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1. EVOLUTIONARY CELL BIOLOGY

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Evolutionary biology encompasses all aspects of life, living and dead, from the molecular level to emergent phenotypes. Like its subject matter, however, evolutionary research has followed a pattern of descent with modification. Four historical contingencies bias and jade our general understanding of evolutionary mechanisms. First, most evolutionary study focuses on aspects of the environment extrinsic to the organism – resource availability, competitors, predators, pathogens, and potential mates. As a consequence, in academic institutions, evolutionary biologists are invariably housed with ecologists and behavioral biologists to the exclusion of molecular, cell, and developmental biologists. Indeed, all too often, there is an unhealthy level of mistrust between these two different camps.

Without question, the community of organisms with which a species interacts is a major driver of evolution, and ecology is central to this field. However, the molecules and structures internal to cells also comprise a sort of community of interacting partners that channel the possible routes of evolutionary descent with modification. Would this kind of disciplinary bias have existed had Darwin spent his life staring down the barrel of a microscope, or had molecular biology existed at the dawn of evolutionary thinking?

A second pervasive problem in biology is the religious adherence to the idea that natural selection is solely responsible for every aspect of biological diversity. Much of the field of evolutionary ecology, for example, seeks simply to determine why particular life-history and/or behavioral strategies are optimized to particular environments, leaving no room for alternative interpretations of phenotypic variation. For traits strongly related to fitness in animals and vascular plants, such models are often quite successful (Charnov 1982, 1993; Roff 1993; Krebs and Davies 1997), leading to conceptual models invoking tradeoffs such as (trait A) $\times$ (trait B) = a constant. This leaves unaddressed deeper questions as to source of the quantitative value of the constant or why such a constant even exists.

Inspired by this way of thinking and digging no deeper, many molecular biologists start with the dubious assumption that natural selection is also the only mechanism of evolution at the cellular level, often asserting that even the most blatantly deleterious features of organisms must actually have hidden favorable effects. Under this view, increased rates of mutation, translation error, and phenotypic aberrations in stressful environments (Galhardo et al. 2007; Jarosz and Lindquist 2010; Schwartz and Pan 2017), aneuploidy in gametes (Wang et al. 2017), and gene location in prokaryotes (Martincorena et al. 2012; Merrikh 2017) are all products of natural selection, maintained to somehow preserve future potential for evolvability. Some have gone so far as to proclaim that virtually any nucleotide that is at least occasionally transcribed or bound to a protein must be maintained by selection

(ENCODE Project Consortium 2012).

Such arguments are inconsistent with substantial theory and empirical work suggesting that many aspects of gene and genome evolution are consequences of the limitations of natural selection (Kimura 1983; Lynch 2007). Remaining, however, is the key question as to the level of biological organization above which selection can be safely assumed to be the only driving force of evolution. Does effectively neutral evolution somehow cease to occur at the level of cellular features or at a higher level of emergent properties in multicellular species?

Third, although evolution is a process of genetic change, and evolutionary biology has long been endowed with a powerful theoretical framework grounded in genetics, a large fraction of what passes as evolutionary research is completely removed from genetics. For example, the optimization hypotheses in evolutionary ecology noted above focus almost exclusively on verbal or semi-quantitative arguments devoid of genetic details. The field of evolutionary developmental biology is often proudly defiant of any association with conventional genetic understanding.

Finally, the vast majority of research in evolutionary biology is focused on multicellular animals and land plants. It is easy to become enamored of biodiversity that is readily visualized on a day-to-day basis. It is also easier to work with organisms that can be seen without the aid of a microscope. Nonetheless, animals and vascular plants are the odd-balls of evolutionary biology – interesting in their own right, and containing the only species capable of writing and rejecting a manuscript, but also constituting only a tiny fraction of the phylogenetic Tree of Life and of the planetary census of individuals.

We now have well-established fields of molecular and genome evolution, and some aspects of evolutionary developmental biology are being integrated with modern evolutionary theory. Yet, despite the extraordinary accomplishments in the field of cell biology, there is as yet no comprehensive field of evolutionary cell biology. Attempts to decipher the Tree of Life, most of which is unicellular, are common, and many aspects of molecular evolution are focused on cell biological issues. However, a general evolutionary framework for explaining the diversity of cell biological structures and processes remains to be developed.

It need not have been this way. Early in the past century, for example, there was an enormous amount of comparative work done on diverse protists. But the late 1900s witnessed a near cessation of this kind of work, as cell biology became increasingly inward looking and medically oriented, focusing on a few model laboratory systems devoid of variation, e.g., the bacterium *Escherichia coli,* the yeast *Saccharomyces cerevisiae,* and mammalian cell cultures. Embracing the many impressive results from these systems, but going well beyond them, the goal here is to plant the seeds for a science of evolutionary cell biology.

The Dominance of Unicellular Life

Taking a phylogenetic perspective, it can be seen that the major foci of life-science research – animals and land plants – comprise only a small fraction of the Tree of Life (Figure 1.1). Most of global diversity at the DNA level resides in prokaryotes, and this is even more true if one further considers the variation of gene functions, as

prokaryotes harbor much more diversity in metabolic pathways than do eukaryotes. Even restricting attention to eukaryotes, the vast majority of phylogenetic diversity resides within lineages consisting entirely of unicellular species.

