GLOBAL EDITION

Evolutionary Analysis

FIFTH EDITION

Jon C. Herron • Scott Freeman

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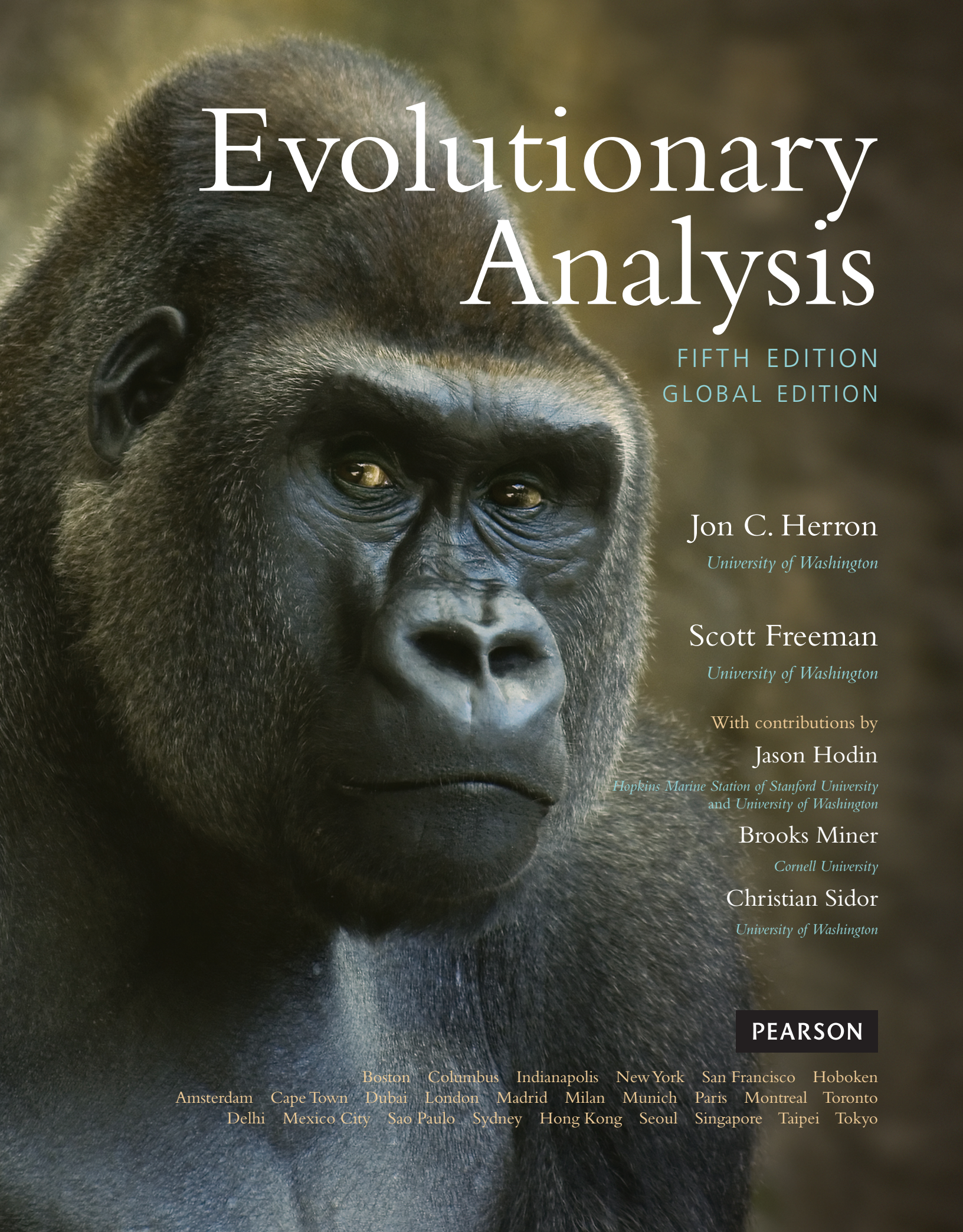

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Authorized adaptation from the United States edition, entitled Evolutionary Analysis, 5th edition, ISBN 978-0-321-61667-8, by Jon C. Herron and Scott Freeman, published by Pearson Education © 2015.

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

10 9 8 7 6 5 4 3 2 1

ISBN 10: 1-292-06127-8
ISBN 13: 978-1-292-06127-6

Typeset by Cenveo Publisher Services
Printed and bound in Slovakia by Neografia

www.pearsonglobaleditions.com

Brief Contents

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Preface

Evolutionary biology has changed dramatically during the 15 years we have worked on *Evolutionary Analysis*. As one measure of this change, consider that when the first edition went to press, the genomes of just five cellular organisms had been sequenced: three bacteria, one archaean, and one eukaryote. As the fifth edition goes to press, Erica Bree Rosenblum and colleagues reported in the *Proceedings of the National Academy of Sciences* (110: 9385–9390) that they had sequenced the genomes of 29 strains of a single species, the chytrid fungus *Batrachochytrium dendrobatidis*. This work was part of an effort to unravel the evolutionary history of an emerging pathogen that has decimated amphibian populations around the world and driven some species to extinction. The avalanche of sequence data has allowed evolutionary biologists to answer long-standing questions with greatly increased depth and clarity. In Chapter 20, *Human Evolution,* for example, we discuss a recent analysis of differences among genomic regions in the evolutionary relationships among humans, chimpanzees, and gorillas. For some questions, the answers have changed completely. In the fourth edition we noted that available sequence data provided no support for the hypothesis that modern humans and Neandertals interbred. But in the fifth edition we describe genomic analyses suggesting that the two lineages interbred after all.

Evolutionary Analysis provides an entry to this dynamic field of study for undergraduates majoring in the life sciences. We assume that readers have completed much or all of their introductory coursework and are beginning to explore in more detail areas of biology relevant to their personal and professional lives. Therefore, throughout the book we attempt to show the relevance of evolution to all of biology and to real-world problems.

Since the first edition, our primary goal has been to encourage readers to think like scientists. We present evolutionary biology not as a collection of facts but as an ongoing research effort. When exploring an issue, we begin with questions. Why are untreated HIV infections typically fatal? Why do purebred Florida panthers show such poor health, and what can be done to save their dwindling population? Why do mutation rates decrease with genome size among some kinds of organisms, but increase with genome size among others? We use such questions to engage students' curiosity and to motivate discussions of background information and theory. These discussions enable us to frame alternative hypotheses, consider how they can be tested, and make predictions. We then present and analyze data, consider its implications, and highlight new questions for future research. The analytical and technical skills readers learn from this approach are broadly applicable, and will stay with them long after the details of particular examples have faded.

New to This Edition

Many of the research areas we cover are advancing at a rate we would not have dreamed possible just a few years ago. We have looked closely at every chapter to both improve how we are teaching today's students and to thoroughly update our coverage.

- We have enhanced our traditional emphasis on scientific reasoning by including a data graph, evolutionary tree, or other piece of evidence to accompany the photo on the first page of every chapter. These one-page case studies engage students as active readers and help them become skilled at working with and interpreting data.
- We have enhanced our strong coverage of tree thinking by thoroughly revising Chapter 4. Consistent with the ever-growing use of phylogenetic analysis by scientists, we incorporate more phylogenies throughout the book. Among the new examples are a tree-based discussion of evolution of vertebrate eyes (Chapter 3); a new case study reconstructing the history of a patient's cancer (Chapter 14); and phylogeny-based reconstructions of the fish-tetrapod transition, the dinosaur-bird transition, and the origin of mammals (Chapter 18). Frequent practice at tree thinking helps students develop this essential skill.

Every chapter contains something new. Most of the new material is from the recent literature.

- Chapter 1 includes updated statistics on the status of the HIV pandemic, newer thinking on how HIV causes AIDS, new data on the origin of HIV, and new ideas and evidence on why HIV is lethal.
- Chapter 2 has a new organization featuring sections on evidence for microevolution, speciation, macroevolution, and common ancestry; discussions of why evolution at each level is relevant to humans outside of textbooks and classrooms; evidence of macroevolution presented using evolutionary trees showing the order in which derived traits are inferred to have evolved; and several new examples, including a terrestrial fish that does not like to swim.
- Chapter 3 brings new evidence on the evolution of development in the beaks of Darwin's finches, a new example of exaptation featuring carnivorous plants, and new coverage of the evolution of complex organs featuring a phylogeny-based discussion of the evolution of vertebrate eyes.
- Chapter 4 has been completely rewritten to offer an improved introduction to tree thinking; more detailed explanations of parsimony, maximum likelihood, and Bayesian phylogeny inference; and new examples of phylogenies used to answer interesting questions—such as identifying the surprising infectious agent responsible for a sexually transmitted tumor in dogs.
- Chapter 5 includes a new section on kinds of variation, featuring new and detailed examples of genetic variation and environmental variation; genotype-by-environment interaction; improved discussion of the mechanisms and consequences of mutation; new examples of gene duplication; and covers rates and fitness effects of mutation in a dedicated section with new examples and data.
- Chapter 6 is bookended with a powerful new example in which genetic engineers made a new gene, accurately predicted its effects on individuals carrying it, introduced it into a population, and used population genetics theory to accurately predict how its frequency would change over a span of 20 generations.
- Chapter 7 is bookended with a new example of conservation genetics involving the Florida panther. The chapter also includes a new example illustrating the founder effect in Polynesian field crickets; improved coverage of the inter-

action of drift and selection, the neutral theory, and the nearly neutral theory; and a new introduction to coalescence.

