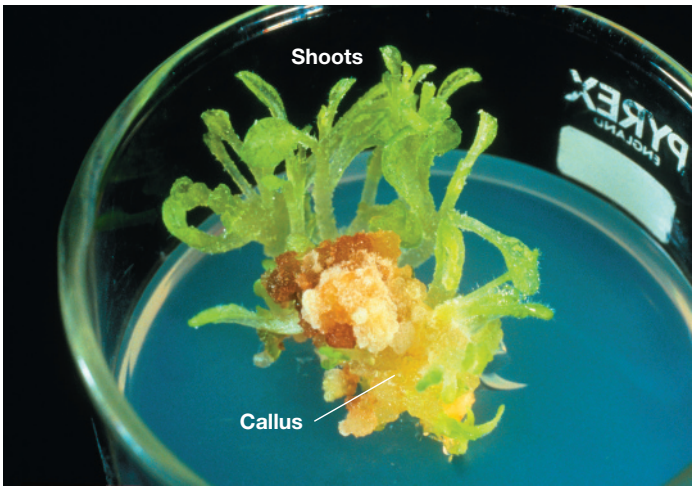


Figure B11.1 Animal and Plant Cells Can Be Grown in the Lab.

Even under optimal conditions, though, normal cells have a finite life span in culture. In contrast, many cultured cancerous cells grow indefinitely. This characteristic correlates with a key feature of cancerous cells in organisms: Their growth is continuous and uncontrolled.

The first human cell type to be grown in culture was isolated in 1951 from a woman with a malignant tumor of the uterine cervix. These cells are called HeLa cells in honor of their donor, Henrietta Lacks, who died soon after from her cancer. HeLa cells continue to grow in laboratories around the world (see the micrograph on the right in Figure B11.1a).

Because of their immortality and relative ease of growth, cultured cancer cells are commonly used in research on basic aspects of cell structure and function.

Plant Tissue Culture Certain cells found in plants are totipotent—meaning that they retain the ability to divide and differentiate into a complete, mature plant, including new types of tissue. These cells, called parenchyma cells, are important in wound healing and asexual reproduction. They also allow researchers to grow complete adult plants in the laboratory, starting with a small number of parenchyma cells.

Biologists who grow plants in tissue culture begin by placing parenchyma cells in a liquid or solid medium containing all the nutrients required for cell maintenance and growth. In the early days of plant tissue culture, as for animal cells, investigators found that successful growth and differentiation depended not only on the presence of specific hormones but also on their relative abundance.

The earliest experiments on hormone interactions in plant tissue cultures were done with tobacco cells in the 1950s. Researchers found that when they added roughly equal amounts of the hormones auxin and cytokinin to the cells, the cells began to divide and eventually formed an undifferentiated mass of parenchyma cells called a callus. By varying the proportion of auxin to cytokinin in different parts of the callus and through time, researchers could stimulate the growth and differentiation of root and shoot systems and produce whole new plants (**Figure B11.1b**).

The ability to grow a whole plant in tissue culture from a single cell has been instrumental in the development of genetic engineering (see Chapter 20). Researchers insert recombinant genes into target cells, test the cells to identify those that successfully express the recombinant genes, and then use tissue culture techniques to grow those cells into adult individuals with novel genotypes and phenotypes.

Model Organisms

Model organisms are chosen because they are convenient to study, and because they each have attributes that make them appropriate for the particular type of research proposed.

They tend to have some common characteristics:

- ***Short generation time and rapid reproduction*** This trait is important because it makes it possible to produce offspring quickly and perform many experiments in a short amount of time—you don't have to wait long for individuals to grow.
- ***Large numbers of offspring*** This trait is particularly important in genetics, where many offspring phenotypes and genotypes need to be assessed to get a large sample size.
- ***Small size and simple feeding and habitat requirements*** These attributes make it relatively cheap and easy to maintain individuals in the lab.

The following examples highlight just a few model organisms supporting current work in biological science.

Escherichia coli Of all model organisms in biology, perhaps none has been more important than the bacterium *Escherichia coli*—a common inhabitant of the human gut. The strain that is most commonly worked on today, called K-12 (**Figure B11.2a**), was originally isolated from a hospital patient in 1922.

During the last half of the twentieth century, key results in molecular biology originated in studies of *E. coli*. These results include the discovery of enzymes such as DNA polymerase, RNA polymerase, DNA repair enzymes, and restriction endonucleases; the elucidation of ribosome structure and function; and the initial characterization of promoters, regulatory transcription factors, regulatory sites in DNA, and operons. In many cases, initial discoveries made in *E. coli* allowed researchers to confirm that homologous enzymes and processes existed in an array of organisms, ranging from other bacteria to yeast, mice, and humans.

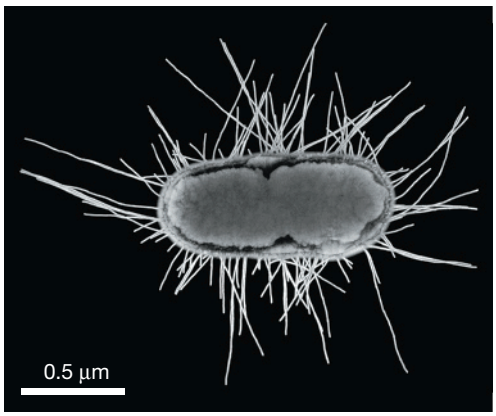
The success of *E. coli* as a model for other species inspired Jacques Monod's claim that "Once we understand the biology of *Escherichia coli*, we will understand the biology of an elephant." The genome of *E. coli* K-12 was sequenced in 1997, and the strain continues to be a workhorse in studies of gene function, biochemistry, and particularly biotechnology.