The conclusion that the vast majority of life on Earth is in the provenance of unicellular organisms is retained if the focus is shifted to total numbers of individuals. Achieving accurate census counts in various groups of organisms is made difficult by the uneven sampling of different global ecosystems, the absence of surveys for many phylogenetic groups, and seasonal fluctuations of population sizes in microbes. However, crude order-of-magnitude estimates are possible. For example, the number of viral particles in the open oceans is estimated to be $\simeq 10^{30}$ (Suttle 2005), and even if there were twice as many viruses on land and in freshwater (unlikely), this would not increase the global estimate beyond $\simeq 10^{31}$. The estimated global number of prokaryotic cells is also $\simeq 10^{30}$ (Flemming and Wuertz 2019), and this sums to a total amount of global biomass that exceeds that of all animals by a factor of ~ 40 (Whitman et al. 1998; Kallmeyer et al. 2012; Bar-On et al. 2018). There may be as many as 10^{12} species of prokaryotes (Locey and Lennon 2016), although an alternative upper-bound estimate is $\sim 10^6$ (Amann and Rosselló-Móra 2016). Taking the logarithmic mean of these two estimates, 10^9, implies an average of $\sim 10^{21}$ individuals per prokaryotic species (although substantial variation in this number must exist among taxa).

The total number of unicellular heterotrophic eukaryotic cells is $\sim 0.1\%$ of that for bacteria in the marine environment (Pernice et al. 2015), and drawing from average estimates in Whitman et al. (1998) and Landenmark et al. (2015), the ratio in terrestrial soils is $\simeq 0.5\%$. This suggests that the total number of unicellular eukaryotic cells on Earth exceeds 10^{27} (Bar-On et al. 2018), as the previous estimates exclude fungi and photosynthetic species. Thus, assuming the average volume of a eukaryotic cell is $\simeq 1000\times$ that of a prokaryote (Chapter 8), the global biomass of unicellular eukaryotes likely exceeds that of prokaryotes. Of the estimated 10^7 eukaryotic species on earth (potentially just 1% of the number for prokaryotes), it has been suggested that $\sim 90\%$ are animals, 6% fungi, 3% plants, and the small remainder protists (Mora et al. 2011). The latter could, however, be vastly underestimated, given the relative lack of attention to the systematics of such groups (Wideman et al. 2020). Assuming 10^6 unicellular eukaryotic species would imply an average $\simeq 10^{21}$ individuals per species, the same order of magnitude as in prokaryotes.

Although crude, these estimates for unicellular organisms dwarf the numbers of individual land plants and metazoans. For example, there are $\sim 10^{13}$ trees on Earth, and $\sim 75,000$ tree species (Crowther et al. 2015; Beech et al. 2017; Gatti et al. 2022), implying an average of $\sim 10^8$ individuals per tree species. Two of the most abundant groups of invertebrates on Earth are the ants, estimated to comprise $\sim 10^{16}$ individuals distributed over $\sim 10^4$ species (Hölldobler and Wilson 1990) and Antarctic krill with $\sim 10^{15}$ individuals in a single species (Atkinson et al. 2008). Nematodes, perhaps the most numerically abundant animal phylum, comprise $\sim 10^{20}$ individuals globally, distributed over some 10^6 species (Kiontke and Fitch 2013; van den Hoogen et al. 2019). As these observations imply that most animals have global population sizes $\ll 10^{15}$ (with a suggested mode of $\sim 10^{10}$; Buffalo 2021), assuming 10^7 animal species suggests that the total number of animals on Earth is $< 10^{20}$, several orders of magnitude below the numbers for both prokaryotes and unicellular

eukaryotes.

An upper-bound estimate to the total number of vertebrate individuals on earth is $\sim 10^{16}$, distributed over $\sim 10^5$ species (mostly fish), implying an average of $\sim 10^{11}$ individuals/species (Bar-On et al. 2018). For birds, the average number is $\sim 10^7$ individuals (Callaghan et al. 2021). Notably, the average human harbors a microbiome of $\sim 10^{13}$ bacterial cells, which exceeds the total number of humans that have ever lived (Sender et al. 2016).

What is Evolutionary Cell Biology?

As all organismal features derive from cell-level processes, an ultimate understanding of the mechanisms of evolution cannot be complete without an appreciation for how cellular features emerge on an evolutionary time scale. As nicely summed up in the timeless quote of E. B. Wilson (1925), "The key to every biological problem must finally be sought in the cell, for every living organism is, or at some time has been, a cell."

Evolutionary cell biology is the fusion of cell biology with evolutionary thinking, informed by the integration of the great engines of theoretical and quantitative biology – biochemistry, biophysics, and population genetics (Lynch et al. 2014). Despite its centrality, especially for the multitude of species for which the individual cell is also the organism, this intrinsically interdisciplinary field is embryonic in almost every way. For example, evolutionary biologists have only rarely incorporated the concepts of biochemistry and biophysics into their thinking, despite some striking similarities between the underlying theoretical frameworks of statistical physics and population genetics (Sella and Hirsh 2005; Lässig 2007; Barton and de Vladar 2009; Zhang et al. 2012). Likewise, although cell biologists commonly remark on the exquisite design of the traits being studied, they almost never consider the feasibility of the evolutionary paths by which such features are imagined to have emerged.