- Chapter 8 carries a new example—on Crohn's disease in humans—showing how linkage disequilibrium due to genetic hitchhiking can lead to spurious associations between genotype and phenotype and a revised and updated section on the adaptive significance of sex, featuring recent experiments using *C. elegans* as a model organism.
- Chapter 9 has improved narrative coherence due to the inclusion throughout the chapter of examples on the quantitative genetics of performance and prize winnings in thoroughbred racehorses.
- Chapter 10 includes an improved primer on statistical hypothesis testing, using research on the evolution of wild barley populations in response to a warming climate, and a new example of comparative research involving color in feather lice.
- Chapter 11 improves our coverage of the evolution of female choice by presenting the Fisher-Kirkpatrick-Lande model as the null hypothesis. New examples and data consider Bateman's principle in a hermaphrodite; female preferences in genetically modified zebrafish; correlated displays and preferences in Hawaiian crickets; and sexual selection in humans.
- Chapter 12 features enhanced coverage, with examples, of the four basic kinds of social behavior; improved coverage of kin selection and spite; a new section on multilevel selection and the evolution of cooperation; and several data sets on human social behavior.
- Chapter 13 has new examples on telomeres and aging; the evolution of menopause; life history traits and biological invasion; and life history traits and vulnerability to extinction.
- Chapter 14 discusses new evidence, from genome architecture, on the origin of influenza A; a new example using phylogenetic analysis to reconstruct the history of a cancer; and updated coverage of diseases of civilization, including a dramatic example from Iceland and new material on obesity.
- Chapter 15 has been completely rewritten, bringing new sections on the evolution of genome architecture; the evolution of mutation rates and gene families; and updated treatment of mobile genetic elements and the molecular basis of adaptation.
- Chapter 16 features new sections on mechanisms of divergence; hybridization and gene flow; and drivers of diversification. The chapter includes new examples illustrating the application of species concepts; updated coverage of vicariance in snapping shrimp; and new examples on mechanisms of isolation—including temporal isolation in a moth and single-gene speciation in snails.
- Chapter 17 incorporates updated coverage of the effort to create self-replicating RNAs and of the prebiotic synthesis of activated nucleotides.
- Chapter 18 has greatly expanded coverage of evolutionary transitions, featuring phylogeny-based reconstructions of the fish-tetrapod transition, the dinosaur-bird transition, and the origin of mammals; a new section on taxonomic and morphological diversity over time; updated treatment of mass extinctions, including the Permian-Triassic extinction; and a new section on fossil and molecular divergence timing.

- Chapter 19 has been completely rewritten. It includes revised coverage of Hox genes and detailed discussions of deep homology, developmental constraints and trade-offs, and the evolution of novel traits.
- Chapter 20 discusses new evidence from complete genomes on incomplete lineage sorting among humans, chimps, and gorillas, and on genetic differences between these species; new evidence, also from complete genomes, on hybridization between modern humans and Neandertals and between modern humans and Denisovans; and updated coverage of the human fossil record and the evolution of spoken language.

Hallmark Features

While fully updating this edition, we also maintained core strengths for which this book is recognized.

- We continue to strive for clarity of presentation, ensuring each chapter contains a coherent, accessible narrative that students can follow.
- We remain committed to strong information design and a tight integration between the text and illustration program. Nearly all phylogenies are presented horizontally, with time running from left to right, because research has shown this makes it easier for students to interpret them correctly.
- Boxes contain detailed explorations of quantitative issues discussed in the main text. These are called *Computing Consequences,* after physicist Richard Feynman's concise description of the scientific method: "First, we guess . . . No! Don't laugh—it's really true. Then we compute the consequences of the guess to see if this law that we guessed is right—what it would imply. Then we compare those computation results to nature—or, we say, to experiment, or experience—we compare it directly with observation to see if it works. If it disagrees with experiment, it's wrong."

All chapters end with a set of questions that encourage readers to review the material, apply concepts to new issues, and explore the primary literature.

Additional Resources for Instructors and Students

At the Pearson Instructor Resource Center, you can download JPEG and PowerPoint files containing all of the line art, tables, and photos from the book. You can access versions with and without labels to best suit your needs.

A thorough test bank and TestGen software is available to help you generate tests. Each chapter has dozens of multiple choice, short answer, and essay questions.

The updated Companion Website has been revised and updated to reflect the new edition. The website can be found at: www.pearsonglobaleditions.com/herron

Activities such as case studies and simulations challenge students to pose questions, formulate hypotheses, design experiments, analyze data, and draw conclusions. Many of these activities accompany downloadable software programs that allow students to conduct their own virtual investigations. Students will also find chapter study quizzes that allow them to check their understanding of key ideas in each chapter.

Acknowledgments

Evolutionary Analysis is a team effort. The book owes its existence and quality to the generosity and talents of a large community of colleagues, students, and friends. They have reviewed chapters; made suggestions; answered our questions; shared their photos, data, and insights; and lent us their expertise in countless other ways. Getting to spend time with and learn from such smart and interesting people is the best part of our job.

For the fifth edition we have had the great fortune to work with three extraordinary contributors.

- Brooks Miner, *Cornell University,* wrote the entirely new Chapter 15 and extensively revised and updated Chapter 16.
- Christian Sidor, *University of Washington,* thoroughly revised and expanded Chapter 18.
- Jason Hodin, *Hopkins Marine Station of Stanford University* and *University of Washington,* wrote the entirely new Chapter 19.

Mark Ong provided the beautiful and rational design of the new edition. Robin Green designed and produced art that is both engaging and effective, and is responsible for the coherent integration of the illustrations with the text.

The editorial, production, and marketing team at Pearson Education has offered steadfast guidance and support: Michael Gillespie, Senior Acquisitions Editor—Biology; Beth Wilbur, VP and Editor-in-Chief of Biology and Environmental Science; Paul Corey, President—Science, Business, and Technology; Lauren Harp, Executive Marketing Manager; Lori Newman, Production Project Manager; Deborah Gale, Executive Director of Development—Biology; Laura Murray, Project Editor; and Eddie Lee, Assistant Editor.

Our preparation of the fifth edition has been guided by thoughtful, detailed, and constructive critiques by

Mirjana Brockett, *Georgia Institute of Technology*
Jeremy Brown, *LSU*
Michael Emerman, *University of Washington*
Charles Fenster, *University of Maryland*
Matthew Hahn, *University of Indiana*
Michael E. Hellberg, *LSU*
Christopher Hess, *Butler University*
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Yale Passamaneck, *University of Hawaii*
Bruno Pernet, *California State University, Long Beach*
Thomas Ray, *University of Oklahoma*
Doug Schemske, *Michigan State University*
Billie J. Swalla, *University of Washington*
Sara Via, *University of Maryland*
Rebecca Zufall, *University of Houston*

Finally, we extend a special thank you to Christopher Parkinson and his students at the University of Central Florida and to Carol E. Lee and her students at the University of Wisconsin. Both groups class-tested preliminary versions of the chapters and provided insightful feedback that improved the final drafts.

Acknowledgments for the Global Edition

Pearson would like to thank and acknowledge Dr. Himender Bharti, Punjabi University for contributing to the Global Edition, and Dr. Krishna Kr. Ojha, Central University of Bihar, and Dr. Deepak Barua, IISER Pune for reviewing the Global Edition.

1

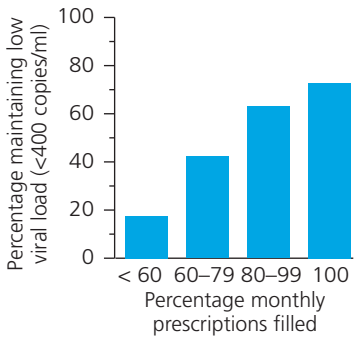
A Case for Evolutionary Thinking: Understanding HIV

Why study evolution? An incentive for Charles Darwin (1859) was that understanding evolution can help us know ourselves. "Light will be thrown," he wrote, "on the origin of man and his history." The allure for Theodosius Dobzhansky (1973), an architect of our modern view of evolution, was that evolutionary biology is the conceptual foundation for all of life science. "Nothing in biology makes sense," he said, "except in the light of evolution." The motive for some readers may simply be that evolution is a required course. This, too, is a valid inducement.

Here we suggest an additional reason to study evolution: The tools and techniques of evolutionary biology offer crucial insights into matters of life and death. To back this claim, we explore the evolution of HIV (human immunodeficiency virus). Infection with HIV causes AIDS (acquired immune deficiency syndrome)—sometimes, as shown at right, despite triple-drug therapy.

Our main objective in Chapter 1 is to show that evolution matters outside of labs and classrooms. However, a deep look at HIV will serve other goals as well. It will illustrate the kinds of questions evolutionary biologists ask, show how an evolutionary perspective can inform research throughout biology, and introduce concepts that we will explore in detail elsewhere in the book.

Multidrug therapies have, for some patients, transformed HIV from fatal to treatable. Such therapies work best for conscientious patients, but still may fail. The data below are from 2,800 patients on triple-drug therapy (Nachega et al. 2007).



HIV makes a compelling case study because it illustrates public health issues likely to influence the life of every reader. It is an emerging pathogen. It rapidly evolves drug resistance. And, of course, it is deadly. AIDS is among the 10 leading causes of death worldwide (Lopez et al. 2006; WHO 2008).

Here are the questions we address:

- What is HIV, how does it spread, and how does it cause AIDS?
- Why do therapies using just one drug, and sometimes therapies using multiple drugs, work well at first but ultimately fail?
- Are human populations evolving as a result of the HIV pandemic?
- Where did HIV come from?
- Why are untreated HIV infections usually fatal?

While one of these questions contains the word *evolution*, some of the others may appear unrelated to the subject. But evolutionary biology is devoted to understanding how populations change over time and how new forms of life arise. These are the issues targeted by our queries about HIV and AIDS. In preparation to address them, the first section covers some requisite background.

As a case study, HIV will demonstrate how evolutionary biologists study adaptation and diversity.

1.1 The Natural History of the HIV Epidemic

AIDS was recognized in 1981, when doctors in the United States reported rare forms of pneumonia and cancer among men who have sex with men (Fauci 2008). The virus responsible, HIV, was identified shortly thereafter (Barré-Sinoussi et al. 1983; Gallo et al. 1984; Popovic et al. 1984). Nearly always fatal, HIV/AIDS was devastating for those infected. But few physicians or researchers foresaw the magnitude of the epidemic to come **(Figure 1.1)**.

