Evolutionary Cell Processes in Primates
Evolutionary Cell Biology

Series Editors
Brian K. Hall—Dalhousie University, Halifax, Nova Scotia, Canada
Sally A. Moody—George Washington University, Washington DC, USA

Editorial Board
Michael Hadfield—University of Hawaii, Honolulu, USA
Kim Cooper—University of California, San Diego, USA
Mark Martindale—University of Florida, Gainesville, USA
David M. Gardiner—University of California, Irvine, USA
Shigeru Kuratani—Kobe University, Japan
Nori Satoh—Okinawa Institute of Science and Technology, Japan
Sally Leys—University of Alberta, Canada

Science Publisher
Charles R. Crumly—CRC Press/Taylor & Francis Group

PUBLISHED TITLES

Cellular Processes in Segmentation
Edited by Ariel Chipman

Cellular Dialogues in the Holobiont
Edited by Thomas Bosch and Michael G. Hadfield

Evolving Neural Crest Cells
Edited by Daniel Meulemans Medeiros, Brian Frank Eames, Igor Adameyko

Development of Sensory and Neurosecretory Cell Types:
Vertebrate Cranial Placodes, Volume 1
Gerhard Schlosser

Evolutionary Origin of Sensory and Neurosecretory Cell Types:
Vertebrate Cranial Placodes, Volume 2
Gerhard Schlosser

Evolutionary Cell Processes in Primates: Bones, Brains, and Muscle, Volume I
Edited by M. Kathleen Pitirri and Joan T. Richardsmeier

Evolutionary Cell Processes in Primates: Genes, Skin, Energetics, Breathing, and Feeding, Volume II
Edited by M. Kathleen Pitirri and Joan T. Richardsmeier

For more information about this series, please visit: www.crcpress.com/Evolutionary-Cell-Biology/book-series/CRCEVOCELBIO
Evolutionary Cell Processes in Primates
Bone, Brains, and Muscle

Volume I

Edited by

M. Kathleen Pitirri
Joan T. Richardsmeier

CRC Press
Taylor & Francis Group
Boca Raton  London  New York

CRC Press is an imprint of the
Taylor & Francis Group, an Informa business
Dedication

We dedicate these volumes to our families, whose love, support, and patience throughout this process and during a worldwide lockdown made these volumes possible.

Ben and Russ – thanks for making this past year in lockdown so much fun, you made it one of the best times of my life.

Thrill, Hannah, Lute, and Faith – you’ll probably never know that these books exist, much less read them. Still, I like to imagine you finding them one day and reading a page aloud to one another Wizard People, Dear Reader-style
Contents

Preface for Volume I ................................................................. ix
Acknowledgments ....................................................................... xi
Editors .................................................................................... xiii
Contributors ............................................................................ xv

Chapter 1  Introduction to Evolutionary Cell Biology in
Anthropological Research ....................................................... 1

Joan T. Richtsmeier and M. Kathleen Pitirri

Chapter 2  The Role of Bone and Cartilage Cells in the Evolution
of Bipedalism ........................................................................ 19

Campbell Rolian

Chapter 3  Cellular Processes in Limb Development and Primate
Evolution ............................................................................... 61

Philip L. Reno, Kelsey M. Kjosness, and
Allison L. Machnicki

Chapter 4  A Muscular Perspective on Human Evolution: Locomotor
Insights from Analyses of Primate Muscle Architecture
and Fiber Type ....................................................................... 97

Andrew S. Deane, Jason M. Organ, and
Magdalena N. Muchlinski

Chapter 5  Evolution of the Encephalized Human Brain: How Did
We become Exceptional? ..................................................... 133

Kevin D. Alloway and Kevin Flaherty

Chapter 6  Primate Cognition: Cellular Processes and the Developmental
Mechanisms in Brain Expansion ............................................. 159

Maria Carolina Marchetto and Katerina Semendeferi

Chapter 7  The Role of Primate Embryogenesis in Encephalization
and Neocortical Expansion .................................................. 181

Andrew C. Halley
Chapter 8  Mouse Models to Test Hypotheses of Human Evolution .......... 203

Neus Martínez-Abadías, Rubèn Gonzàlez, Roger Mateu,
Jaume Sastre, Alexandre Robert-Moreno, Jim Swoger,
Susan M. Motch Perrine, Kazuhiko Kawasaki,
Joan T. Richtsmeier, and James Sharpe

Chapter 9  Cellular Dynamics and the Developmental Basis for
Craniofacial Variation in Human Evolution and
Disease ........................................................................................................... 227

Nathan M. Young, Ralph S. Marcucio, Benedikt Hallgrímsson,
Heather A. Richbourg, and Rebecca M. Green

Index ............................................................................................................. 245
Preface for Volume I

What you are about to read represents the first of a two-volume series focused on cell processes in the evolution of primate characteristics. Chapters in these volumes focus on the role of cells in establishing traits that characterize the primates. Our aim in organizing these volumes was fourfold: (1) to present the current relevant work in such a way as to be of interest to specialists who study specific complex traits and to those more generally interested in a cellular biology approach in anthropology, (2) to provide a broad body of work enabling a general evaluation of this approach for the study of fundamental questions of primate evolution, (3) to assess modes of analyses and current knowledge of cellular biology underlying the evolution of primate-specific complex traits, and (4) to promote further interest and investigation of cell biology as the basis for understanding variation in and evolution of primate characteristics.

The study of morphological change over evolutionary time is the backbone of biological anthropological research. Chronicling patterns of morphological change is a required, though preliminary, step in understanding those patterns that figure prominently in primate evolution. Contributors were recruited to provide chapters that would inform anthropologists about work being done to uncover cellular behaviors and processes that impact the building of primate phenotypes. Our intent is to introduce the essential role of cellular processes in the production of some of the primates’ most celebrated complex traits and highlight this approach as a potential niche for novel anthropological research.