Understanding evolution at the cellular level requires consideration of three major aspects of the environment, each of which subdivides into at least three other domains (Figure 1.2). First, as noted above, the classical intellectual domain of evolutionary biology is ecology, where the usual focus is on challenges imposed by factors outside of the organism. The central issues here include the procurement of resources, the avoidance of predators and pathogens, the acquisition of mates, and various aspects of mutualism and cooperation.

Second, we must consider the cellular environment, which imposes historical contingencies, biophysical constraints, and molecular stochasticity. All cells are endowed with an array of features fundamentally unmodified since the last universal common ancestor of life. These include: the use of double-stranded DNA as genomic material; the expression of genes through intermediate transcriptional products (made of RNA), and in the case of proteins followed by translation at ribosomes; the use of lipid membranes; and the deployment of highly conserved mechanisms for ATP production. The strengths of bonds associated with C, H, N, O, and P atoms, life's favored elements, dictate the stability of intermolecular interactions. Small cell size imposes an upper limit on the number of molecules that can be housed. One can imagine other possible forms of cellular organization, but

on planet Earth these are the indelible backgrounds upon which all other cellular modifications must develop.

Finally, there is the population-genetic environment. Owing to the imperfections in all molecular interactions, DNA replication is naturally error-prone. Although this ensures the recurrent input of the genetic variation upon which all evolutionary change ultimately depends, the privilege of evolutionary potential comes at a cost – most mutation are deleterious. Recombination assorts variation within and among chromosomes, further generating genetic diversity, which promotes some kinds of evolutionary change while inhibiting others. Random genetic drift, a consequence of finite numbers of individuals within populations and genes being linked on chromosomes, creates noise in all evolutionary processes, more so in smaller populations.

The joint operation of these three dimensions of the population-genetic environment defines the limits to what natural selection can and cannot accomplish in various phylogenetic lineages (Chapters 4, 5, and 6), thereby dictating the mechanisms and directions by which evolution proceeds at the cellular level.

Although cell biology has not been the traditional domain of evolutionary biology, it offers powerful opportunities for identifying the explicit biological connections between genotypes, phenotypes, and fitness, which are essential to the development of a mature field of evolutionary biology. With these matters in mind, the following chapters focus on the degree to which selection, effectively neutral processes, historical contingencies, and/or constraints at the biochemical and biophysical levels jointly influence patterns of evolutionary diversification. This way of thinking may ultimately find use in the applied fields of agriculture, medicine, environmental science, and synthetic biology.

The Completeness of Evolutionary Theory

Before proceeding, some comments on the use of theory in biology are in order. Without an explanatory framework, science is reduced to a fact-collecting enterprise. Of course, the emergence of facts from consistent observations is central to science, but theory provides a mechanistic explanation of the facts. A theoretical framework can motivate the development of predictions in areas where observations have not been made previously. Ideally, such a reach is not simply based on statistical extrapolation, but on arguments from first principles. In particular, mathematical theory allows the construction of logical arguments from well-defined assumptions, whereas verbal theorizing can easily go awry in the analysis of complex systems.

Fortunately, evolutionary biology has a well-established framework of quantitative principles from which to draw. In one of the most important scientific papers ever written, Fisher (1918) convincingly elucidated a simple connection between the Mendelian inheritance of segregating genetic factors and the near continuous range of phenotypic variation for complex traits within populations, closing a long-standing controversy about the material basis of evolution (Provine 1971). In this same paper, Fisher established one of the primary pillars upon which modern statistics relies, the analysis of variance. Emanating from these roots, the century-old field of popu-

lation genetics now forms the foundation for all of evolutionary theory (Walsh and Lynch 2018). Most of the principles for integrating selection, mutation, and random genetic drift were laid down in the first half of the last century, while key findings with respect to recombination were generated over the next fifty years. With the further integration of diffusion theory and statistical aspects of gene genealogies now commonplace, the field of theoretical population genetics is as well-grounded as any other area of quantitative biology.

Notably, the establishment of the primary roots of evolutionary theory substantially preceded any knowledge of the details of the genetic material. Starting in the 1950s, dramatic findings emerged in the field of molecular genetics. These included the discovery of DNA as the ultimate genetic material, the basic structure of genes and their component parts, the processes of transcription and translation, and the molecular mechanisms of recombination. Yet, none of these discoveries led to any alteration in the basic structure of evolutionary theory. The discovery of mitochondrial DNA did not alter our basic understanding of maternal effects, and the discovery of transposable elements did not alter our appreciation of the mutational process. Such observations simply provided a deeper molecular explanation of modes of production of phenotypic variation. This robustness of evolutionary theory in the face of revolutionary changes in our understanding of genetics at the molecular level speaks volumes. Important specific applications may remain to be developed, but the theoretical foundations of evolutionary genetics provide a solid framework for defining the conditions under which various evolutionary scenarios are possible and not possible.

This optimistic viewpoint is periodically confronted with claims that evolutionary biology is in a phase of turmoil. However, the bearers of such messages seldom offer a solution to the field's imagined short-comings, and without exception, these episodes have gone badly. Most notable are Goldschmidt's (1940) argument that large changes in evolution are products of macromutations with coordinated developmental effects and Lysenko's rejection of Mendelian genetics in favor of the inheritance of acquired characteristics.

