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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 research focuses on aspects of the environment extrinsic to the organism – resource availability, competitors, predators, pathogens, and potential mates. In academic institutions, evolutionary biologists are invariably housed with ecologists and behavioral biologists to the exclusion of molecular, cell, and developmental biologists. Would this bias have existed had scanning electron microscopes and molecular biology existed at the dawn of evolutionary thinking? Without question, the community of organisms within which a species exists 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.

Second, a pervasive problem in biology is the religious adherence to the idea that natural selection is solely responsible for every feature 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).

Inspired by this way of thinking and digging no deeper, many molecular biologists start with the dubious assumption that natural selection is 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) 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). This raises 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.

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 arguments 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, as noted below, 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 small 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. The goal here is to plant the seeds for such an enterprise.

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 total branch length of the Tree of Life (Figure 1.1). Most of global diversity at the DNA level resides in prokaryotes, and this would be even more true if one were to further account for the diversity of gene types, as prokaryotes harbor most of the global diversity in metabolic pathways. Even restricting attention to eukaryotes, the vast majority of phylogenetic diversity resides within lineages consisting nearly entirely of unicellular species.

Shifting the focus to total biomass or number of cells, the conclusion that the vast majority of life on Earth is in the provenance of unicellular organisms is retained. Achieving accurate estimates of the numbers of individuals 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 $\simeq 10^{30}$ (Suttle 2005), and supposing there are twice as many viruses on land and in freshwater (unlikely) 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 $\simeq 40$ (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 mean of these two estimates, 10^9, implies an average number of individuals per prokaryotic species of $\simeq 10^{21}$ (although substantial variation in this number must exist among taxa).

Of the estimated 10^7 eukaryotic species on earth (potentially just 1% of the number for prokaryotes), it is thought that approximately 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. The total number of unicellular heterotrophic eukaryotes 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 4), the global biomass of unicellular eukaryotes likely exceeds that of prokaryotes. Further 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, Crowther et al. (2015) estimate that there are $\simeq 10^{13}$ trees on Earth, so even generously allowing 10^4 additional plants for every tree, there would be a total of $< 10^{17}$ land plants on Earth, with an average of $< 10^{12}$ individuals per species (again, with a wide range of variation around the mean).

Two of the most abundant groups of invertebrates on Earth are the ants, estimated to comprise a total $\simeq 10^{16}$ individuals distributed over $\simeq 10^4$ species (Hölldobler and Wilson 1990) and Antarctic krill with $\simeq 10^{15}$ individuals in a single species (Atkinson et al. 2008). Perhaps the most numerically abundant animal phylum, the nematodes, comprises $\simeq 10^{20}$ 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 $\ll 10^{15}$, 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 $\simeq 10^{16}$, distributed over $\simeq 10^5$ species (mostly fish), implying an average of $\simeq 10^{11}$ individuals/species (Bar-On et al. 2018). Notably, the average human harbors a microbiome of $\simeq 10^{13}$ 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 ultimately grow out of cell-level processes, an ultimate

understanding of the mechanisms of evolution cannot be complete without an understanding of how cellular features emerge on an evolutionary time scale. The salient point is 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." With historical connection to population-genetic theory, the fields of molecular and genome evolution provide an entrée to this line of thought. They come up short, however, in explaining the assemblage of molecular building blocks into higher-order structures and of the latter into emergent subcellular features.

Evolutionary cell biology can be defined as 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 of the underlying theoretical frameworks in statistical physics and population genetics (Sella and Hirsh 2005; Lässig 2007; Barton and de Vladar 2009; Zhang et al. 2012). Although commonly remarking on the exquisite design of the traits being studied, cell biologists almost never consider the evolutionary paths by which such features might 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 domains (Figure 1.2). First, as noted above, the classical intellectual domain of evolutionary biology is ecology, where the usual focus is on challenges existing outside of the organism. The central issues here concern 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, e.g., 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. One can imagine other possible forms of cellular organization, but on this particular planet 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 mutations upon which all evolutionary change depends, the privilege of evolutionary potential comes at a cost – the deleterious nature of most mutations. Recombination assorts variation within and among chromosomes, further generating genetic diversity, promoting some kinds of evolutionary change and inhibiting others. And finally, random genetic drift, a consequence of finite numbers of individuals within populations and the stochasticity resulting from genes being linked on chromosomes, creates noise in all evolutionary

processes. 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 3 – 5).