Indeed, many were optimistic about the prospects for containing HIV (Walker and Burton 2008). Smallpox had been declared eradicated in 1980 (Moore et

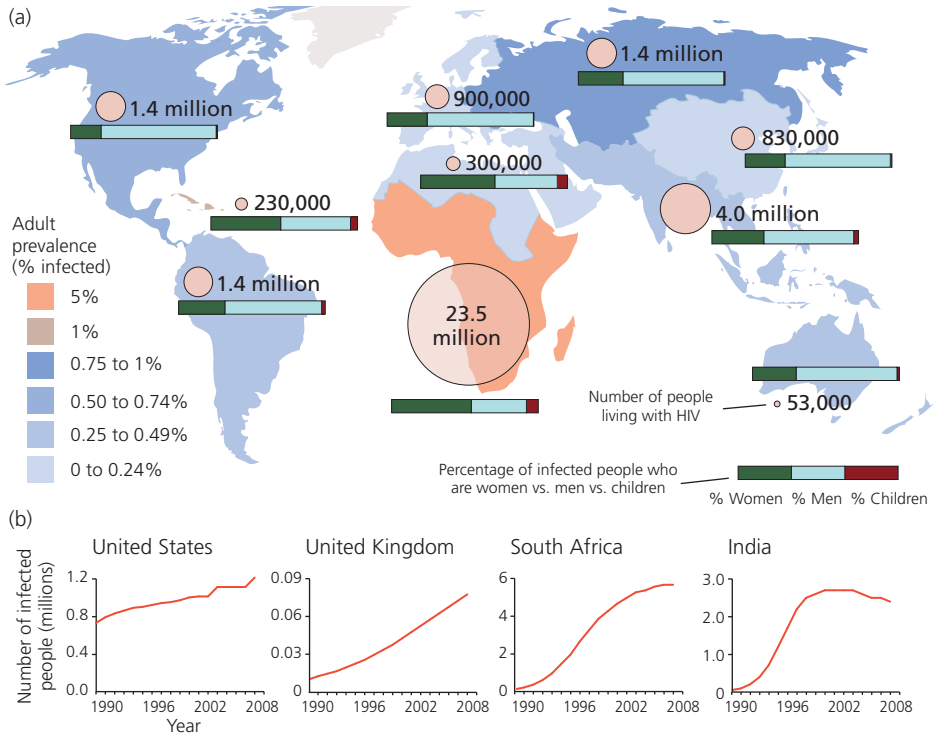


Figure 1.1 The HIV/AIDS pandemic The map (a) shows the geographic distribution of HIV infections. The color of each region indicates the fraction of adults infected with HIV (UNAIDS 2012b). The areas of the circles are proportional to the number of individuals living with HIV (UNAIDS 2012b). The bars divide individuals living with HIV by sex and age (UNAIDS 2008). The graphs (b) show the growth of the epidemic from 1990 to 2008 in four countries. Prepared with data from UNAIDS (2008).

al. 2006), and vaccines and antibiotics had brought many other infectious diseases under control. In 1984 the U.S. Secretary of Health and Human Services, Margaret Heckler, predicted that an AIDS vaccine would be ready for testing in two years. Actual events have, of course, played out rather differently.

HIV has infected over 65 million people (UNAIDS 2010, 2012a). Roughly 30 million have died of the opportunistic infections that characterize AIDS. The disease is the cause of about 3.1% of all deaths worldwide (WHO 2008/2011). AIDS is responsible for fewer deaths than heart disease (12.8%), strokes (10.8%), and lower respiratory tract infections (6.1%)—common agents of death among the elderly. But it causes more deaths than tuberculosis (2.4%), lung and other respiratory cancers (2.4%), and traffic accidents (2.1%).

Figure 1.1 summarizes the global AIDS epidemic. The map reveals substantial variation among regions in the number of people living with HIV, the percentage of the population infected, and the proportion of infected individuals who are women versus men versus children. The graphs show that the number of people infected has peaked in some countries but continues to climb in others.

The epidemic has been most devastating in sub-Saharan Africa, where 1 in 20 adults is living with HIV (UNAIDS 2008). Worst hit is Swaziland, with 26% of adults infected, followed by Botswana at 24%; Lesotho, 23%; and South Africa, 18%. Across southern Africa, life expectancy at birth has dropped below 50, a level last seen in the early 1960s **(Figure 1.2a)**. The good news is that the annual rate of new infections in sub-Saharan Africa has been falling for over a decade (UNAIDS 2012). This has meant that the global rate of new infections has been falling as well (Figure 1.2b).

In developed countries, overall infection rates are much lower than in sub-Saharan Africa (UNAIDS 2008). In western and central Europe, 0.3% of adults are infected. In Canada the rate is 0.4%, and in the United States it is 0.6%. For certain risk groups, however, infection rates rival those in southern Africa. Among men who have sex with men, the infection rate is 12% in London, 18% in New York City, and 24% in San Francisco (CDC 2005; Dodds et al. 2007; Scheer et al. 2008). Among injection drug users, the infection rate is 12% in France, 13% in Canada, and 16% in the United States (Mathers et al. 2008).

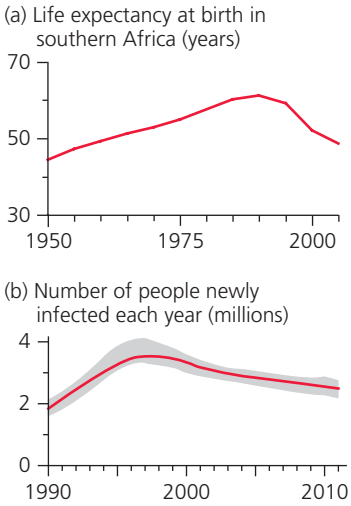


Figure 1.2 Long-term trends in HIV/AIDS (a) In southern Africa, the epidemic has caused a sharp reduction in life expectancy at birth. From UNAIDS (2008). (b) Worldwide, the annual number of new infections has been falling since the late 1990s. Red line shows best estimate; gray band shows range of estimates. From UNAIDS (2012).

How Does HIV Spread, and How Can It Be Slowed?

An HIV infection can be contracted only from someone else who already has it.

A new HIV infection starts when a bodily fluid carries the virus from an infected person directly onto a mucous membrane or into the bloodstream of an uninfected person. HIV travels via semen, vaginal and rectal secretions, blood, and breast milk (Hladik and McElrath 2008). It can move during heterosexual or homosexual sex, oral sex, needle sharing, transfusion with contaminated blood products, other unsafe medical procedures, childbirth, and breastfeeding.

HIV has spread by different routes in different regions **(Figure 1.3, next page)**. In sub-Saharan Africa and parts of south and southeast Asia, heterosexual sex has been the most common mode of transmission. In other regions, including Europe and North America, male–male sex and needle sharing among injection drug users have predominated. Certain activities are particularly risky. For example, data on men who have sex with men in Victoria, Australia, show that having receptive anal intercourse with casual partners without the protection of a condom is a dangerous behavior. Individuals who report practicing it are nearly 60 times as likely to be infected with HIV as individuals who do not report practicing it (Read et al. 2007).

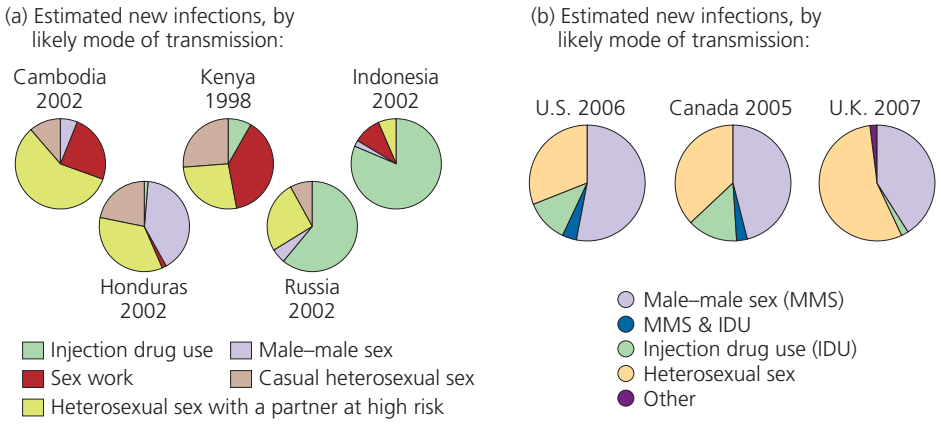


Figure 1.3 HIV's main routes of transmission in various regions (a) From Pisani et al. (2003). (b) From Hall et al. (2008), Public Health Agency of Canada (2006), Health Protection Agency (2008). The authors of the reports on Canada and the United Kingdom note that many of the individuals who contracted HIV through heterosexual sex likely did so in sub-Saharan Africa. See also UNAIDS (2008).

Clinical studies in which volunteers are randomly assigned to treatment versus control groups have identified medical interventions that reduce the rate of HIV transmission. Use of antiviral drugs, for example, lowers the risk that infected mothers will pass the virus to their infants by about 40% (Suksomboon et al. 2007). Antivirals are similarly effective in reducing transmission among men who have sex with men (Grant et al. 2010). Circumcision reduces the risk that men will contract HIV by about half (Bailey et al. 2007; Gray et al. 2007). Antiviral vaginal gels are comparably beneficial for women (Abdool Karim et al. 2010).

The value of encouraging people to change their behavior is less clear. Behavioral change undoubtedly has the potential to curtail transmission. Consistent use of condoms, for example, may reduce the risk of contracting HIV by 80% or more (Pinkerton and Abramson 1997; Weller and Davis 2002). And there are apparent success stories. In Uganda, for instance, a campaign discouraging casual sex and promoting condom use and voluntary HIV testing is thought to have substantially reduced the local AIDS epidemic (Slutkin et al. 2006; but see Oster 2009). On the other hand, the results of randomized controlled trials have been somewhat disappointing. A study of over 4,000 HIV-negative men who have sex with men in the United States offered extensive one-on-one counseling to members of the experimental group and conventional counseling to the control group (Koblin et al. 2004). As hoped, the experimental subjects engaged in fewer risky sexual behaviors than the controls. However, the rates at which the experimentals versus the controls contracted HIV were not statistically distinguishable.

There is clearly no room for complacency. The graph in Figure 1.4 tracks the number of new infections each year among men who have sex with men in the United States. After falling from the mid 1980s to the early 1990s, the annual number of new infections has since been rising steadily. The same thing seems to be happening elsewhere (Hamers and Downs 2004; Giuliani et al. 2005). Results of surveys suggest that the introduction of effective long-term drug therapies, which for some individuals has at least temporarily transformed HIV into a manageable chronic illness, has also prompted an increase in risky sexual behavior (Crepaz, Hart, and Marks 2004; Kalichman et al. 2007).