Unlike the laws of physics, biology is subject to historical contingencies, and for virtually every set of general observations, one can find some kind of exception. Discoverers of such exceptions sometimes claim that their observations are sufficient to dismantle previous theoretical frameworks for broadscale patterns. More often than not, however, a deeper look almost always reveals underlying explanations for oddities that are fully compatible with the rules of life.

One of the more recent promotional exercises involves a clamor for an "extended evolutionary synthesis" or EES (Gerhart and Kirschner 1997; Pigliucci and Müller 2010; Goldenfeld and Woese 2011; Shapiro 2011; Laland et al. 2014, 2015). Asserting that population genetics provides an antiquated and inadequate framework for evolution, the claim is that "the number of biologists calling for change in how evolution is conceptualized is growing rapidly," and that there is a current "struggle for the very soul of the discipline." The nature of this discourse is reminiscent of the distant "bean-bag genetics" diatribe of Mayr (1959, 1963), which was promptly disemboweled by Haldane (1964). No glaring errors in contemporary evolutionary theory have been correctly pointed out by the EESers, little evidence of familiarity with current theory has been provided, and no novel predictions have been offered

(Stoltzfus 2017; Welch 2017). There is just a warning that once qualified theoreticians come on board, the revolution will begin.

A particularly extreme claim is that the discovery of various epigenetic effects amounts to a game-changer in evolutionary biology, imposing the need to revamp our general understanding of inheritance and its evolutionary implications (Jablonka and Lamb 2005; Caporale 2006; Danchin et al. 2011; Shapiro 2011). Highlighted phenomena include base modifications on DNA, histone modifications on nucleosomes, and mechanisms of gene regulation by small RNAs, all of which can in principle have transient trans-generational effects without imposing permanent changes at the level of genomic DNA. Advocates of epigenetic inheritance as an enhancer of evolvability commonly argue that such phenomena promote beneficial phenotypic responses to environmental induction, which then allows for an acceleration in the rate of adaptive phenotypic evolution, in effect resurrecting the concept of the inheritance of acquired characteristics.

The logic underlying the entire subject has been masterfully dismantled by Charlesworth et al. (2017), and just two points will be made here. First, to appreciate the implausibility of a long-term contribution of nongenetic effects to phenotypic evolution, one need only recall the repeated failure of inbred (totally homozygous) lines to respond to persistent strong selection. Many such experiments dating back to the beginning of the 20th century provided formal support for the necessity of genetic variation for evolutionary progress (Lynch and Walsh 1998). Second, countering the claim that evolutionary theory is incapable of addressing the matter of epigenetic inheritance, one need only point to models for the inheritance of environmental maternal effects developed well before the discovery of the molecular basis of any epigenetic effects (Table 1.1). Existing theory readily demonstrates that variance in maternal effects can contribute to the response to selection, but unless such effects reside at the DNA level, the response is bounded, owing to the fact that trans-generational effects are progressively diluted out. Moreover, if epigenetic effects are sufficiently stochastic, they will reduce rather than enhance the response to selection, owing to the reduction in the correspondence between genotype and phenotype.

Although a persistent claim of the EESers is that the environmental induction of a trait in a novel situation can enhance the exposure of the trait to selection, thereby magnifying the response to selection, this is by no means a novel insight. Such effects are central to the concept of genotype $\times$ environment interaction, the theory of which dates back decades (Lynch and Walsh 1998). Indeed, breeders have long exploited this concept to determine the optimum environmental setting in which to select for particular phenotypes (Walsh and Lynch 2018). Thus, the idea that evolutionary theory needs to be remodeled to account for phenotypic plasticity is without merit.

The most remarkable EESer claim is that the key flaw of contemporary evolutionary theory is the assumption that change in allele frequencies is a necessary component of the response to selection (Laland et al. 2014). Their counter view is that "the direction of evolution does not depend on selection alone, and need not start with mutation." Whereas it has long been appreciated that evolution can and sometimes does occur in the absence of selection (for example, by random genetic drift of neutral traits), we await an explanation as to how any form of evolu-

tion (aside from cultural) can occur in the absence of genetic variation. Technically speaking, evolution can occur in the absence of allele-frequency change, but only via changes in the form of allelic associations across loci (e.g., via linkage disequilibrium, which necessarily implies transient genotype-frequency change).

Far from providing a weak and/or incomplete caricature of evolving genetic systems, population- and quantitative-genetic theory has generated powerful, general, and sometimes unexpected mechanistic explanations for trait variation and phenotypic evolution, several of which are noted in Table 1.1. Few of these issues would have ever been resolved with simplistic verbal arguments. Indeed, it was Fisher's paper (1918) that rescued the previously verbal debate over evolutionary mechanisms from the high seas of obfuscation. Inspired by quantitative thinking derived from first principles in genetics, most subfields in evolutionary biology were rapidly transformed by the emergence of population-genetic theory. Developmental biology is somewhat of an exception, remaining in many respects in a pre-population-genetics mode of confusion, a condition that evolutionary cell biology need not emulate.