A central goal in the following pages is to establish the theoretical foundations for how these various aspects of the environment conspire to dictate the mechanisms by which evolution proceeds at the cellular level. Throughout, the focus will be on the degree to which selection, effectively neutral processes, historical contingencies, and/or constraints at the chemical and biophysical levels jointly determine 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 a theoretical framework, science is reduced to a fact-collecting enterprise. Although the emergence of facts from consistent observations is progress, theory provides a mechanistic explanation of the facts. A theoretical framework also motivates the making of predictions in areas where observations have not previously been made. Ideally, such a reach is not simply based on statistical extrapolation, but on arguments from first principles. Mathematical theory allows the development 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 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, Fishser established one of the primary pillars upon which modern statistics relies, the analysis of variance. Descending from these roots, the century-old field of population 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 and linkage disequilibrium were generated over the next fifty years. With the application of diffusion theory and statistical aspects of gene genealogies now commonplace, the field of theoretical population genetics is at least as well-grounded as any other area of quantitative biology.

Notably, the earliest period of evolutionary theory development substantially preceded any knowledge of the molecular basis of genes. 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 mechanisms of recombination, the basic structure of genes and their component parts, the central roles of transcription and translation, the existence of mobile genetic elements, and various nuances related to epigenetic inheritance. Yet, none of these discoveries led to any alteration in the basic structure of evolutionary theory. The discovery of mitochondrial DNA and nuclear epigenetic effects 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. Such robustness 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 biology are up to the task. That is, we have in place a well-established framework for defining the conditions under which various evolutionary scenarios are 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 previously unappreciated problem. Without exception, these episodes have gone badly, the most notable being 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. Aficionados of such exceptions sometimes claim that their observations are sufficient to dismantle the previous theoretical framework for broadscale patterns. More often than not, however, a deeper look almost always reveals underlying explanations for exceptions 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. 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 current "struggle for the very soul of the discipline" (Gerhart and Kirschner 1997; Pigliucci and Müller 2010; Goldenfeld and Woese 2011; Shapiro 2011; Laland et al. 2014, 2015). 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 dramatic 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 trans-generational effects without direct changes at the level of genomic DNA. Advocates of epigenetic inheritance have generally argued that such phenomena respond in beneficial ways 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 here just two points will be made. First, to appreci-

ate 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). Absence of genetic variation does not mean an absence of environmental or epigenetic sources of phenotypic variation, yet there is no permanent response to selection unless there is variation at the DNA level.

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. The response to selection on environmental (and/or epigenetic) maternal effects is also transient, decaying away if the selective pressure is eliminated. 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 × environment interaction, the theory of which dates back decades. Indeed, breeders have long exploited this concept to determine the optimum environmental setting in which to select for particular phenotypes. Thus, the idea that evolutionary theory needs to be remodeled to account for phenotypic plasticity is without merit.

Still another argument against the adequacy of evolutionary theory proposes that the field is incapable of dealing with the possibility that evolution " is guided along specific routes opened up by the processes of development" (Laland et al. 2014). This claim again reveals a lack of awareness of a substantial area of evolutionary theory. Although models concerned with the role of mutation in phenotypic evolution commonly assume a symmetrical distribution of mutational effects, it is relatively easy to modify any evolutionary-genetic model to allow for biased mutational effects. Indeed, many existing models have a structure in which mutation bias is intrinsic – here there is a fixed set of possible allelic effects, so there is always a directional bias to mutation unless the mean genotypic value is positioned at exactly the intermediate location along the axis of possibilities. As for the role of developmental bias in evolution, one need only consider the substantial theory devoted to multivariate evolution explicitly focused on pleiotropy and the ways in which this influences the evolutionary trajectories of complex traits (e.g., Jones et al. 2003, 2007, 2012; Hansen and Houle 2008).

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. Their counter view is that "the direction of evolution does not depend on selection alone, and need not start with mutation"

(Laland et al. 2014). 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 evolution (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 genotype-frequency change, and does not appear to be what the dissenters have in mind).

Far from providing a weak and/or incomplete caricature of evolving genetic systems, population- and quantitative-genetic theory has generated powerful, general, and sometime 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 (1918) paper 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, from which many of the pleas for novel theory emanate, remains in many respects in a pre-population-genetics mode of confusion. There are potential lessons for evolutionary cell biology here.

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. As outlined in Table 1.1 and expanded upon in Chapters 3 – 5, the structure of evolutionary theory developed over the past century has found no boundaries in terms of applications and continues to make predictions that are bolstered by empirical observations. All of this being said, it must be emphasized that 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. 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 the theory of population and quantitative genetics has enhanced our understanding of the mechanistic basis of trait variation and provided novel predictions. This list is by no means complete.