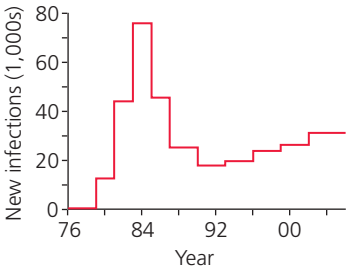


Figure 1.4 New HIV infections among men who have sex with men in the United States From Hall et al. (2008).

What Is HIV?

Like all viruses, HIV is an intracellular parasite incapable of reproducing on its own. HIV invades specific types of cells in the human immune system. The virus hijacks the enzymatic machinery, chemical materials, and energy of the host cells to make copies of itself, killing the host cells in the process.

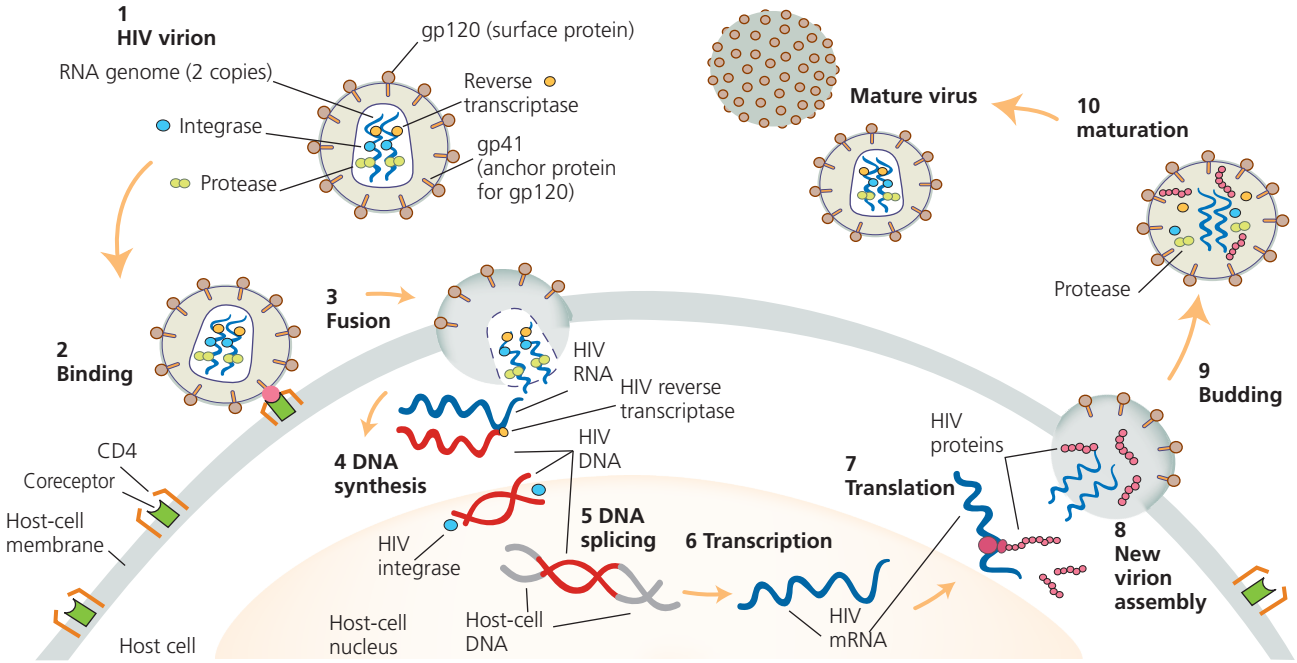


Figure 1.5 The life cycle of HIV **(1**, upper left**)** HIV's extracellular form, known as a virion, encounters a host cell (usually a helper T cell). **(2)** HIV's gp120 surface protein binds first to CD4, then to a coreceptor (usually CCR5; sometimes CXCR4) on the surface of the host cell. **(3)** The HIV virion fuses with the host cell; HIV's RNA genome and enzymes enter the host cell's cytoplasm. **(4)** HIV's reverse transcriptase enzyme synthesizes HIV DNA from HIV's RNA template. **(5)** HIV's integrase enzyme splices HIV's DNA genome into the host cell's genome. **(6)** HIV's DNA genome is transcribed into HIV mRNA by the host cell's RNA polymerase. **(7)** HIV's mRNA is translated into HIV precursor proteins by host cell's ribosomes. **(8)** A new generation of virions assembles at the membrane of the host cell. **(9)** New virions bud from the host cell's membrane. **(10)** HIV's protease enzyme cleaves precursors into mature viral proteins, allowing the new virions to mature.

Figure 1.5 outlines HIV's life cycle in more detail (Nielsen et al. 2005; Ganser-Pornillos et al. 2008). The life cycle includes an extracellular phase and an intracellular phase. During the extracellular, or infectious phase, the virus moves from one host cell to another and can be transmitted from host to host. The extracellular form of a virus is called a virion or virus particle. During the intracellular, or replication phase, the virus replicates.

HIV initiates its replication phase by latching onto two proteins on the surface of a host cell. After adhering first to CD4, HIV attaches to a second protein, called a coreceptor. This leads to fusion of the virion's envelope with the host's cell membrane and spills the contents of the virion into the cell. The contents include the virus's genome (two copies of a single-stranded RNA molecule) and two viral enzymes: reverse transcriptase, which transcribes the virus's RNA genome into DNA; and integrase, which splices this DNA genome into the host cell's genome.

HIV is a parasite that afflicts cells of the human immune system. HIV virions enter host cells by binding to proteins on their surface, then use the host cells' own machinery to make new virions.

Once HIV's genome has infiltrated the host cell's DNA, the host cell's RNA polymerase transcribes the viral genome into viral mRNA. The host cell's ribosomes synthesize viral proteins. New virions assemble at the host cell's membrane, then bud off into the bloodstream or other bodily fluid. Inside the new virions, HIV's protease enzyme cleaves precursors of various viral proteins into functional forms, allowing the virions to mature. The new virions are now ready to invade new cells in the same host or to move to a new host.

A notable feature of HIV's life cycle is that the virus uses the host cell's own enzymatic machinery—its polymerases, ribosomes, and tRNAs, and so on—in

almost every step. This is why HIV, and viral disease in general, is so difficult to treat. It is a challenge to find drugs that interrupt the viral life cycle without also disrupting the host cell's enzymatic functions and thus causing debilitating side effects. Effective antiviral therapies usually target enzymes specific to the virus, such as reverse transcriptase and integrase.

How Does the Immune System React to HIV?

A patient's immune system mobilizes to fight HIV the same way it moves to combat other viral invaders. Key aspects of the immune response appear in Figure 1.6.

Sentinels called dendritic cells patrol vulnerable tissues, such as the lining of the digestive and reproductive tracts (Banchereau and Steinman 1998). When a dendritic cell captures a virus, it travels to a lymph node or other lymphoid tissue and presents bits of the virus's proteins to specialized white blood cells called naive helper T cells (Sprent and Surh 2002).

Naive helper T cells carry highly variable proteins called T-cell receptors. When a dendritic cell presents a helper T cell with a bit of viral protein that binds to the T cell's receptor, the helper T cell activates. It grows and divides, producing daughter cells called effector helper T cells. Effector helper T cells help coordinate the immune response.

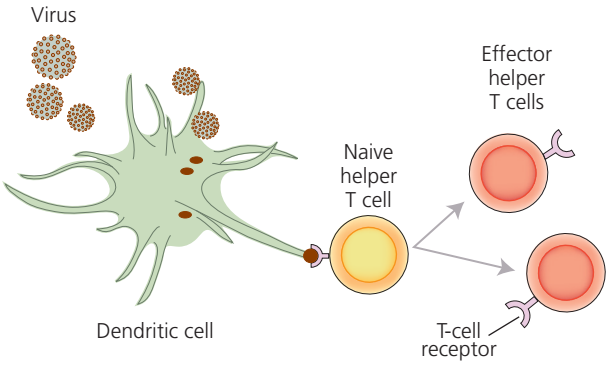
Effector helper T cells issue commands, in the form of molecules called cytokines, that help mobilize a variety of immune cells to join the fight. They induce B cells to mature into plasma cells, which produce antibodies that bind invading virions and mark them for elimination (McHeyzer-Williams et al. 2000). They activate killer T cells, which destroy infected host cells (Williams and Bevan 2007). And they recruit macrophages (not shown), which destroy virus particles or kill infected cells (Seid et al. 1986; Abbas et al. 1996).

Most effector helper T cells die within a few weeks. However, a few survive and become memory helper T cells (Harrington et al. 2008). If the same pathogen invades again, the memory cells produce a new population of effector helper T cells.

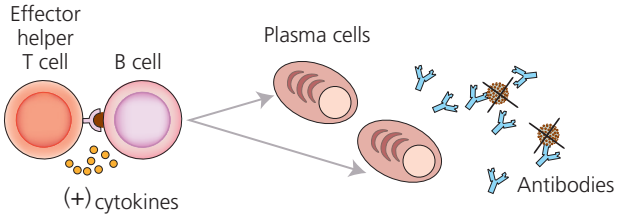
How Does HIV Cause AIDS?

As we noted earlier, HIV invades host cells by first latching onto two proteins on the host cell's surface. The first of these is CD4; the second is a called a coreceptor. Different strains of HIV exploit different coreceptors, but most strains responsible for new infections use a protein called CCR5. Cells that carry both CD4 and CCR5 on their membranes, and are thus vulner-

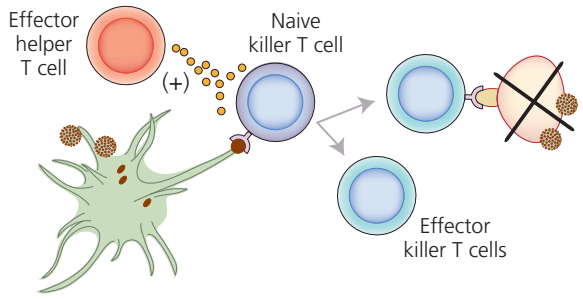
(a) Dendritic cells capture the virus and present bits of its proteins to naive helper T cells. Once activated, these naive cells divide to produce effector helper T cells.



(b) Effector helper T cells stimulate B cells displaying the same bits of viral protein to mature into plasma cells, which make antibodies that bind and in some cases inactivate the virus.