Volume I opens with an introductory chapter meant to demonstrate how the discovery of a quantifiable pattern of bone microstructure, whose presence is fully and satisfactorily explained by behavioral changes, still lacks a mechanistic explanation. A cellular biology approach is proposed as a potential route for identifying the mechanism that enables a behavioral change to be translated into a skeletal phenotype, demonstrating how explanation can be sought at varying levels.

Because topics in cell biology have not been a focus of anthropological research, the subsequent chapters are organized by the phenotypic characteristics that differentiate primates from other mammals. Contributors of chapters in the first section focus on primate dexterity and locomotion. Evolutionary trends in posture, dexterity, and locomotion are almost certainly behaviorally linked and require changes in the functions of numerous cell types, perhaps most obviously modifications in how cells contribute to the musculoskeletal system. Campbell Rolian (Chapter 2) focuses on the evolution of bipedal locomotion, discussing how osteoprogenitor cells modulate key cellular activities, including differentiation, proliferation, apoptosis, extracellular matrix production, motility, and orientation. He shows how changes in cellular activity provide the mechanism underlying changes in skeletal growth and development, skeletal proportions, joint morphology, and internal bone architecture of long bones. Phil Reno and colleagues (Chapter 3) use the vertebrate limb to show how developmental patterning is accomplished by cellular processes that assign positional information during development. Chapter 4 by Andrew S. Deane and colleagues focuses
on muscle cells showing how analyses of skeletal muscle architecture and fiber type can provide critical information on force generation, speed, and range of the specific movements that define locomotor adaptations. More on skeletal cells can be found in Chapter 4 of Volume II by Genevieve Housman, which evaluates the contribution of gene regulation in skeletal cells to primate evolution.

The second section of Volume I focuses on encephalization and craniofacial form. Encephalization is a hallmark of primate, and especially human, evolution. Chapter 5 by Kevin D. Alloway and Kevin Flaherty focuses on changes that made the human brain different, including expansion of some areas, reduction of others, functional changes, shifts in cortical connectivity, and the role of the association cortex in modulating and integrating impulses. Maria Carolina Marchetto and Katerina Semendeferi (Chapter 6) review the significant aspects of hominoid brain reorganization, acknowledging that most of the neurodevelopmental qualities responsible for primate brain expansion are not recovered in the fossil record. The contributors introduce induced pluripotent stem cell (iPSC) technologies and organoids, reviewing their use in the study of neurodevelopmental mechanisms of primate brain expansion that may have implications for the study of hominoid brain evolution. Andrew C. Halley (Chapter 7) shows how comparative embryology and experimental neurobiology can be used to reveal developmental mechanisms responsible for both primate encephalization and neocortical expansion and explains how those mechanisms may be linked to a broader suite of primate phenotypes.

Volume I is completed by two chapters that focus on the developmental basis of craniofacial features. Both contributors use data from mouse models, an approach gaining wider acceptance among anthropologists. Neus Martínez-Abadías and colleagues (Chapter 8) use experimental work with embryonic mice to show that changes in the location and timing of gene expression can induce correlated changes in brain and craniofacial morphology. This work is accomplished by using a mouse model for a human disease, but the contributors show how the data can be used for testing hypotheses about the shared developmental basis for facial retraction and encephalization in human evolution. In the final chapter of Volume I, Nathan M. Young and colleagues (Chapter 9) focus primarily on their own work to demonstrate how the integration of imaging, morphometrics, and developmental biology is helping to build a coherent picture of the developmental basis for variation in craniofacial morphology. The chapter shows how work with laboratory mice and model building can help expose the contribution of developmental processes to our evolutionary history and to the production of our distinctive morphology. For more on the skull, Chapter 5 of Volume II of this series by Nandini Singh includes a quantitative study of morphological changes in the mouse skull produced by upregulation of Sonic Hedgehog signaling and describes how changes in signaling can structure modularity of the skull.

Contributors of the chapters in this volume have applied new methods to old data sets, shown how novel types of data can reveal insights into old problems, and analyzed new types of data with new methods. We hope these chapters inspire new students of anthropology and experienced researchers to look to cellular processes as an avenue for explanation and discovery of the mechanistic basis for traits that have been studied since the dawn of our discipline.
Acknowledgments

We greatly appreciate the efforts of our chapter contributors and of the reviewers of each of these chapters who persevered in the face of challenging topics that cross disciplines and overcame obstacles created by the COVID-19 pandemic. We thank the editorial staff of CRC Press/Taylor & Francis for guiding us through the final stages of manuscript preparation and editing. Individuals who provided informative and supportive reviews of the chapters in Volume I of this series are listed in the following.

Neal Anthwal
Postdoctoral Scholar
Department of Ecology and Evolutionary Biology
University of California, Los Angeles

Dean Falk
Hale G. Smith Professor
Department of Anthropology
Florida State University

Brian K. Hall
Professor Emeritus
Department of Biology
Dalhousie University

Kazuhiko Kawasaki
Associate Research Professor
Department of Anthropology
Pennsylvania State University

Philipp Mitteröcker
Associate Professor
Department of Evolutionary Biology
Universität Wien

Alex Pollen
Assistant Professor
Weill Institute for Neurosciences
University of California, San Francisco

Barbara Finlay
W. R. Kenan Jr. Professor Emerita
Department of Psychology
Cornell University

Andrew Halley
Postdoctoral Scholar
Department of Psychology
University of California, Davis

Kate Lesciotto
Assistant Professor of Anatomy
Osteopathic Medicine
Sam Houston State University

Christopher Percival
Assistant Professor
Department of Anthropology
Stony Brook University