Topic:	References:
Quantitative-trait variation:	
Phenotypic resemblance between relatives, and how this scales 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 the 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 and 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

Evolutionary features of alleles:

Low probability of fixation of mutant alleles.	Kimura 1962
Ages of alleles.	Kimura and Ohta 1973
Conditional time to fixation of deleterious mutations equaling that of beneficial mutations.	Maruyama and Kimura 1964
Enhanced evolutionary divergence under uniform selection.	Cohan 1984; Lynch 1986
Doubling the effective population size by equalizing family sizes.	Crow and Kimura 1970

Nonadaptive Hypotheses and Our Understanding of Evolution

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. They were so convincing that most who think about the subject of evolution simply view all aspects of biology 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. Such noise 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 3). Biased mutation pressure can also modify evolutionary trajectories if mutation is sufficiently strong relative to the efficiency of selection, and even nonbiased mutation can strongly influence the impact of selection on mean phenotypes (Chapter 4).

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. Under such models, the three nonadaptive mechanisms of evolution – random genetic drift, mutation, and recombination – are the sole evolutionary determinants. The resultant formulations then provide null models 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).

The construction of neutral models becomes more challenging in the case of more complex cellular/organismal features, where the baseline features of mutations can be more difficult to define. This, however, is not justification for ignoring the matter. Indeed, such models have been particularly useful in attempts to understand long-term phenotypic changes recorded in the fossil record, where dramatic changes seemingly 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; Kern and Hahn 2018), with Hahn (2008) 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 has no logical basis. Moreover, as outlined in Chapters 3 – 5, selection never operates in isolation, but in the context of the background population-genetic environment. When properly constructed, neutral models make very explicit predictions, without which arguments for the role of natural selection in evolution are reduced to qualitative hand-waving. The measurement of deviations between observations and neutral expectations yields a deeper and more defensible understanding of evolutionary processes.

The second problem with invoking selection as the null model is that one can concoct a selection argument for essentially any observed pattern, rendering such a hypothesis unfalsifiable. If one form of selection does not adequately fit the data, then one can try another, and failing that, still another, never abandoning the pan-selection view.

A third problem with criticisms of neutral theory is their frequent reliance on

incorrect biological assumptions. For example, Lewontin (1974) long ago 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 3). Although this argument continues to be made (Hahn 2008), the postulated pattern ignores the fact (unknown at Lewontin's time) that mutation rates evolve to be inversely correlated with N_e (Chapter 3), naturally leading to weak dependence of standing variation on N_e (Lynch et al. 2016).

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 dictated by the relative power of the nonadaptive forces of evolution – mutation, recombination, and random-genetic drift (Walsh and Lynch 2018).

In summary, although those who promote the need for an urgent overthrow of current evolutionary theory are unlikely to read the preceding comments, we offer them primarily for the benefit of outsiders with only a peripheral understanding of what might appear to be a meaningful controversy. Conflict and cooperation are the engines that keep science running. Conflict engineered under false pretenses 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 what needs to be explained. In some cases, where there is a decent fossil record, comparative biology has also provided insight into rates of 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). However, if devoid of attention to the mechanisms giving rise to variation, comparative biology is a far cry from evolutionary biology.

Here the challenges are even greater, for owing to cell biology's focus on just a few model organisms, there is no comparative cell biology. As a consequence of this void, the range of existing variation is often unclear, leaving even the question of what needs to be explained unsettled. This being said, evolution is still part of the mindset of many cell biologists focused on a single species throughout their careers, as one can see 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 is unlikely to 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 through the integration of comparative cell biology and evolutionary theory 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 make all of the necessities for evolution possible: 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, or whether competing forms of life initially coexisted and 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, and may yield useful predictions as to where life might have originated independently elsewhere in the universe (Chapter 2). Fortunately, a lack of clarity on these matters does not bear in any significant way on our ability to understand the mechanisms by which current life forms evolve.

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? Why has the apex of biological complexity, multicellularity at the level found in land plants and animals only evolved twice? One might argue that there is something fundamentally lacking in prokaryotes that prevents such evolution, yet despite any imagined deficiencies, microbes comprise much of the earth's biomass. To put things in broader perspective, from the standpoint of metabolism, prokaryotes are the cradles of diversity, whereas eukaryotes are relatively bland. Such disparities reveal the intrinsic biases in evolutionary thinking confined to visually perceived morphological differences.

As noted above, there is substantial support for the idea that much of evolution at the DNA and genomic levels 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.

Although 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 is quite uneven and 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 some are quite arcane. As a modern analogy, 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. A number of aspects of eukaryotic cellular complexity may have arisen in this manner.

Molecular stochasticity. Messenger RNAs are often present in fewer than ten copies per cell, sometimes with a mean less than one, especially in small-celled species. Proteins have longer half lives and tend to be more abundant, but still can frequently have on the order of only hundreds of copies per cell. Transcription factors are among the rarest of proteins, leading to issues of how they reliably find their DNA targets to produce their cognate proteins. Collectively, these features and more (including asymmetries in cell division) lead to stochasticity in cellular composition and presumably associated phenotypes, even among cells with identical genotypes inhabiting homogenous environments.