Although the preceding railing on the EES movement may be offensive to some and/or pandering to trivia to others, the implication that a century's worth of theoreticians has been woefully mislead is a misrepresentation of the facts, and as Darwin (1871) pointed out, a reliance on false facts is "highly injurious to the progress of science." As outlined in Table 1.1 and expanded upon in Chapters 4 to 6, evolutionary theory developed over the past century has made predictions that are consistent with a wide range of empirical observations. This being said, however, because evolution is a stochastic process, no theoretical framework can ever be expected to predict the exact trajectories of evolution at the molecular, cellular, or developmental levels in any specific lineage. As Haldane (1964) pointed out, if population genetics could make such specific predictions, it would not be a branch of biology – it would be the entirety of biology.

Table 1.1. A few key areas in evolutionary biology where theory has enhanced our understanding of the mechanistic basis of trait variation, and in doing so has provided novel predictions. Many of these issues will be covered in depth in subsequent chapters, although this list is by no means complete. LW denotes Lynch and Walsh (1998).

Topic:	References:
Quantitative-trait variation:	
Phenotypic resemblance between relatives, and its scaling with the degree of relationship.	Fisher 1918; Kempthorne 1954; LW Chapter 7
Inbreeding depression, and how this scales with parental relatedness.	Crow 1948; LW Chapter 10
Quasi-inheritance of familial (including maternal) effects, and transient selection response.	Willham 1963; Falconer 1965; LW Chapter 23
Expression of all-or-none traits as a function of underlying determinants.	Wright 1934a,b; LW Chapter 25
Pleiotropy and genetic correlation between traits.	Mode and Robinson 1959; LW Chapter 21
Long-term patterns of evolution:	

Sudden (saltational) transitions from one discrete character state to another.	Lande 1978
Rates / patterns of evolution in the fossil record.	Charlesworth et al. 1982; Charlesworth 1984a,b
Rapid evolution across adaptive valleys by stochastic tunneling.	Lynch 2010; Weissman et al. 2010
Mutation bias and the inability of a mean phenotype to attain an optimal state.	Lynch 2013; Lynch and Hagner 2014
Spatial variation in genotypic values in the absence of underlying ecological variation	Higgins and Lynch 2001
Genome evolution:	
The fate of duplicate genes.	Force et al. 1998; Lynch and Force 2000
Conditions for the spread of mobile elements.	Charlesworth and Charlesworth 1983; Charlesworth and Langley 1986
Evolution of codon bias.	Bulmer 1991
Evolution of transcription-factor binding sites.	Lynch and Hagner 2014
The illusion of evolutionary robustness.	Frank 2007; Lynch 2012
Evolution of the genetic machinery:	
Evolution of the mutation rate.	Lynch 2011; Lynch et al. 2016
Evolutionary consequences of sexual reproduction.	Kondrashov 1988; Charlesworth 1990; Otto and Barton 2001
Evolutionary deterioration of sex chromosomes.	Charlesworth and Charlesworth 2000

Evolution Via Nonadaptive Pathways

Darwin's (1859) and Wallace's (1870) grand views about selection as a natural force for the emergence of adaptive change marked a watershed moment in the history of biology. Their narrative has been so convincing that most who now think about evolution simply view all aspects of biology, current and historical, as necessary products of natural selection. However, whereas natural selection is one of the most powerful forces in the biological world, it is not all powerful. As will be seen repeatedly in the following pages, the genetic paths open to exploitation by selection are strongly influenced by another pervasive force – the noise in the evolutionary process imposed by random genetic drift.

Evolutionary stochasticity is an inevitable consequence of finite numbers of individuals within populations and the physical linkage of different nucleotide sites on chromosomes. If the power of selection is weak relative to that of drift, as is often the case at the molecular level, evolution will proceed in an effectively neutral manner (Chapter 4). Biased mutation pressure can also modify evolutionary trajectories, but even nonbiased mutation can strongly influence the distribution of mean phenotypes if mutation is sufficiently strong relative to the efficiency of selection (Chapter 5).

To understand the degree to which natural selection molds the features of populations, it is essential to know what to expect in the absence of selection. For

this reason, neutral models have been repeatedly exploited in evolutionary analyses. The three nonadaptive mechanisms of evolution – random genetic drift, mutation, and recombination – are the sole evolutionary mechanisms under such models. The resultant formulations then provide null hypotheses for testing for natural selection. Neutral models are relatively easy to develop for DNA-level features, as mutation can be explicitly defined in terms of the six possible nucleotide substitutions, and such constructs are fundamental to most studies in molecular evolution (Kimura 1983; Jensen et al. 2019). Although the construction of neutral models becomes more challenging in the case of complex cellular/organismal traits, where the phenotypic features of mutations can be more difficult to define (Lynch and Walsh 1998), this is not justification for ignoring the matter. Indeed, neutral models have been particularly useful in attempts to understand long-term phenotypic divergence recorded in the fossil record, where dramatic changes that might seem only achievable by selection are found to be not so impressive when evaluated in the proper context of drift and mutation (Lande 1976; Charlesworth 1984a; Lynch 1990).