Timothy Ryan
Professor
Department of Anthropology
Pennsylvania State University
Karen Sears
Professor
Department of Ecology and Evolutionary Biology
University of California, Los Angeles

Chet Sherwood
Professor
Department of Anthropology
George Washington University

Paul Trainor
Investigator
Stowers Institute for Medical Research

Licia Selleri
Professor
Orofacial Sciences
School of Dentistry
University of California, San Francisco

Andrea Taylor
Professor
College of Osteopathic Medicine
Touro University, California

Jesse Young
Associate Professor
Anatomy and Neurobiology
Northeast Ohio Medical University
Editors

M. Kathleen Pitirri, PhD, is a postdoctoral scholar in the Department of Anthropology at Pennsylvania State University. She received her PhD in 2019 from the University of Toronto, where she studied primate evolution, focusing specifically on the taxonomic, ontogenetic, and functional basis of mandibular shape variation in living and fossil primates. During her PhD research, Dr. Pitirri developed a novel methodology for studying shape variation of mandibular fragments that are part of the primate fossil record. She found a strong relationship between the shape of the mandibular corpus and molar crypt formation in great apes, suggesting that mandibular shape is linked to an extended period of development in great apes, representing an important evolutionary shift in primates. Upon joining the Richtsmeier Lab, Dr. Pitirri began using mouse models to study the cellular mechanisms involved in transferring information from the genotype to the phenotype. The changes observed in mouse models can be used to interpret the cellular basis for changes observed in skull shape in primates because mechanisms that build the craniofacial skeleton during development also drive variation in disease and evolution. Dr. Pitirri is particularly interested in the evolutionary consequences of change in developmental processes driving the patterning of cellular activities involved in embryogenesis of skull bones, the role of the chondrocranium in skull development, and the genetic pathways regulating the relationship between tooth and bone formation during embryonic development.

Joan T. Richtsmeier, PhD, is Distinguished Professor of Anthropology at the Pennsylvania State University. She received her PhD from Northwestern University in 1985 and joined the faculty of the Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, in 1986. There, she focused on establishing new quantitative methods for studying change in biological shape through time, especially in primates, with Professor Subhash Lele. In 1999, she became the 55th woman to achieve the rank of professor at Johns Hopkins University School of Medicine since the school opened in 1893. In 2000, Dr. Richtsmeier moved her lab to the Pennsylvania State University. There, her focus turned to joining developmental biology with evolutionary biology, and with collaborators and students, she has worked to integrate the study of mouse models carrying known genetic variants with understanding the biological basis of patterns of craniofacial disease and evolutionary change. She is particularly interested in early formation of the chondrocranium and how and why cells decide to become osteoblasts and make bone. Dr. Richtsmeier was elected Fellow of the American Association of Anatomists (AAA) in 2018, received the Henry Gray Scientific Achievement Award of the AAA in 2019, and received the David Bixler Excellence in Craniofacial Research Award of the Society for Craniofacial Genetics and Developmental Biology in 2019. She was elected Fellow of the AAAS (Section on Biological Sciences) in 2020. Her work is supported by grants from the National Science Foundation, the National Institutes of Health, and the Wellcome Trust.
Contributors

Kevin D. Alloway
Department of Neural and Behavioral Sciences
Center for Neural Engineering
Pennsylvania State University
University Park, Pennsylvania

Andrew S. Deane
Department of Anatomy, Cell Biology, and Physiology
Indiana University School of Medicine
Indianapolis, Indiana

Kevin Flaherty
School of Health Sciences
Stephens College
Columbia, Missouri

Rubén González
GREAB-Research Group in Biological Anthropology
Department of Evolutionary Biology, Ecology and Environmental Sciences
BEECA Universitat de Barcelona
Barcelona, Spain

Rebecca M. Green
Department of Cell Biology and Anatomy
University of Calgary
Calgary, AB, Canada
and
Department of Oral Biology
Center for Craniofacial and Dental Genetics
University of Pittsburgh
Pittsburgh, Pennsylvania

Andrew C. Halley
Center for Neuroscience
University of California
Davis, California

Benedikt Hallgrímsson
Department of Cell Biology and Anatomy
University of Calgary
Calgary, AB, Canada

Kazuhiko Kawasaki
Department of Anthropology
Pennsylvania State University
University Park, Pennsylvania

Kelsey M. Kjosness
Department of Bio-Medical Sciences
Philadelphia College of Osteopathic Medicine
Philadelphia, Pennsylvania

Allison L. Machnicki
Center for Functional Anatomy & Evolution
Johns Hopkins University School of Medicine
Baltimore, Maryland

Maria Carolina Marchetto
Department of Anthropology
Center for Academic Research and Training in Anthropogeny (CARTA)
University of California, San Diego
La Jolla, California

Ralph S. Marcucio
Department of Orthopedic Surgery
University of California, San Francisco
San Francisco, California

Neus Martínez-Abadías
GREAB–Research Group in Biological Anthropology
Department of Evolutionary Biology, Ecology and Environmental Sciences
BEECA Universitat de Barcelona
Barcelona, Spain
Roger Mateu
GREAB–Research Group in Biological
Anthropology
Department of Evolutionary Biology,
Ecology and Environmental Sciences
BEECA Universitat de Barcelona
Barcelona, Spain

Magdalena N. Muchlinski
Anatomical Sciences Center
Oregon Health & Science University
Portland, Oregon

Jason M. Organ
Department of Anatomy, Cell Biology,
and Physiology
Indiana University School of Medicine
Indianapolis, Indiana

Susan M. Motch Perrine
Department of Anthropology
Pennsylvania State University
University Park, Pennsylvania

M. Kathleen Pitirri
Department of Anthropology
Pennsylvania State University
University Park, Pennsylvania

Philip L. Reno
Department of Bio-Medical Sciences
Philadelphia College of Osteopathic
Medicine
Philadelphia, Pennsylvania