In the lab, *E. coli* is usually grown in suspension culture, where cells are introduced to a liquid nutrient medium, or on plates containing agar—a gelatinous mix of polysaccharides. Under optimal growing conditions—meaning before cells begin to get crowded and compete for space and nutrients—a cell takes just 30 minutes on average to grow and divide. At this rate, a single cell can produce a population of over a million descendants in just 10 hours. Unless they have new mutations, all of the descendant cells are genetically identical.

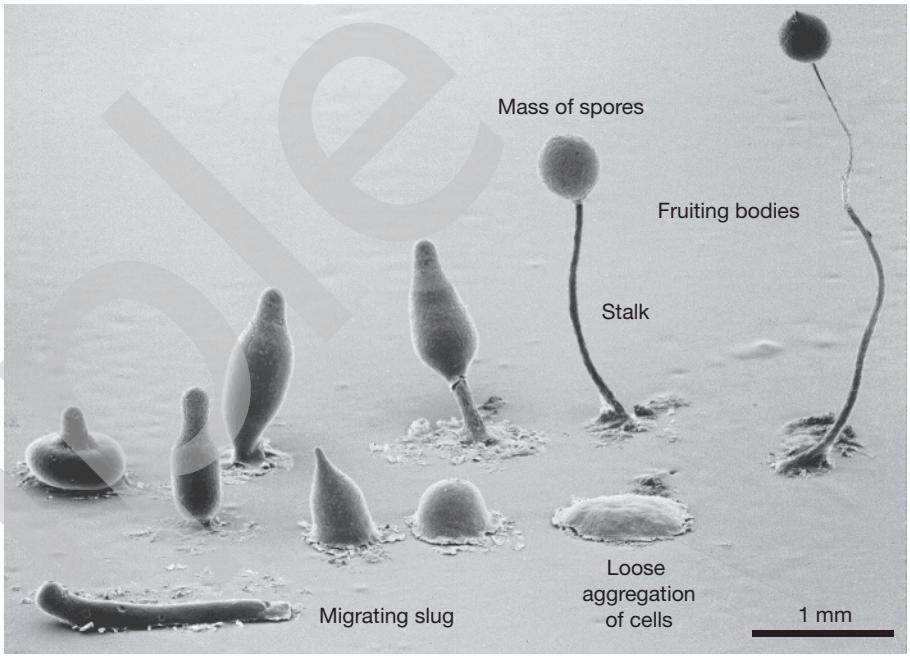
Dictyostelium discoideum The cellular slime mold *Dictyostelium discoideum* is not always slimy, and it's not a mold—which is a type of fungus. Instead, it is an amoeba. Amoeba is a general term that biologists use to characterize a unicellular eukaryote that lacks a cell wall and is extremely flexible in shape. *Dictyostelium* has long fascinated biologists because it is a social organism. Independent cells sometimes aggregate to form a multicellular structure.

Under most conditions, *Dictyostelium* cells are haploid (n) and move about in decaying vegetation on forest floors or other habitats. They feed on bacteria by engulfing them whole. When these cells reproduce, they can do so sexually by fusing with another cell and then undergoing meiotic cell division, or asexually by mitotic cell division, which is more common. If food begins to run out, the cells begin to aggregate. In many cases, tens

(a) Bacterium *Escherichia coli* (strain K-12)



(b) Slime mold *Dictyostelium discoideum*

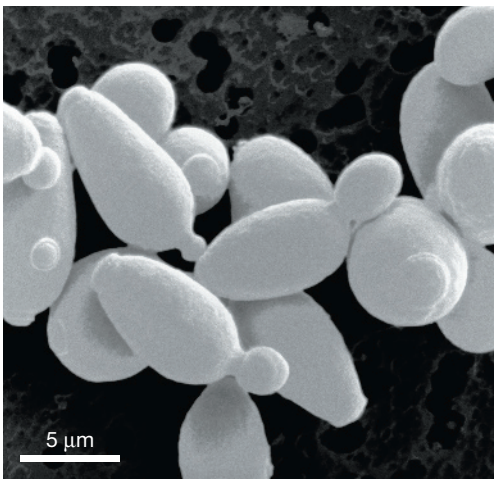


(c) Thale cress *Arabidopsis thaliana*

(e) Fruit fly *Drosophila melanogaster*

(f) Roundworm *Caenorhabditis elegans*

(d) Yeast *Saccharomyces cerevisiae*



(g) Mouse *Mus musculus*

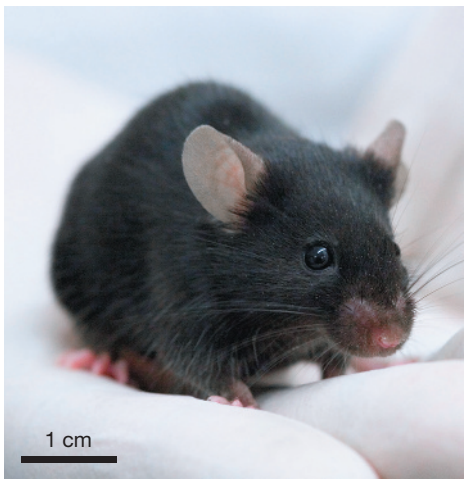


Figure B11.2 Model Organisms.