Some have suggested that so much evidence for selection has emerged that we should abandon the use of neutral theory (Pigliucci and Kaplan 2000; Hahn 2008; Kern and Hahn 2018), in one case going so far as to argue that "the implications of our continued use of neutral models are dire," and "can positively mislead researchers and skew our understanding of nature." No one argues that selection is unimportant, but the proposition of a selection theory as a null model for hypothesis testing presents a logical challenge. One can concoct a selection argument for essentially any observed pattern, rendering such a restrictive view unfalsifiable. If one form of selection does not adequately fit the data, then one can invoke another, and failing that, still another, never abandoning the pan-selection view. In contrast, when properly constructed, neutral models make very explicit predictions, rescuing arguments for the role of natural selection from an endless loop of qualitative handwaving. By offering a formal means for testing for the influence of selection, the measurement of deviations between observations and neutral expectations yields a deeper and more defensible understanding of evolutionary processes.

An additional problem with criticisms of neutral theory is their frequent reliance on incorrect biological assumptions. For example, Lewontin (1974) invoked the fact that standing variation in natural populations is only weakly associated with effective population size (N_e) as a dramatic violation of the neutral theory, as standing levels of variation at silent sites in populations should scale with $N_e u$, where u is the mutation rate per nucleotide site (Chapter 4). Although this argument continues to be made (Hahn 2008; Buffalo 2021), the postulated pattern ignores the fact (unknown at Lewontin's time) that mutation rates evolve to be inversely correlated with N_e, rendering the product $N_e u$ relatively constant (Lynch et al. 2016; Chapter 4). Thus, Lewontin's observation is not so paradoxical after all.

Like the call for an extended evolutionary synthesis, the call for a selection theory of evolution has not resulted in any theoretical upheaval. No offering of a novel theory of selection has been presented, and none is likely to emerge for the very simple reason that we already have such a theory. From the very beginning, population- and quantitative-genetic theory has fully embraced selection as a central force in evolution, with the understanding that what selection can accomplish is modulated by the relative power of the nonadaptive forces of evolution (Walsh and

Lynch 2018).

The preceding comments have been offered primarily for the benefit of outsiders with only a peripheral understanding of what might appear to be substantive controversies. Conflict and cooperation are the engines that keep science running. Conflict engineered and incessantly repeated with no evidence sometimes has other motivations (Gupta et al. 2017).

The Grand Challenges

Comparative biology has made substantial contributions to evolutionary biology, telling us what has evolved, and hence revealing the facts that evolutionary theory needs to explain. In some cases, where there is a decent fossil record, comparative biology has also provided insight into rates of phenotypic evolution. Where there is compelling phylogenetic information, ancestral phenotypic states can sometimes be predicted, and in the case of simple molecular features even resurrected and evaluated (Hochberg and Thornton 2017). Good comparative biology can be done in the complete absence of knowledge of evolution. However, when disconnected from the mechanisms driving genetic change, comparative biology is a far cry from evolutionary biology.

The challenges for evolutionary cell biology are substantial. Owing to cell biology's focus on just a few model organisms, there is no expansive field of comparative cell biology. As a consequence, the range of existing variation for cellular traits is often unclear, leaving even the question of what needs to be explained unsettled. Even so, evolution is still part of the mindset of many cell biologists who focus on a single species throughout their careers. This can be seen from the final paragraphs of numerous papers in cell biological journals where adaptive hypotheses are commonly offered for the phenomenon observed.

The ultimate goal of any area of science is to provide compelling, mechanism-based answers to all of the central questions in the field, bringing things to the point at which all future observations have a pre-existing explanation. No scientific area has yet reached that point, and evolutionary biology might not be the first. What follows is a brief list of some of the major challenges that will have to be surmounted for evolutionary cell biology to achieve a reasonably mature state. Ways in which their solution might be achieved will be explored in detail in the following chapters.

The origin of life. More than three billion years ago, cellular biochemistry became established in such a way as to provide all of the necessities for evolution: metabolism, growth, replication, and variation. Deciphering the ways in which this happened would go a long way towards explaining the seemingly idiosyncratic features shared by all of life. Unfortunately, owing to the absence of fossils for the simplest of cells, we will probably never attain a precise understanding of the first steps by which the ancestor of all life emerged. We may never know whether competing forms of life initially coexisted and/or fused to form the most distant ancestor of us all. However, hypotheses focused on potentially plausible scenarios, combined with research in biochemistry, can help narrow down the alternative possibilities, in turn yielding useful predictions as to where life might have originated independently

elsewhere in the universe (Chapter 2), and offering up possibilities for designing synthetic life forms. Fortunately, a lack of clarity on these matters does not bear in any significant way on our ability to understand the mechanisms of evolution in contemporary organisms.

The roots of organismal complexity. Although it is commonly asserted that added layers of cellular complexity make for more robust and evolutionary successful organisms, evidence for this is entirely lacking. If complexity is entirely driven by natural selection, then why has only one lineage (eukaryotes) evolved complex internal cell structure? And why has the apex of biological complexity, multicellularity at the level found in land plants and animals, evolved only twice (or three or four times if one wishes to include kelps and fungi)? One might argue that there is something fundamentally lacking in prokaryotes that prevents such evolution, yet as noted above, despite any imagined deficiencies, microbes comprise much of the earth's biomass. Indeed, from the standpoint of metabolism, prokaryotes are the cradles of diversity, whereas eukaryotes are relatively bland. Such disparities reveal the intrinsic biases that arise if evolutionary thinking is confined to visually perceived morphological differences.