Effector helper T cells also help activate killer T cells, which destroy host cells infected with the virus.



(c) Most effector T cells are short lived, but a few become long-lived memory helper T cells.

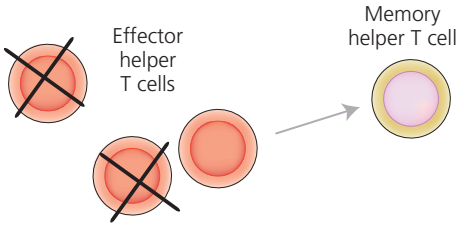


Figure 1.6 How the immune system fights a viral infection After NIAID (2003) and Watkins (2008).

able to HIV, include macrophages, effector helper T cells, and memory helper T cells **(Figure 1.7)**.

The progress of an HIV infection can be monitored by periodically measuring the concentration of HIV virions in the patient's bloodstream and the concentration of CD4 T cells in the patient's bloodstream and in the lymphoid (immune system) tissues associated with the mucous membranes of the gut. A typical untreated infection progresses through three phases.

In the acute phase, HIV virions enter the host's body and replicate explosively. The concentration of virions in the blood climbs steeply **(Figure 1.8)**. The concentrations of CD4 T cells plummet—especially in the lymphoid tissues of the gut. During this time, the host may show general symptoms of a viral infection. The acute phase ends when viral replication slows and the concentration of virions in the bloodstream drops. The host's CD4 T-cell counts recover somewhat.

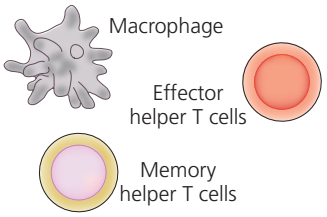


Figure 1.7 Immune system cells that carry both CD4 and CCR5 on their membranes, and are thus vulnerable to HIV. Data from UNAIDS (2008).

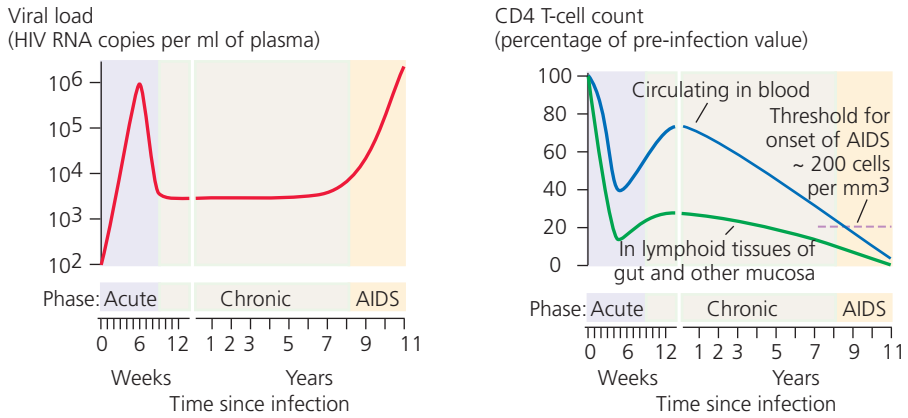


Figure 1.8 Typical clinical course of an untreated HIV infection By the time the concentration of CD4 T cells in the blood stream falls below about 200 cells per cubic millimeter, the patient's immune system begins to collapse. After Bartlett and Moore (1998), Brenchley et al. (2006), Pandrea et al. (2008).

During the chronic phase, the patient usually has few symptoms. HIV continues to replicate, however. The concentration of virions in the blood may stabilize for a while, but eventually rises again. Concentrations of CD4 T cells fall.

The AIDS phase begins when the concentration of CD4 T cells in the blood drops below 200 cells per cubic millimeter. By now the patient's immune system has begun to collapse and can no longer fend off a variety of opportunistic viruses, bacteria, and fungi that rarely cause problems for people with robust immune systems. Without effective anti-HIV drug therapy, a patient diagnosed with AIDS can expect to live less than three years (Schneider et al. 2005).

The mechanisms by which an HIV infection depletes the patient's CD4 T cells and undermines the patient's immune system are complex. Despite a quarter century of research, they remain incompletely understood (Pandrea et al. 2008; Douek et al. 2009; Silvestri 2009). The simple infection and destruction of host CD4 T cells may explain their precipitous loss during the acute phase of infection. But the immune system has an impressive capacity to regenerate these cells. Furthermore, during the chronic phase no more than one CD4 T cell in a hundred is directly infected. There must be more to the story.

Figure 1.9 (next page) outlines key events thought to lead from HIV infection to AIDS (Appay and Sauce 2008; Pandrea et al. 2008; Douek et al. 2009; Silvestri 2009). HIV's attack on the CD4 T cells in the gut (top) initiates a vicious cycle. This attack not only destroys a large fraction of the patient's helper T cells, it also damages other tissues in the gut that help provide a barrier between gut bacteria

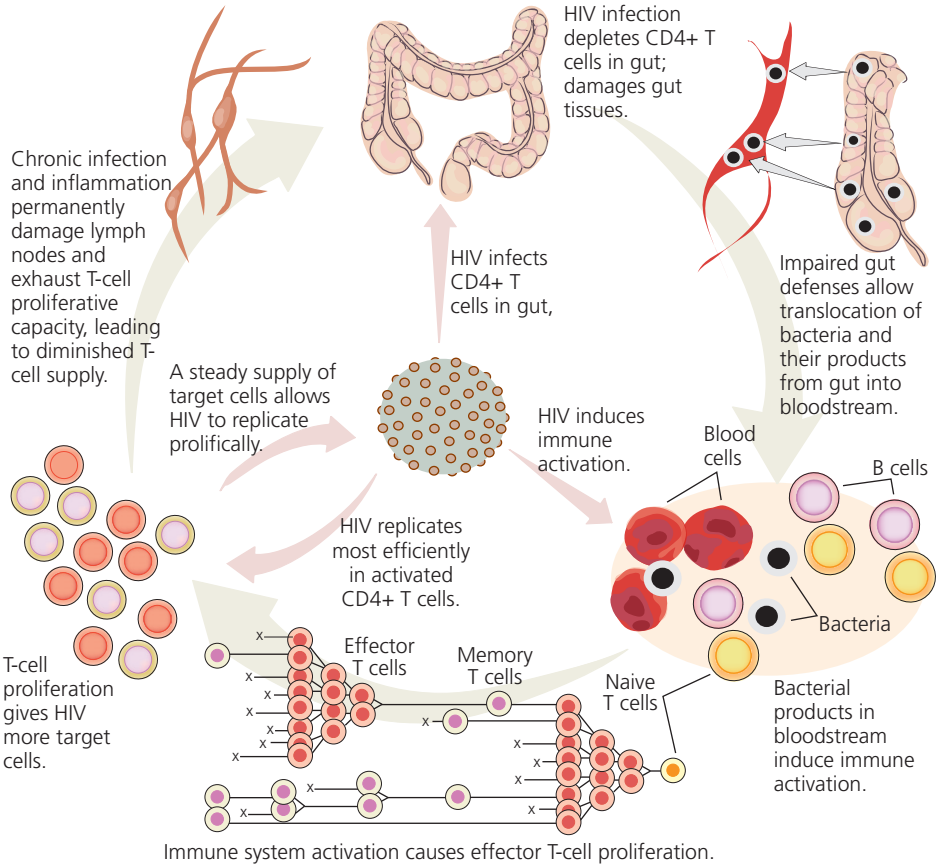


Figure 1.9 A model for how HIV causes AIDS To read the figure, start at the top, with HIV depleting CD4+ T cells in the gut. Then proceed clockwise. Direct effects of the virus are indicated by smaller pink arrows in the center; indirect effects by larger tan arrows around the outside. After Appay and Sauce (2008); Pandrea et al. (2008); Douek et al. (2009); Silvestri (2009).

and the bloodstream. The weakening of this barrier lets bacteria and their products move (translocate) from the gut into the blood (Figure 1.9, upper right).

The translocation of bacterial products into the blood triggers a high level of immune activation, to which the HIV infection itself also contributes (Biancotto et al. 2008). As we saw in Figure 1.6, activation of the immune system induces B cells and T cells to proliferate. This aggressive immune response has benefits, at least temporarily. For example, the anti-HIV killer T cells it yields help restrain HIV's replication. This and the production of new helper T cells allow the patient's concentrations of CD4 T cells to recover somewhat (Figure 1.8). But in the case of HIV, a strong immune response comes with heavy costs. The reason is that HIV replicates most efficiently in activated CD4 T cells. In other words, the immune system's best efforts to douse the HIV infection just add fuel to the fire.

HIV directly and indirectly induces immune activation, then replicates in activated immune system cells. When the ongoing battle damages the immune system to the point that it can no longer produce enough T cells to function properly, AIDS begins.

A major battleground in the ongoing fight between HIV and the immune system is the patient's lymph nodes (Lederman and Margolis 2008). The lymph nodes are, among other things, the places where naive T cells are activated. Chronic infection and inflammation eventually damages the lymph nodes irreversibly and exhausts the immune system's capacity to generate new T cells. As the patient's T-cell concentrations inexorably fall, the immune system loses its ability to fight other pathogens. The ultimate result is AIDS.

How might HIV be stopped before it leads to AIDS? The obvious answer is to prevent it from replicating. The first drug to do so, azidothymidine, or AZT, was approved for therapeutic use in 1987 (De Clercq 2009). Clinical experience with AZT, and every antiviral developed since, brings us to the first of our organizing questions. Why do single drugs offer only temporary benefits?

1.2 Why Does HIV Therapy Using Just One Drug Ultimately Fail?

To fight a virus, researchers often look for drugs that inhibit enzymes that are special to the virus and crucial to its life cycle. Such drugs should, in principle, hobble the virus and have limited side effects. For HIV, potential targets include the virus's protease, integrase, and reverse transcriptase (see Figure 1.5). AZT, the first drug approved to fight HIV, interferes with reverse transcriptase.