Heather A. Richbourg
Department of Orthopedic Surgery
University of California, San Francisco
San Francisco, California

Joan T. Richtsmeier
Department of Anthropology
Pennsylvania State University
University Park, Pennsylvania

Alexandre Robert-Moreno
EMBL–Barcelona
European Molecular Biology
Laboratory
Barcelona, Spain

Campbell Rolian
Department of Comparative Biology
and Experimental Medicine
University of Calgary
Calgary, AB, Canada

Jaume Sastre
Universitat de les Illes Balears
Palma de Mallorca
Illes Balears, Spain

Katerina Semendeferi
Department of Anthropology
Kavli Institute for Brain and Mind
Center for Academic Research and
Training in Anthropogeny (CARTA)
Program in Neuroscience
University of California, San Diego
La Jolla, California

James Sharpe
EMBL–Barcelona
European Molecular Biology
Laboratory
Barcelona, Spain

Jim Swoger
EMBL–Barcelona
European Molecular Biology
Laboratory
Barcelona, Spain

Nathan M. Young
Department of Orthopedic Surgery
University of California, San Francisco
San Francisco, California
Introduction to Evolutionary Cell Biology in Anthropological Research

Joan T. Richtsmeier and M. Kathleen Pitirri

CONTENTS

1.1 Purpose of the Volumes: Why Cells? ...............................................................1
1.2 Patterns, Processes, Mechanisms .................................................................2
1.3 How Do Cells Make Traits? ..............................................................................4
1.4 How Do Cells Make Bone? ..............................................................................5
1.5 Osteoblast Lineage Cells and the Evolution of Skeletal Tissues ......................7
1.6 The Cellular Basis for Variation in the Primate Skeleton ..............................10
  1.6.1 Influence of Mechanical Environment on Cell Activity and Bone Structure .........................................................11
  1.6.2 Genetic Information and Information Transfer in Mechanotransduction ..........................................................11
1.7 Summary ........................................................................................................14
1.8 Acknowledgments ...........................................................................................14
1.9 References .......................................................................................................15

1.1 PURPOSE OF THE VOLUMES: WHY CELLS?

Much of anthropology is built on the accumulation of evidence from material artifacts, both cultural and biological. The biological material most commonly studied consists of fossilized remains of our primate ancestors, of which there are relatively few, and comparative collections of skeletal series, of which there are many due to the efforts of 19th- and 20th-century collectors. Given the nature of the evidence, biological anthropology is a discipline built on comparative studies of morphological characteristics. The laws of preservation yield fossilized evidence limited primarily to mineralized tissues like bone, enamel, and dentin—and these are the materials that have been used to reconstruct our evolutionary path. Because fossils are rare and usually found as individual specimens providing no evidence of variation, they are subject to the utmost scrutiny to get every conceivable piece of information out of
what might be only a small bit of bone or tooth—and this has led to decisions about who begat whom that are based on slim direct evidence.

Phylogenetic analysis of skeletal series of extant species rests on the identification of homologous traits assumed to be derived from an original structure that emerged once and was retained after species diverged (Arendt 2005). The course of evolution has traditionally been interpreted through the identification of homologous traits and the study of novel, derived traits. As a result, fossil specimens are often considered separate lineages on the basis of minor anatomical differences. But new sources of data have brought clarity, highlighted subtleties, and provided surprising avenues of evidence for the study of primate evolution. DNA sequenced from fossils has shown that some of us modern humans carry traces of the Neanderthal and Denisovan genomes and that more mating occurred among groups of extinct hominids (orangutan, gorilla, chimpanzee, and human lineages) than previously thought—even among yet-to-be-discovered extinct hominin groups (chimpanzee and human lineages) described as “ghost” lineages (Gibbons 2020; Rogers et al. 2020). The reconstruction of ancient human oral microbiomes from calcified dental plaque has informed us about regional differences in Neanderthal ecology, diet, and disease (Weyrich et al. 2017, 2015). Comparative analyses of microCT images of the skeletons of individuals from primate species and behaviorally distinct human populations reveal a correspondence between human behavior and bone structure, revealing surprising patterns of trabecular bone organization in more highly mobile human populations (Ryan and Shaw 2015). These examples highlight how the creative application of novel methods has produced information previously thought to be unrecoverable.

Primates exhibit a large suite of characteristics that facilitated behaviors allowing them to survive and thrive in challenging environments, including large brains, color vision, altered shoulder girdle anatomy, and dexterous hands. Each of these characteristics has its own evolutionary history and exhibits marked differences in morphology and physiology across species, but the cellular basis for differences in these characteristics across species, and for their evolution, is unknown. By focusing on cellular processes in the production of primate characteristics, this volume is intended to disseminate data and approaches but, more importantly, stimulate ideas about how anthropologists can contribute to knowledge of the cellular basis of the evolution of primate characteristics. Our goal in organizing these two volumes is to demonstrate the fundamental importance of cell structure and function in the production of phenotypic variation and to inspire anthropologists to look to the cell when contemplating the study of mechanisms driving morphological variation across primates, extant and extinct. These chapters provide an impetus for anthropologists to investigate and adopt foundational knowledge of cellular biology, to join this knowledge with cutting-edge methods, and to push the study of the evolution of primate characteristics forward through collaboration, re-training, and/or reorientation of career goals.

1.2 PATTERNS, PROCESSES, MECHANISMS

Many changes have taken place in biological anthropology in recent years that have affected the types of evidence and instrumentation available, the directions in which our discipline is heading, and the speed at which anthropological science
is progressing. Traditionally, advances in anthropology consisted primarily of the observation, and in many cases quantification, of the distribution and variation of traits across populations. Anthropologists have used these types of analyses to define *morphological patterns* that are used to propose *processes* that might produce those patterns, their variation, and their evolution (Lesciotto and Richtsmeier 2019). These approaches have typically resulted in post-hoc and adaptationist explanations for the existence of traits, patterns of trait variation, and the emergence of behaviors associated with those traits (see Gould and Lewontin 1979; Losos 2011; Grine and Daegling 2017).