As noted above, there is substantial support for the idea that much of evolution at the genomic level has proceeded by effectively neutral processes, guided largely by the forces of mutation and random genetic drift. Moving to higher and higher levels of organization, e.g., protein structure, protein-complex architecture, cellular features, and the emergent properties of multicellular organisms, one might expect that the likelihood of neutral evolution would be dramatically diminished (Zhang 2018). We will see in subsequent chapters, however, that because there are often many ways to achieve the same phenotype, the paths open to neutral evolution at the cellular level may often be more plentiful than at lower levels of organization. This means that rather than being products of adaptive promotion, various aspects of cellular complexity may have arisen via nonadaptive pathways of evolution.

The types of mutations that arise in any particular interval are a matter of chance, not summoned by selective demand, the molecular spectrum of mutations strongly depends on organismal background. Thus, owing to the combined forces of mutation pressure and genetic drift, biological structures and functions need not evolve in directions that would be most economical from an engineering perspective, and as will be seen in the following pages, some are quite arcane. As a modern analogy to the passive emergence of complexity, consider how software companies modify their computer code over time – not by full-scale rewriting of the code, but by inserting patches for old problems. This slow accrual can lead to a complexity ratchet, whereby a general function is retained despite an irreversible series of cumulative changes at the component level.

Molecular stochasticity. Messenger RNAs are often present in fewer than ten copies per cell, sometimes with a mean less than one for specific genes, especially in small-celled species. Proteins have longer half lives and tend to be more abundant, but still are often present as only hundreds of copies per gene per cell. Transcription factors are among the rarest of proteins, leading to questions as to how they reliably find their DNA targets. Collectively, these features and more (including

asymmetries in cell division; Chapters 7, 9, and 21) lead to substantial stochasticity in cellular composition, even among cells with identical genotypes inhabiting homogenous environments.

Natural selection operates on phenotypic variance, and is more efficient when most of the variance is due to genetic differences. Thus, stochastic cellular noise must impose a speed limit on the rate of evolution, as it blurs the reliability of the phenotype as an indicator of the genotype. How does this problem with phenotypic variation vary across cellular life forms? On the one hand, small cells might be expected to exhibit more phenotypic variation associated with internal and external environmental factors (Chapters 7, 9, and 21). But on the other hand, large populations of small cells may harbor more genetic variation, which combined with short cell-division times might enhance the rate of evolution (Chapters 4 to 6).

Molecular complexes. The rules of life are such that each messenger RNA almost always encodes for one amino-acid chain. However, the majority of proteins organize into higher-order consortiums, e.g., dimers, tetramers, etc. Such complexes are frequently comprised of subunits derived from the same genetic locus (homomers), often with the multimer having no different function than the subunit components. Heteromers consisting of nonidentical components also exist, many of which are higher-order complexes that are more than the sum of their parts, e.g., the ribosome and the nuclear pore complex. The number of subunits underlying the same protein can vary across species, but not always in ways that reflect organismal complexity. This weak connection is very unlike the situation in genome evolution, where genome architecture becomes enormously complex in large multicellular species (Lynch 2008). Observations on the phylogenetic diversity of molecular complexes raise the suspicion that natural selection is unlikely to be the sole driving force (Chapter 13).

Cellular networks. Very few of the molecular constituents of cells operate alone. Examples of molecular networks include the cell cycle, transport systems, circadian clocks, and pathways in metabolism, transcription regulation, and signal transduction (Chapters 10, 18, 15, 19, 21, 22). The structural features of cellular pathways often border on the baroque, e.g., larger numbers of steps than seemingly necessary, linear chains of enhancing vs. suppressing steps, etc. Given that each component added to a pathway imposes an energetic cost of production on the cell, how do such architectures emerge? Do significantly profitable kinetic and/or dynamical properties emerge with some structures, or do they again just represent evolutionary sojourns along effectively neutral paths? Sometimes different lineages have similar network topologies, but with entirely different underlying protein participants or orders of steps. How does rewiring of an underlying structure evolve without leading to catastrophic intermediate consequences?

Intracellular and extracellular communication systems consist of at least one signaling molecule and one receptor. How does the language of such systems coevolve so as to avoid crosstalk between parallel pathways? When are there sufficient degrees of freedom to allow cellular communication systems to drift over time in an effectively neutral fashion, much like the human languages have diversified across the planet?

Cellular surveillance systems. The internal cellular environment introduces a wide variety of challenges associated with the accuracy of a wide array of cellular functions: errors introduced at the levels of replication, transcription, and translation, enzyme promiscuity with respect to substrate utilization, protein-folding problems, etc. There are often multiple layers of surveillance and correction for intracellular errors, suggesting highly refined and robust systems. Yet, the error rates for some of these functions can vary by at least 1000-fold among organisms, and some layers of surveillance are lost in some phylogenetic lineages (Chapter 20). Such observations raise numerous questions. High rates of surveillance are costly, but low fidelity can be catastrophic, so what are the limits to the burden of manageable intracellular error proliferation? Owing to the power of random genetic drift, there are limits to the level of molecular perfection that can evolve for any particular cellular function. Does this encourage the expansion of complexity by the evolutionary layering of surveillance mechanisms, e.g., proofreading, and if so, are there any long-term advantages to such embellishments or does the overall performance regress to its original state?