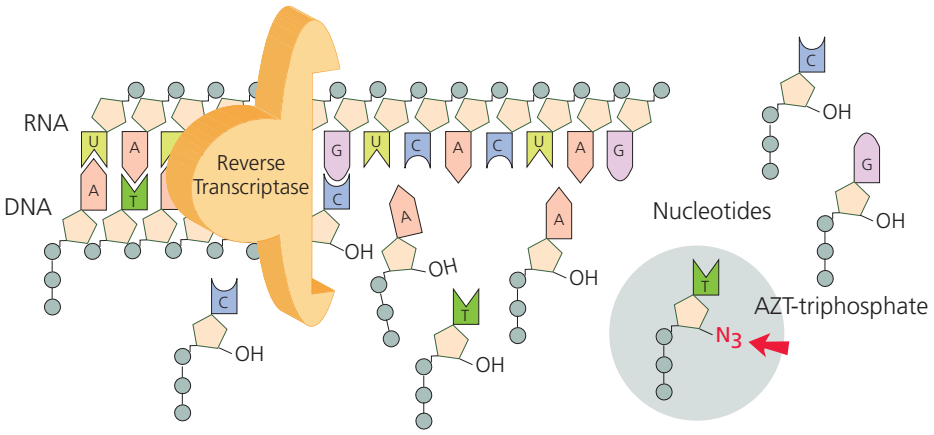


Figure 1.10 How AZT blocks reverse transcription HIV's reverse transcriptase enzyme uses nucleotides from the host cell to build a DNA strand complementary to the virus's RNA strand. AZT mimics a normal nucleotide well enough to fool reverse transcriptase, but lacks the attachment site for the next nucleotide in the chain.

Figure 1.10 shows what reverse transcriptase does. The enzyme uses the virus's RNA as a template to construct a complementary strand of viral DNA. Reverse transcriptase makes the DNA with building blocks—nucleotides—stolen from the host cell.

The figure also shows how AZT stops reverse transcription. Azidothymidine is similar in its chemical structure to the normal nucleotide thymidine—so similar that AZT fools reverse transcriptase into picking it up and incorporating it into the growing DNA strand. There is, however, a crucial difference between normal thymidine and AZT. Where thymidine has a hydroxyl group (–OH), AZT has an azide group ($-N_3$). The hydroxyl group that AZT lacks is precisely where reverse transcriptase would attach the next nucleotide to the growing DNA molecule. Reverse transcriptase is now stuck. Unable to add more nucleotides, it cannot finish its job. AZT thus interrupts the pathway to new viral proteins and new virions.

AZT is incorporated by HIV's reverse transcriptase into the viral DNA strand, where the drug prevents the enzyme from adding more nucleotides. However, alterations in the structure of reverse transcriptase can make viral replication less vulnerable to disruption.

In early tests AZT worked, halting the loss of T cells in AIDS patients. The drug caused serious side effects, because it sometimes fools the patient's own DNA polymerase and thereby interrupts normal DNA synthesis. But it appeared to promise substantially slower immune deterioration. By 1989, however, after only a few years of use, patients stopped responding to treatment. Their T-cell counts again began to fall. What went wrong?

Does AZT Alter the Patient's Physiology?

In principle, AZT could lose its effectiveness in either or both of two ways. One is that the patient's own cellular physiology could change. After it enters a cell, AZT has to be phosphorylated by the cell's own thymidine kinase enzyme to become biologically active. Perhaps long-term exposure to AZT causes a cell to make less thymidine kinase. If so, AZT would become less effective over time.

Patrick Hoggard and colleagues (2001) tested this hypothesis by periodically checking the intracellular concentrations of phosphorylated AZT in a group of patients taking the same dose of AZT for a year. The data refute the hypothesis. The concentrations of phosphorylated AZT did not change over time.

Does AZT Alter the Population of Virions Living in the Patient?

The other way AZT could lose its effectiveness is that the population of virions living inside the patient could change so that the virions themselves would be resistant to disruption by AZT.

To find out whether populations of virions become resistant to AZT over time, Brendan Larder and colleagues (1989) repeatedly took samples of HIV from patients and grew the virus on cultured cells in petri dishes. Figure 1.11 shows data for two patients the researchers monitored for many months. Each colored curve in the graphs represents a particular sample. Each curve falls to show how rapidly HIV's ability to replicate is curbed by increasing concentrations of AZT.

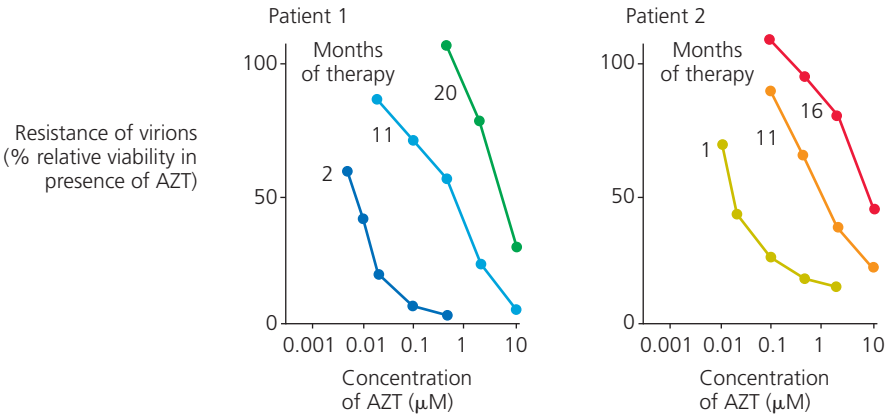


Figure 1.11 HIV populations evolve resistance to AZT within individual patients As therapy continued in these patients, higher concentrations of AZT were required to curtail HIV's replication. Redrawn from Larder et al. (1989).

Examine the three curves for Patient 1. Virions sampled from this patient after he had been taking AZT for two months were still susceptible to the drug. At moderate concentrations of AZT, the virions lost their ability to replicate almost entirely. Virions sampled from the patient after 11 months on AZT were partially resistant. They could be stopped, but it took about 10 times as much AZT to do it. Virions taken after 20 months on AZT were highly resistant. They were completely unaffected by AZT concentrations that stopped the first sample and could still replicate fairly well at concentrations that stopped the second sample.

The data for Patient 2 tell the same story. Populations of virions within individual patients change to become resistant to AZT. In other words, the populations evolve.

In many patients taking AZT, drug-resistant populations of HIV evolve within just six months (Figure 1.12).

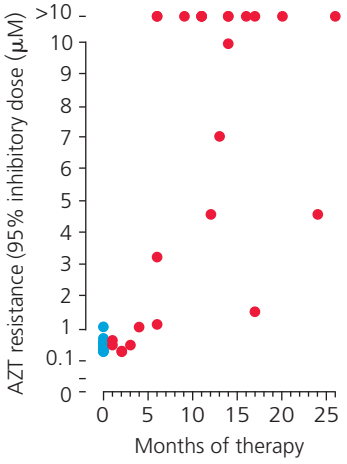


Figure 1.12 In many patients, AZT resistance evolves within six months This graph plots resistance in 39 patients checked at different times. Redrawn from Larder et al. (1989).

What Makes HIV Resistant to AZT?

What is the difference between a resistant virion versus a susceptible one? To answer this question, consider a thought experiment. If we wanted to engineer an HIV virion capable of replicating in the presence of AZT, what would we do? We would have to modify the virus's reverse transcriptase enzyme so that it either avoids inserting AZT molecules into the growing DNA strand in the first

place or, having inserted an AZT molecule, is more likely to take it back out so that the DNA strand can continue to grow (Figure 1.13).

In practice, we could expose large numbers of HIV virions to a mutagenic chemical or ionizing radiation. This would generate strains of HIV with altered nucleotide sequences in their genomes—and thus altered amino acid sequences in their proteins. If we generated enough mutants, at least a few would carry changes in the active site of the reverse transcriptase molecule—the part that recognizes nucleotides, adds them to the growing DNA strand, and corrects mistakes. If one of the reverse transcriptases with an altered binding site were less likely to mistake AZT for the normal nucleotide, or more likely to remove AZT after insertion, then the mutant variant of HIV would be able to continue replicating in the presence of the drug. If we treated our population of mutant virions with AZT, HIV strains unable to replicate in the presence of AZT would decline in numbers, and the resistant strain would become common.

The steps involved in this thought experiment are just what happens inside the bodies of HIV patients like the ones followed by Larder and colleagues. How do we know? In studies similar to Larder's, researchers took repeated samples of HIV virions from patients receiving AZT. The researchers found that viral strains present late in treatment were genetically different from viral strains that had been present before treatment in the same hosts. The mutations associated with AZT resistance were often the same from patient to patient (St. Clair et al. 1991; Mohri et al. 1993; Shirasaka et al. 1993) and caused amino acid changes in reverse transcriptase's active site (Figure 1.14).

The altered reverse transcriptase enzymes still pick up AZT and insert it into the growing DNA strand, but they are more likely to subsequently remove the AZT and, therefore, be able to continue building the DNA copy (Boyer et al. 2001). Possession of such a modified reverse transcriptase enables HIV virions to replicate in the presence of AZT.

Note that, unlike the situation in our thought experiment, no conscious manipulation took place. How, then, did the change in the viral strains occur?

The answer is that, despite having some ability to correct transcription errors, reverse transcriptase is prone to mistakes. Over half the DNA transcripts it makes contain at least one error—one mutation—in their nucleotide sequence (Hübner et al. 1992; Wain-Hobson 1993). Because thousands of generations of HIV replication take place within each patient during an infection, a single strain of HIV produces enormous numbers of reverse transcriptase variants in every host.

Simply because of their numbers, it is a virtual certainty that one or more of these variants contains an amino acid substitution that improves reverse transcriptase's ability to recognize and remove AZT. If the patient takes AZT, the replication of unaltered HIV variants is suppressed, but the resistant mutants will still be able to synthesize some DNA and produce new virions. As the resistant virions propagate and the nonresistant virions fail, the fraction of the virions in the patient's body that are resistant to AZT increases over time. Furthermore, each new generation in the viral population contains virions with additional mutations, some of which may further enhance the ability of reverse transcriptase to function in the presence of AZT. Because they reproduce faster, the virions that carry these new mutations will also increase in frequency.

This process of change over time in the composition of the viral population is called evolution by natural selection. It has occurred so consistently in patients taking AZT that use of AZT alone has long been abandoned as an AIDS therapy.