We are now at the point where we can strive to determine *mechanism* underlying trait distribution, dispersal, and variation, thereby providing testable explanations for trait evolution. But to identify, define, and confirm mechanism, we need to understand what we mean by the term. Mechanism can evoke many definitions but is commonly associated with genes. Genetics was embraced by many in biological anthropology because it was widely believed that the identification of the genes associated with phenotypes would provide a mechanistic explanation, answering the question of how complex traits are produced and how they evolve (Weiss 2018), and that those answers would be more reliable than what could be gained through more traditional approaches (Szathmáry 2018).

Although we have witnessed tremendous progress in genetics and related fields over the past 50 years, mechanistic explanations have not emerged effortlessly through the identification of genes. Though most serious researchers acknowledge the complexity of biological causation, genes are still labeled as being “for” all sorts of traits, even traits that are central to the history of anthropological inquiry (e.g., intelligence, social or sexual deviation, and race) (Weiss 2018). We know that even traits whose characteristics are linked primarily with a single gene are affected by variants in that gene or in its regulatory network(s). To complicate matters more, non-genetic factors like environmental inputs, human cultural diversity, cultural evolution, and human history contribute to trait variation, and the estimated impact of each of these varies across individuals and populations (Weiss 2018). In other words, genes have provided critical information but are not *the* answer.

Even if you argue that biological mechanism ultimately lies at the level of the gene—with changes in genes and in the temporospatial distribution of their expression domains as the basis for variation in complex traits—you must also concede that since genes provide instructions for cells to construct those traits, genes have little meaning without cells (omitting viruses). Mechanisms that underlie traits and behaviors require changes at the cellular level. The genetic underpinnings of traits whose appearance and maintenance are often considered a result of natural selection can be fairly complicated, involving functional associations between genetic variants that lead to unexpected outcomes (Moore 2013). For example, in epistatic interactions, the effect of one non-allelic gene is masked or otherwise influenced by the effect of another gene(s) (Miiko 2008). The result can vary from total suppression of the effect of a gene to a situation where the combined genetic effects produce a new trait. We are now moving from an era of thinking about interactions among two or perhaps several genes to interacting networks of genes on a genome-wide scale (Moore 2013). Advances in sequencing technology and computational methods have
delivered loads of information regarding systems of genetic interactions. Knowledge gained from these analyses leads to a definition of mechanism as the cellular behaviors driven by the interactions of gene products (i.e., biochemical material like RNA and/or proteins) that underlie the production of phenotypes. Although these interactions take place during development, they are capable of producing evolutionary change (Salazar-Ciudad et al. 2003).

Salazar-Ciudad and colleagues (2003) classified developmental mechanisms capable of generating pattern and form in animals into three basic categories that can act independently or in combination. The proposed categories are:

1. **Cell autonomous** mechanisms in which cells organize into patterns without interacting;
2. **Inductive** mechanisms in which cell communication is associated with change in cell phenotypes (defined by cell structure and function) that leads to changes in pattern; and
3. **Morphogenetic** mechanisms in which patterns change by means of cell interactions that do not change cell phenotypes.

This classification understands mechanism as cellular activity that responds to genetic and microenvironmental input in different ways. Differences in ‘variational properties’ lead to mechanisms that can be characterized as morphostatic (a developmental mechanism where inductive mechanisms act first and the morphogenetic mechanism follows) and morphodynamic (a developmental mechanism in which inductive and morphogenetic mechanisms interact with one another in a reciprocal fashion, continuously revising each other) (Salazar-Ciudad et al. 2003). Each type of mechanism is present to different extents in early and late stages of development, and each is capable of contributing in distinct ways to morphological modifications in evolution (Salazar-Ciudad et al. 2003).

### 1.3 HOW DO CELLS MAKE TRAITS?

To understand how phenotypes are produced during development and how they change or are maintained by evolution, we need to understand the functions of cells and the gene networks that regulate their activity. *Genes* evolve through processes like duplication, mutation, and transposition, and gene activity is adjusted by modifier genes that can change the intensity, timing, or position of expression domains (Ohno 1970). *Complex traits* change through evolution, but if the tissue(s) from which they are constructed remain(s) relatively stable, the genetic instructions that likely co-evolved with the tissues and the developmental programs that underlie their construction are conserved (Brinkley et al. 2016; Kawasaki and Weiss 2003; Ohno 1970; Richtsmeier et al. 2000).

Gene expression is a complex process responsible for the conversion of information encoded in a gene into a functional product. The process of turning genes on and off is called gene regulation, is responsible for cells taking on specific functions, and can also help an organism respond to its environment (Mitsis et al. 2020; Spitz and Furlong 2012). The primary regulators of gene expression are transcription factors that bind specific
DNA sequences and instruct cells to augment, increase, and/or repress the production of specific gene products (Mitsis et al. 2020). Transcription factors include a multitude of proteins involved in the process of transcribing DNA into RNA. Transcription factors have DNA-binding domains that enable them to bind to promoter and enhancer sequences that initiate (promoter sequence) and regulate (enhancer sequence) the transcription of genes and thus play a key role in the supervision of unique cell- and tissue-specific gene expression during development (Spitz and Furlong 2012).