Growth regulation. So-called "growth laws" have been invoked for years by microbial physiologists, and substantial theoretical work has been devoted to explain these. However, the empirical work has been largely confined to a single species (*E. coli*), leaving open many questions about generality (Chapter 9). Moreover, the models that have been developed are largely phenomenological, leaving mechanistic issues unresolved. When species evolve under different resource conditions, does the evolved "growth-law" pattern recapitulate the more transient (plastic) pattern found within a genotype in response to varying nutrient availability? That is, do patterns of evolutionary response reflect patterns of physiological response? Are the rules for eukaryotes the same as those for prokaryotes?

Biological scaling laws. Cell biologists have identified a number of "scaling laws" that transcend species boundaries (Chapter 8), whereby specific cellular features can be approximated as power functions of cell size. The traits involved range from cell division rates to total lifetime energy budgets to internal organelle sizes to swimming speeds. Such patterns provide convincing statistical descriptions of the rules of life. But what are the underlying mechanisms leading to the observed slopes and intercepts of such functions, and why do they often appear to be universal across the Tree of Life?

Summary

- The fact that all evolutionary change begins at the cellular level motivates the need for eliminating the intellectual disconnect between cell biology (including microbiology) and evolutionary theory. Together, these subdisciplines provide the key connections between genotype, phenotype, and fitness that are essential to understanding all evolutionary processes.

- The need for a field of evolutionary cell biology is further justified by the composition of the biosphere. The total number of prokaryotic cells on earth outnumbers that of unicellular eukaryotes by several orders of magnitude, and the latter exceeds the number of animals and land plants by a similar degree.

- A mature field of evolutionary cell biology will ultimately need to integrate the three big engines of quantitative biology (population genetics, biophysics, and biochemistry) with comparative and experimental analyses across the Tree of Life.

- Evolutionary theory, grounded in principles of Mendelian genetics and stochastic transmission of gene frequencies, is as well-established as any area of quantitative biology. Thus, an essential platform is in place for developing a mechanistic understanding of the origin and diversification of cellular features by the progressive fixation of new mutations.

- Although natural selection is the most powerful force in the biological world, it is not all powerful. Rather, the efficiency of selection is dictated by the population-genetic environment – defined by the magnitudes of mutation, recombination, and random genetic drift, all of which vary by orders of magnitude among phylogenetic lineages. Many aspects of molecular and genome evolution reflect the inability of natural selection to act, as opposed to being reflections of adaptive refinement. The following chapters will demonstrate that this is also commonly true at the cell biological level.

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Figure 1.1. A broad overview of the Tree of Life. The overall structure is presented in an idealized fashion, some details of which are covered in Chapter 3. The main points are that: 1) the overall tree is primarily prokaryotic, with eukaryotes being derived from one small lineage (highlighted by the small blue ball) within the major domain called the Euryarcheota; and 2) the land-plant (green) and animal (red) lineages comprise only a small fraction of the diversity within eukaryotes, as shown by the expansion of the total eukaryotic lineage to the left (dashed lines).

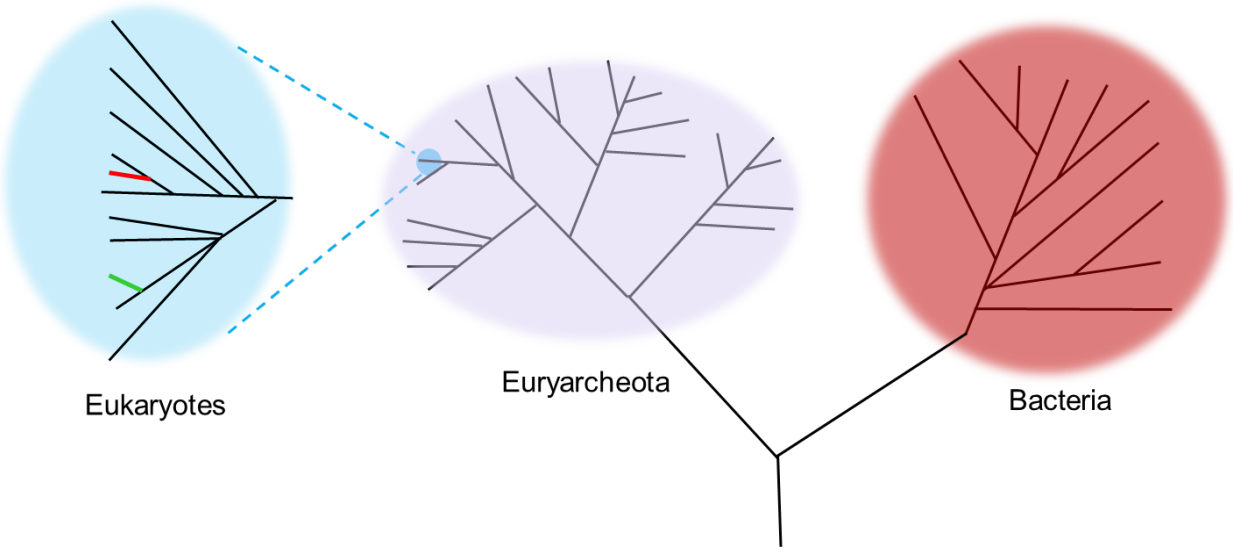

Figure 1.2. Summary of the major environmental components influencing the tempo and mode of evolution.