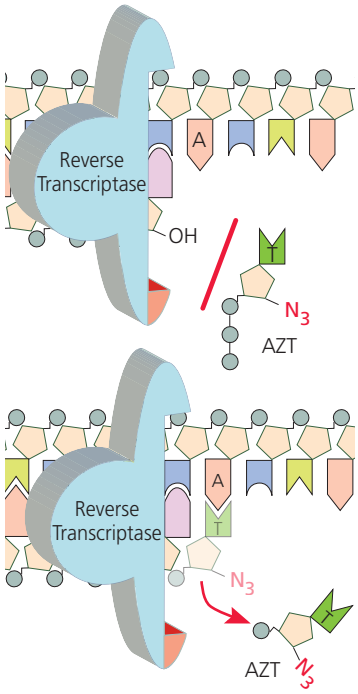


Figure 1.13 Two ways reverse transcriptase could resist AZT A resistant enzyme might avoid AZT (top) or, having inserted AZT into the growing DNA strand, take it back out (bottom).

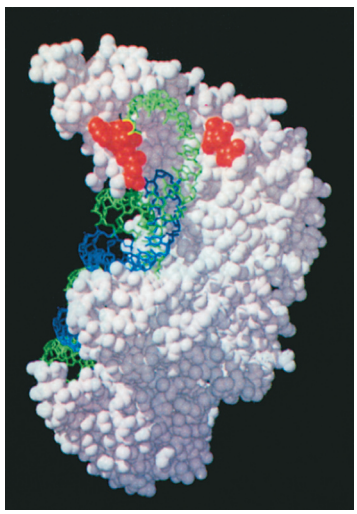


Figure 1.14 AZT-resistant reverse transcriptase The green and blue strands are the template RNA and growing DNA. The solid spheres show the structure of reverse transcriptase. Amino acid changes correlated with resistance to AZT are in red. They are in the enzyme's active site. By Lori Kohlstaedt; from Cohen (1993).

Evolution by Natural Selection

The process we have described involves four steps **(Figure 1.15)**:

1. Replication errors produce mutations in the reverse transcriptase gene. Virions carrying different reverse transcriptase genes produce versions of the reverse transcriptase enzyme that vary in their resistance to AZT.
2. The mutant virions pass their reverse transcriptase genes, and thus their AZT resistance or susceptibility, to their offspring. In other words, AZT resistance is heritable.
3. During treatment with AZT, some virions are better able to survive and reproduce than others.
4. The virions that persist in the presence of AZT are the ones with mutations in their reverse transcriptase genes that confer resistance.

Heritable traits that lead to survival and abundant reproduction spread in populations; heritable traits that lead to reproductive failure disappear. This is evolution by natural selection.

The result is that the composition of the viral population within the host changes over time. Virions resistant to AZT comprise an ever larger fraction of the population; virions susceptible to AZT become rare. There is nothing mysterious or purposeful about evolution by natural selection; it just happens. It is an automatic consequence of heritable differences in replication.

Because evolution by natural selection is an automatic consequence of cold arithmetic, it can happen in any population in which the four steps occur. That is, it can happen in any population in which there is heritable variation in reproductive success. We will see many examples in the chapters to come.

One measure of whether we understand a process is whether we can control it. If we truly understand the mechanism of evolution by natural selection as it operates inside the bodies of HIV patients, we should be able to find a way to

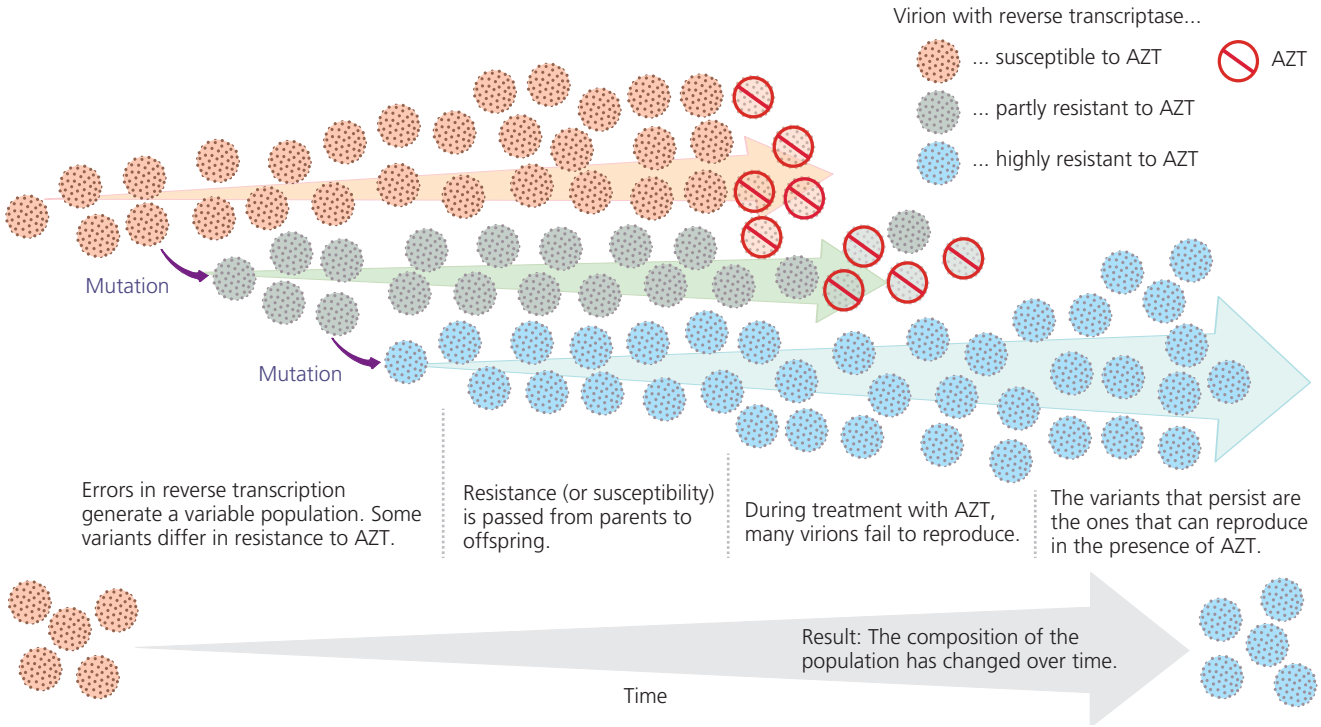


Figure 1.15 Evolution by natural selection, as illustrated by the evolution of resistance to AZT in an HIV population After Richman (1998).

stop it—or at least slow it down. We next consider how understanding the evolution of resistance allowed researchers to devise more effective therapies.

Understanding Evolution Helps Researchers Design Better Therapies

Since AZT was introduced, the number of drugs approved for treatment of HIV has grown to over two dozen (De Clercq 2009). The categories of drugs in use, in order of the stage of HIV's life cycle they are intended to disrupt, include

- **Coreceptor inhibitors.** These bar HIV from entering host cells in the first place by preventing them from latching onto the host cell's CCR5 molecules.
- **Fusion inhibitors.** These bar HIV from entering host cells by interfering with HIV's gp120 or gp41 proteins.
- **Reverse transcriptase inhibitors.** Some, like AZT, inhibit reverse transcriptase by mimicking the normal building blocks of DNA. Others inhibit reverse transcriptase by interfering with the enzyme's active site.
- **Integrase inhibitors.** These block HIV's integrase from inserting HIV's DNA into the host genome, preventing the transcription of new viral RNAs.
- **Protease inhibitors.** These prevent HIV's protease enzyme from cleaving viral precursor proteins to produce mature components for new virions.

Experience so far indicates that when any antiretroviral drug is used alone, the outcome will be the same as we have seen with AZT. The virus population in the host quickly evolves resistance (see, for example, St. Clair et al. 1991; Condra et al. 1996; Ala et al. 1997; Deeks et al. 1997; Doukhan and Delwart 2001).

Why do HIV populations evolve resistance so easily? With any single drug, just one or a few mutations in the gene for the targeted viral protein can render the virus resistant. With its high mutation rate, short generation time, and large population size, HIV generates so many mutant genomes that variants with the crucial combination of mutations are likely to be present much of the time. When the HIV population in a patient harbors genetic variation for replication in the presence of a drug, and the patient takes the drug, the population evolves.

This analysis suggests that the way to improve anti-HIV therapy is to increase the number of mutations that must be present in a virion's genome to render the virion resistant. The more mutations needed for resistance, the lower the probability that they will occur together in a single virion. In other words, we need a strategy to reduce the genetic variation for resistance. Without genetic variation—without differences in survival and reproduction that are passed from one generation to the next—the viral population cannot evolve.

By reducing the genetic variation for resistance in populations of virions, cocktails of drugs that target different points in HIV's life cycle limit the evolution of resistant strains. This, in turn, has dramatically improved patient survival.

The simplest way to raise the number of mutations required for resistance is to use two or more drugs at once. For this to work, a mutation that renders a virion resistant to one drug must not render it resistant to the others. Indeed, in the best scenario, a mutation that makes HIV resistant to one of the drugs will simultaneously make the virus more susceptible to the others (see St. Clair et al. 1991).

Treatment cocktails using combinations of drugs have, in fact, proven much more effective than single drugs used alone. Robert Murphy and colleagues (2008) tracked the viral loads of 100 patients who, as their first treatment for HIV, used a cocktail including two reverse transcriptase inhibitors and a protease inhibitor. Seven years later, 61 of the patients were still participating in the study, and 58 had viral loads under 50 copies per ml of blood—low enough to be

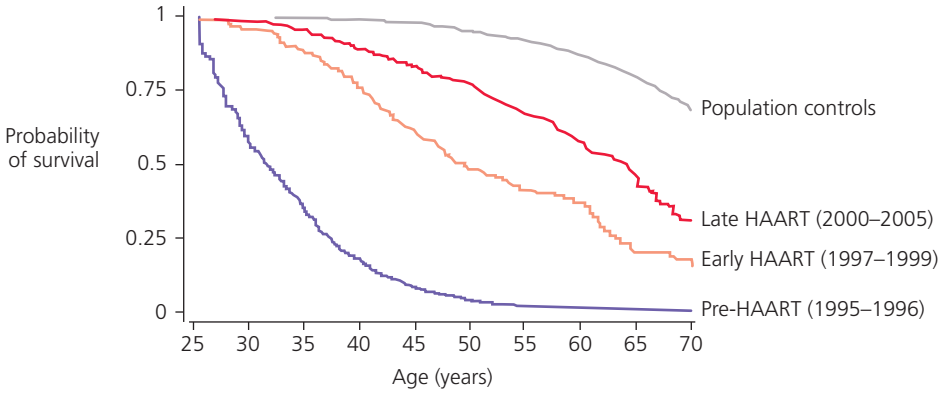


Figure 1.16 Treatment with multiple drugs prolongs the lives of patients with AIDS Redrawn from Lohse et al. (2007).

undetectable in standard tests. Results like these have earned regimens including three or more drugs that block HIV in two or more different ways the nickname highly active antiretroviral therapy, or HAART. (For more information on drug combinations used in HAART, see Hammer et al. 2008.)