The tissue(s) that make up traits are formed by cells early in development. Evolutionary changes in the size, shape, and material properties of these tissues occur at the cellular level. Certain measurable tissue parameters can change their phenotype according to environmental input. This is interpreted as phenotypic plasticity or the expression of multiple phenotypes from one genome and is a common adaptation to short-term environmental fluctuations (Oostra et al. 2018). Whether plasticity facilitates evolutionary adaptation remains contentious, but for tissue properties to change, cell properties and behaviors must change. By studying changes in cell differentiation, patterning, and function during development and throughout an organism’s lifetime, we can begin to understand how phenotypic variation occurs. Advanced techniques in developmental cell biology (e.g., Eames and Schneider 2008) and novel methods from fields like mechanobiology (e.g., Ambrosi et al. 2019) provide numerous approaches for the analysis of how the collective behavior of cells shape tissues. Thus, it is now appropriate—and may prove extremely valuable—for anthropologists to consider the evolution and variation of primate characteristics through the investigation of how cells behave to make structures and how environmental influences affect the behavior of those cells.

So how would such an approach work? And what exactly can we learn? The chapters in this volume are assembled to provide relevant illustrations of what can be gained by infusing cell biology into anthropological research. Here, we provide an abbreviated example to introduce the approach.

1.4 HOW DO CELLS MAKE BONE?

Bone and some cartilages have been traditional targets for evolutionary studies because mineralized tissues are more likely to preserve and potentially fossilize. We know that the main bone-forming cells (osteoblasts) and cartilage-forming cells (chondrocytes) derive from a population of mesenchymal progenitors generally referred to as osteoblast lineage cells (OLCs) (Long 2012). As a cell matures, it changes from a less specialized cell to a more specialized cell that is distinct in form and in function through a genetically driven process known as differentiation. As differentiation progresses, the structure of the cell, the function of the cell, and the genes expressed within the cell change. OLCs are indeed the building blocks of the skeleton and can differentiate into chondrocytes, preosteoblasts, osteoblasts, bone-lining cells, and osteocytes (Hall 2015; Long 2012), but certain populations of cells are more likely chondrogenic (those that form Meckel’s cartilage) or osteogenic (those that form the frontal bone).

OLCs that give rise to osteoblasts and chondrocytes are initially marked by the transcription factor SRY-box 9, or SOX9 (Lefebvre and Dvir-Ginzberg 2017), and
the Sox9 protein is critical to the development of the skeleton. As OLCs differentiate along a path towards making bone, SOX9 expression is followed by the expression of Runt-related transcription factor 2 (RUNX2) (Schroeder et al. 2005) and then Osterix (OSX) (Sinha and Zhou 2013), leading to the differentiation of mature osteoblasts (Figure 1.1). Additional evidence suggests that cells expressing SOX9 (SOX9-positive, or SOX9+, cells) are bipotential and can switch fates under certain conditions, differentiating along the osteoblast pathway or into chondrocytes (Bahney et al. 2014; Hu et al. 2017; Long 2012). Each of the stages that together define the OLC differentiation trajectory (Figure 1.1) confer varying functions to the cell that play critical roles in how a given cell type contributes to the construction of skeletal morphology.

Cells work individually, but their group effort accounts for the development of tissues and the shapes they assume. Skeletal histogenesis, the histological differentiation of skeletal tissue, is initiated when a dispersed population of undifferentiated cells gathers together to form a condensation, marking a pivotal stage in the development of a tissue (Eames et al. 2012; Hall and Miyake 2000). Condensations are the aggregations of cells from which cartilages and bones form during embryonic development and within which the processes of chondrogenesis and osteogenesis are initiated (Hall and Miyake 2000). Condensation triggers the upregulation of tissue-specific genes that establish the boundaries, size, shape, and position of the newly formed tissue.

![FIGURE 1.1](image)

**FIGURE 1.1** Stages of osteoblast lineage cell (OLC) differentiation. Osteoblasts are derived from mesenchymal progenitor cells that initially express the transcription factor SOX9, followed by expression of runt transcription factor 2 (RUNX2), and then osterix (OSX). Osteocytes are derived from a subset of osteoblasts. Early in OLC differentiation, cells marked by SOX9 are bipotential and can differentiate into chondrocytes and osteocytes. Additionally, deletion of OSX (Nakashima et al. 2002) or the gene encoding β-catenin (Day et al. 2005; Hill et al. 2005; Rodda and McMohan 2006) causes cells marked by RUNX2 or RUNX2/OSX to differentiate into chondrocytes, suggesting that these cells may be capable of switching fates under certain conditions (dashed lines). (Figure by Kevin Flaherty, modified from Long 2012.)
of condensations and promotes the differentiation of a particular cell/tissue type (Eames and Schneider 2008; Hall and Miyake 2000). Skeletal morphogenesis contributes to the ongoing process through morphogenetic movements that arrange cells in proximity to local molecular and biomechanical signals (Arendt 2008, 2005; Cole 2011) and to the production of phenotypes by guiding skeletal element location, size, and shape (Eames and Schneider 2008). As skeletal elements mature, osteoblasts become trapped within the bone matrix they produce. Once entombed, the trapped osteoblasts differentiate into osteocytes and change their function from producing bone matrix: to being able to respond to mechanical strain, to detect fatigue-induced microdamage and signal to osteoclasts to induce replacement of damaged bone through remodeling, to send signals of bone formation or resorption to the bone surface, and to regulate both local and systemic mineral homeostasis (Bellido et al. 2014).