Nicolai Lohse and colleagues (2007) followed 3,990 HIV-infected patients in Denmark from 1995 to 2005. Figure 1.16 tracks the survival of the patients during three treatment eras and compares them to the survival of 379,872 controls from the general population. HAART dramatically improved patient survival, and it got better over time—as more drugs became available, and as researchers, doctors, and patients learned how best to deploy them. Understanding how resistance evolves has helped prolong lives.

Unfortunately, even the best drug cocktails do not cure HIV infection. A reservoir of viable HIV genomes remains in the body, hidden in resting white blood cells and other tissues (Maldarelli et al. 2007; Brennan et al. 2009). As a result, when patients go off HAART, their viral loads climb rapidly (Chun et al. 1999; Davey et al. 1999; Kaufmann et al. 2004).

The Evolution of HIV Strains Resistant to Multiple Drugs

Because HAART cannot eradicate HIV, the evolution of strains resistant to multiple drugs is a constant threat for patients. Richard Harrigan and colleagues (2005) followed 1,191 patients on HAART. By the end of three years, the HIV populations in 25% of the patients had evolved resistance to at least one antiretroviral drug. The HIV populations in some patients were resistant to both reverse transcriptase inhibitors and protease inhibitors. Not surprisingly, patients with drug-resistant strains of HIV face a higher risk of death (Hogg et al. 2006).

Some patients have the bad luck to become infected with HIV strains that are already drug resistant (Johnson et al. 2008). Other patients inadvertently allow their HIV populations to evolve by failing to follow their treatment regimens strictly enough. Any time the concentration of a drug in the patient's body falls to levels that allow partially resistant virions to replicate, there is an opportunity for fully resistant mutants to appear. When the concentration of the drug rises again, such mutants will enjoy a strong selective advantage.

Harrigan and colleagues (2005) gauged patient adherence to treatment by calculating the percentage of prescription refills each patient picked up. Figure 1.17 plots the hazard ratio for the evolution of multidrug-resistant HIV as a function of refill percentage. The hazard ratio is the fraction of patients in a given adherence category who evolved resistant HIV divided by the fraction of patients in

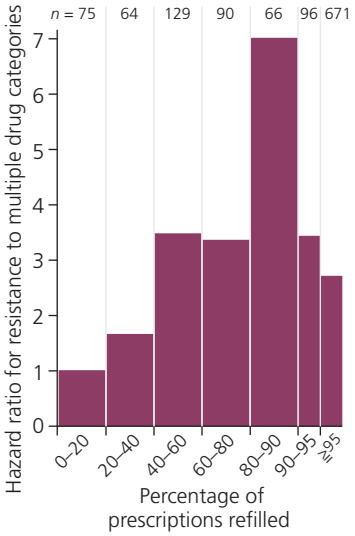


Figure 1.17 HIV evolves resistance to multiple drugs most readily in patients who take most, but not all of their prescribed doses Redrawn from Harrigan et al. (2005).

the 0 to 20% refill category who evolved resistant HIV. For an example of how to read the graph, patients who picked up 40% to 60% of their refills were between three and four times as likely to evolve resistant HIV. Patients who refilled most, but not all, of their prescriptions evolved resistant HIV at the highest rate. Given what we know about how evolution by natural selection works, the explanation is straightforward. Patients who took few of their doses subjected their HIV populations to weak selection. Patients who took all their doses shut down virtually all viral replication. Patients who took most, but not all, of their doses subjected their HIV populations to strong selection, but allowed some viral replication—thus creating permissive conditions for evolution.

Sometimes HIV populations evolve resistance even in patients taking multidrug cocktails. The risk is highest in patients who fill most, but not all, of their prescriptions.

One reason patients fail to take all of their prescribed doses is that antiretrovirals cause serious side effects. Among the reasons patients dropped out of Murphy's (2008) study were changes in body fat distribution, liver damage, elevated cholesterol, diarrhea, and joint pain. Anti-HIV therapies that are easily tolerated and that permanently suppress viral replication remain a goal of ongoing research.

We noted earlier that evolution by natural selection will happen in any population in which there are differences among individuals that are passed from parents to offspring and that influence survival and procreation. Variants associated with reproductive success automatically become common while variants associated with failure disappear. This broad applicability brings us to our next question.

1.3 Are Human Populations Evolving as a Result of the HIV Pandemic?

In Section 1.2, we saw how the HIV population inside a patient evolves in response to AZT. The drug influences which genetic variants of HIV survive and reproduce. Strains that do well despite the drug become common; strains that do poorly because of the drug become rare. In Section 1.1, we saw that the HIV pandemic is influencing which members of the human population survive and reproduce—particularly in southern Africa, where infection rates are high. This raises the question: Will human populations change over time in response to the pandemic? That is, will we evolve?

The answer depends on whether the humans who survive the pandemic owe their good fortune, at least in part, to genetic characteristics they can transmit to their offspring. If there are heritable differences among those who succumb versus those who live on, then traits conducive to surviving HIV will rise in frequency. Whether such differences exist is of more than academic interest. If we can identify genetic variants that confer resistance to HIV, then understanding how they work might suggest strategies for fighting the virus.

For a population to evolve, it must harbor genetic differences among individuals.

How might we discover genetic variants that make their carriers resistant to HIV? One way is to look for people who have not contracted the virus despite repeated exposure, or who remain healthy despite being infected. In the early 1990s, several laboratories demonstrated that both kinds of individuals exist (see Cao et al. 1995). By studying them, researchers have uncovered genetic variants that offer at least some protection against HIV (see An and Winkler 2010).

A Missing Coreceptor

In 1996, several groups of researchers identified the cell surface protein CCR5 as an important coreceptor for HIV (Deng et al., 1996, and Dragic et al., 1996,

were first into print). Rong Liu and coworkers (1996) and Michel Samson and associates (1996), among others, immediately guessed that resistant individuals might have unusual forms of CCR5 that thwart HIV's entry into host cells.

To test this hypothesis, Liu and colleagues examined the gene that encodes CCR5 from two individuals who had been repeatedly exposed to HIV but remained uninfected. Samson and colleagues looked at the gene from three HIV-infected individuals who were long-term survivors. As predicted, both of Liu's subjects were homozygotes for a mutant form of the gene and one of Samson's subjects was a heterozygote. Because the mutant form is distinguished by a 32-base-pair deletion in the normal sequence of DNA, it has come to be known as the *Δ32* allele (*Δ* is the Greek letter delta).

Investigating further, Liu showed that the version of CCR5 encoded by the mutant allele fails to appear on the surface of the cell. Samson showed that cells making only the *Δ32* form are nearly impervious to invasion by the strains of HIV responsible for most new infections. Samson also found that individuals carrying one or two copies of the *Δ32* allele were substantially less common among Europeans infected with HIV than among the European population at large. Together these results indicated that the *Δ32* allele confers strong (though not perfect) protection against HIV, a conclusion later confirmed by research that followed initially uninfected high-risk subjects over time (Marmor et al. 2001).

In human populations, some individuals carry alleles that make them resistant to infection with HIV.

To find out how common the *Δ32* allele is in various human populations, Samson and colleagues took DNA samples from a large number of individuals of northern European, Japanese, and African heritage, examined the gene for CCR5 in each individual, and calculated the frequency of the normal and *Δ32* alleles in each population. The mutant allele turned out to be present at a relatively high frequency of 9% in the Europeans, but was completely absent among the individuals of Asian or African descent. Subsequent research has confirmed this result. The *CCR5-Δ32* allele is common in northern Europe and declines dramatically in frequency toward both the south and the east **(Figure 1.18)**.

Curiously, the frequency of the best-known protective allele is highest in regions with low rates of HIV infection.

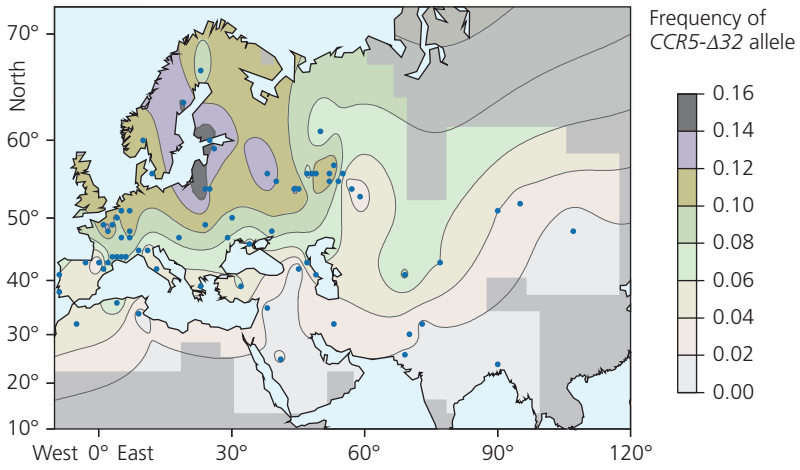


Figure 1.18 The frequency of the *CCR5-Δ32* allele in the Old World Blue dots indicate populations analyzed; colors between contour lines indicate inferred allele frequencies. Areas masked with dark gray are too far from the sources of data for reliable inferrence. From Novembre et al. (2005).

The data on the *CCR5-Δ32* allele show that human populations harbor heritable variation for resistance to HIV, but this variation will influence who lives and who dies only if HIV is present. Comparing the map of *Δ32* frequency in Figure 1.18 with the map of HIV prevalence in Figure 1.1 reveals a striking disconnect. The *Δ32* allele is common in a part of the world where HIV is rare,