1.5 OSTEOBLAST LINEAGE CELLS AND THE EVOLUTION OF SKELETAL TISSUES

Condensations that signal formation of a skeletal tissue typically consist of a homogeneous population of cells marked by a specific set of gene activity that provides details of the cells’ current function. The complement of active genes, usually determined by some type of RNA sequencing (RNA-seq) technology, provides details of the cells’ current functional state and is referred to collectively as a molecular fingerprint of a cell (Arendt 2008, 2005), a measure of transcriptome-wide gene expression and thus of the functional state of a cell (Ozsolak and Milos 2011; Stark et al. 2019; Van den Berge et al. 2019). Research has shown that molecular fingerprints of cell types are highly conserved across the animal kingdom for cells as diverse as neurons (Arendt 2005; Krienen et al. 2020) and those that build the skeleton (Eames et al. 2012; Eames and Schneider 2008; Kawasaki et al. 2020; Long 2012; Nguyen and Eames 2020). Molecular fingerprints can be used to identify homologous cell types over evolutionary time in order to reconstruct phylogenies and determine how cells diversify in evolution (Arendt 2008; Krienen et al. 2020).

In general, RNA-seq allows evaluation of the levels of RNA molecules (that carry information of amino acid sequence from the genes) expressed in a cell, providing data for the analysis of differential gene expression within and across tissues. The broader applications of RNA-seq have shaped our current understanding of many aspects of biology (Stark et al. 2019), but the data produced are vast and complex and require substantial training and computational power for analysis. There are, however, additional methods for obtaining data about molecular fingerprints that can be used in place of or in combination with RNAseq to determine similarity in cell function across extant and extinct groups and explore the cellular basis of complex traits used in phylogenetic analyses.

In a series of studies, Eames and colleagues (Eames et al. 2012; Nguyen and Eames 2020) targeted the expression of the structural collagen genes (Col1a1, Col2a2, Col10a1, Col11a2) in well-formed cartilage and the expression of skeletal regulators (transcription factor genes Sox9 and Runx2) during mesenchymal condensation to demonstrate how gene expression in the cells of skeletons of extant
animals might reveal the evolutionary path of skeletal cells over the past 500 million years. The fossil record suggests that cartilage preceded bone in evolutionary time (Figure 1.2) (Cervantes-Diaz et al. 2017; Gans and Northcutt 1983; Pitirri et al. 2020; Smith and Hall 1990), so how did osteoblasts evolve? As detailed previously, chondrocytes and osteoblasts of modern organisms develop from common progenitor cells, the OLCs (Nakahara et al. 1990; Wagner and Lynch 2010), though certain populations of these cells are more prone to produce chondrogenic or osteogenic lineages in part because of the independent evolution of specific cell populations like the neural crest (Hall 2012). Cartilage and bone cells express some of the same genes as they differentiate, but there are molecular signatures that distinguish these two cell types.

Starting with the knowledge that the molecular fingerprints of teleost osteoblasts differ in key ways from that of tetrapod osteoblasts (Cole 2011; Hecht et al. 2008), Eames and colleagues reviewed osteoblast evolution in the major vertebrate clades by comparing expression patterns for cartilage and bone between later-diverging vertebrates (represented by experimental animals, mice and chicks) and earlier-diverging vertebrates (represented by modern frogs, gar, zebrafish) (Eames et al. 2012; Eames and Schneider 2008; Nguyen and Eames 2020). Comparison of the molecular fingerprints of embryonic cartilage and bone focused on the expression of

![FIGURE 1.2](image)

FIGURE 1.2 Major events in the evolution of cranial cartilage and bone, hypothesizing the approximate origins of chondrocytes and osteocytes. The phylogeny of these vertebrate clades is based on Janvier (2015) and Brazeau and Friedman (2015). (Adapted from Pitirri et al. 2020; figures of cyclostomes, osteostracans, and placoderms are adapted from Romer 1959.) * Indicates a paraphyletic group; † denotes extinct groups.
genes known to contribute to skeletogenic condensations: structural collagen genes and skeletal regulators, including Sox9 and Runx2. Osteoblasts in zebrafish and gar expressed Col10a1 (which is not expressed in tetrapod osteoblasts), as well as Sox9 and Col2a1, suggesting that osteoblasts of a teleost and a basally diverging ray-finned fish differ from tetrapod osteoblasts in their expression of components of the chondrocyte molecular fingerprint. The authors also reported the expression of genes normally seen in chondrocytes in osteoblasts of fish and frogs. These observations, and the fact that the common ancestor of frogs and fish is evolutionarily older than the common ancestor they both share with land animals, yielded the hypothesis that certain chondrocyte genes have been repressed during evolution of the osteoblast. Although Eames et al.’s hypothesis assumes a single origin and lineage of skeletal cells that requires critical evaluation in future analyses, this work clearly demonstrates the ability of cells and their regulatory networks to change over time, offering a source of variation on which evolution can operate.

Can molecular fingerprints be used to tell us about how cells that perform similar functions change the details of the phenotypes they produce? Or how cells produce morphological variation that we recognize as species-specific characters on which evolution acts? And how might information from cells tell us about variation in primate and human skeletal traits? As mentioned previously, chondrogenic gene expression of earlier diverging clades can be differentiated from osteoblasts of later diverging clades on the basis of their “molecular fingerprint” (Eames et al. 2012; Nguyen and Eames 2020). Importantly, the division between earlier-diverging and later-diverging vertebrates also corresponds to the emergence of vertebrates from sea to land. One major change that would have occurred with the emergence of terrestrial forms from aquatic life is the nature of the forces on growth and development of skeletal tissue, as well as the influence of these forces on a tissue as it performs a function.

Cells respond to instructions given by genes, but modern cellular biology reveals that skeletal cells are also regulated by epigenetic factors, of which the mechanical environment is one of the most influential and well studied (Kong et al. 2005; Li et al. 1987; Mauney et al. 2004; Sathi et al. 2015) (see also Rolian [this volume, Chapter 2], Deane et al. [this volume, Chapter 4], and Housman [Volume II, Chapter 4]). When mechanical forces change, cytoskeletal changes ensue that can influence cell growth, cell cycle progression, and gene expression (Burger and Klein-Nulend 1999; Currey 2003; Lanyon 1993; McCreadie et al. 2004; Sugawara et al. 2011). In this way, the mechanical inputs received are connected to important cellular processes (e.g., differentiation, proliferation) and properties of the tissues to which the cells contribute (e.g., strength, rigidity). The formation, development, modeling, and remodeling of bone are informed by genetic information but also depend on the mechanical environment and the associated mechanobiological responses at the cellular level (Ambrosi et al. 2019). The process by which bones sense and convert external mechanical stimuli into a biochemical response, ultimately integrating these signals into a functional response, is an example of mechanotransduction (Gusmão and Belangero 2009; Stewart et al. 2020).

Mineralized bone matrix is permeated by an interconnected network of microscopic channels (canaliculi), which contain cellular processes of osteocytes, allowing
neighboring osteocytes to communicate. Starting with the knowledge that the molecular fingerprints of teleost osteoblasts differ in key ways from that of tetrapod osteoblasts (Cohen et al. 2012), and adding that the biomechanical loads experienced under water are substantially different from those experienced in a gravitational world, the transition from water to land would undoubtedly confront OLCs in the skeletons of developing terrestrial vertebrates with novel mechanical forces never before sensed by aquatic vertebrates and only temporarily (evolutionarily speaking) by amphibians. Would the inherent changes in forces sensed by OLCs on land—forces that we know respond to and trigger molecular signals—contribute to how OLCs differentiate and how those tissues are formed, impacting the molecular fingerprint?

1.6 THE CELLULAR BASIS FOR VARIATION IN THE PRIMATE SKELETON

It has long been known that modern humans have relatively lightly built (gracile) skeletons relative to other primate species and to our early human ancestors. Explanations for observed skeletal gracility have included many factors (Ryan and Shaw 2015), including reduced levels of physical activity (Nowlan et al. 2011; Ruff 2005; Ruff et al. 1993), the dissipation of load through enlarged joint surfaces (Cotter et al. 2011; Latimer 2005), and selection for systemic physiological characteristics that differentiate modern humans from other primates (Alexander 1998; Stock 2006). Ryan and Shaw (2015) integrated multiple streams of evidence to address the effects of reduced mobility, diet, and ancestry on human skeletal variation and found that the relative gracility of the modern Homo sapiens skeleton results from decreased biomechanical loading. Their study demonstrates a correspondence between behavior and bone structure in the human femur and the effectiveness of trabecular bone structure as an indicator of activity patterns among prehistoric hominins and nonhuman primates. Their result reveals a proximate behavioral explanation for a recognized pattern. The authors interpreted the traits that showed variability to represent the plastic response of bone to the loading environment, while other more stable traits are believed to be more canalized (Ryan and Shaw 2015).

This study opens new avenues for anthropological investigation. For example, can we use comparative studies of trabecular structure of early hominin remains to propose or confirm dates in the transition from a fully mobile lifestyle towards sedentism? Among modern human populations, how would the timing of changing trabecular patterns correspond with the development of agriculture? Of animal husbandry? Is there a similar pattern of gracilization of the skeleton in any other nonhuman primate species, and, if so, what are the behavioral correlates? Can such patterns be used as an additional line of evidence for the transition from living and growing in a specific habitat (e.g., arboreal, terrestrial) or evidence of a habitual locomotor posture or adaptation (e.g., scansorial, saltatorial) across primate species? Or can these types of trabecular indicators only be used within species to reveal subtle changes in activity patterns?

The information gained from understanding the association between trabecular architecture and activity patterns is valuable. But this association is correlative and cannot tell us how the skeleton transfers information about mechanical input into interpretable patterns of skeletal variation. Knowing how mechanical information
is translated into a biological response at the level of the cell provides an explanation for how the skeleton adapts in real time and over generations to environmental inputs. This knowledge could produce hypotheses that can be tested experimentally and explored more fully with existing skeletal collections.

1.6.1 **Influence of Mechanical Environment on Cell Activity and Bone Structure**

It is well known that loading of bone through weight-bearing exercise causes increase in bone mass density, while lack of use, either from immobilization or hypogravity environments, results in bone loss. How does a tissue like bone sense these environmental changes and modify its mass to meet the requirements of a skeleton functioning in its environment? Bone’s ability to convert one type of information into another, mechanotransduction, involves a complex interplay of processes orchestrated by cells (Figure 1.3). Mechanical stimuli can prompt cells to generate biochemical signals that initiate intracellular processes (e.g., activation/repression of signaling pathways; up/down regulation of gene expression) that change the intra- and extracellular environments and can cause cells to die, proliferate, migrate, differentiate, or produce any of a number of biochemical reactions (Stewart et al. 2020). Mechanotransduction is not limited to bone but also occurs in cartilage, muscle, and blood vessels.

For bone, the cellular physiology of mechanotransduction is complex, involving four distinct phases (Figure 1.3):

1. **Mechanocoupling**—processes that result in bone cells being deformed by mechanical loading;
2. **Biochemical coupling**—processes by which bone cells communicate the deformation they experience by affecting intracellular signaling pathways;
3. **Signal transmission**—the process by which a sensor cell transmits a biochemical signal to an effector cell; and
4. **Effector cell response**—how the effector cell’s response to the signal results in changes in bone formation.

(Duncan and Turner 1995; Stewart et al. 2020)

The end result of these four phases is changes in bone architecture and morphology, interpreted as an adaptive response of the skeleton to its external environment. An understanding of the association between trabecular architecture and activity patterns entails a detailed dissection of mechanotransduction for trabecular bone formation but can be further informed by an understanding of how changes in the cell’s response to mechanical loading is registered in the DNA of an individual to be passed onto succeeding generations.

1.6.2 **Genetic Information and Information Transfer in Mechanotransduction**

A mechanical stimulus generates a biochemical signal, which in turn initiates several intracellular processes. These can include a response of a specific receptor (i.e., a