Natural selection operates on phenotypic variance, and is more efficient when most of the variance among cells 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 itself 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 (Chapter 6). But on the other hand, populations of small cells may harbor more genetic variation, which combined with short cell-division times will enhance the rate of evolution (Chapter 3).

Molecular complexes. The rules of life are such that each messenger RNA almost always encodes for one form of monomeric protein. However, the majority of proteins organize into higher-order consortiums, e.g., dimers, tetramers, etc. (Chapter 12). Such complexes are often 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 can vary across species, but not always in ways that reflect organismal complexity (very unlike the situation in genome evolution, where genome architecture becomes enormously complex in large multicellular species). One of the greatest mysteries of evolutionary biology, the origin of molecular complexes presents a number of scenarios where natural selection is unlikely to be the driving force.

Cellular networks. Very few of the molecular constituents of cells operate alone. Examples of molecular networks include the cell cycle, circadian clocks, the vesicle-transport system of eukaryotes, and pathways in metabolism, transcription regulation, and signal transduction (Chapters 6, 14, 18, 20, 21, respectively). The structures of pathways often border on the baroque, including larger numbers of steps than seemingly necessary, linear chains of enhancing vs. suppressing steps, etc. Each

component added to a pathway imposes an energetic cost of production on the cell, so how do such architectures emerge? Do significantly profitable kinetic and/or dynamical properties evolve with some structures, or do they again just represent evolutionary sojourns along effectively neutral paths? Sometimes different lineages have similar network topologies, but with different underlying protein participants or orders of steps. How does rewiring of the underlying structure evolve without leading to catastrophic intermediate consequences? Intracellular and extracellular communication systems consist of at least one signaling molecule and one receptor, which necessarily interact in unique ways. How does the language of such systems coevolve so as to avoid crosstalk? When are there sufficient degrees of freedom that cellular communication systems can 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: replication fidelity, errors introduced at the transcriptional and translational levels, enzyme promiscuity with respect to substrates, protein-folding problems, etc. There are often multiple layers of surveillance / editing for intracellular errors, suggesting highly refined and robust systems. Yet, the error rates for some of these functions can vary about 1000-fold among organisms, and some layers of surveillance are lost in some phylogenetic lineages. 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. Does this encourage the expansion of complexity by the evolutionary layering of surveillance mechanisms, and if so, are there any long-term advantages to such embellishments?

Growth regulation. So-called "growth laws" have been discussed for years in microbiology, and substantial theoretical work has been devoted to explain these, but the empirical work has been essentially confined to a single species (*E. coli*), leaving open many questions about generality (Chapter 8). 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 among different genotypes recapitulate the more transient (plastic) pattern found within a genotype in response to nutrient availability? Are the rules for eukaryotes the same as those for prokaryotes?

Biological scaling laws. Cell biologists have identified a number of "scaling laws" across species (Chapter 7), whereby specific cellular features can be approximated as power functions of a cell size feature. 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 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.

- 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 a few orders of magnitude, and the latter exceeds that 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, and provides an essential platform 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, and the following chapters will demonstrate that this is also commonly true at the cell biological level.

Literature Cited

Amann, R., and R. Rosselló-Móra. 2016. After all, only millions? mBio 7: e00999-16.

Atkinson, A., V. Siegel, E. A. Pakhomov, M. J. Jessopp, and V. Loeb. 2009. A re-appraisal of the total biomass and annual production of Antarctic krill. Deep Sea Research Part I: Oceanogr. Res. Papers 56: 727-740.

Bar-On, Y. M., R. Phillips, and R. Milo. 2018. The biomass distribution on Earth. Proc. Natl. Acad. Sci. USA 115: 6506-6511.

Barton, N. H., and H. P. de Vladar. 2009. Statistical mechanics and the evolution of polygenic quantitative traits. Genetics 181: 997-1011.

Bulmer, M. 1991. The selection-mutation-drift theory of synonymous codon usage. Genetics 129: 897-907.

Caporale, L. H. (ed.) 2006. The Implicit Genome. Oxford Univ. Press, Oxford, UK.

Charlesworth, B. 1984a. Some quantitative methods for studying evolutionary patterns in single characters. 10: 308-318.

Charlesworth, B. 1984b. The cost of phenotypic evolution. Paleobiol. 10: 319-327.

Charlesworth, B. 1990. Mutation-selection balance and the evolutionary advantage of sex and recombination. Genet. Res. 55: 199-221.

Charlesworth, B., and D. Charlesworth. 1983. The population dynamics of transposable elements. Genet. Res. 42: 1-27.

Charlesworth, B., and D. Charlesworth. 2000. The degeneration of Y chromosomes. Phil. Trans. Roy. Soc. Lond. B Biol. Sci. 355: 1563-1572.

Charlesworth, B., R. Lande, and M. Slatkin. 1982. A neo-Darwinian commentary on macroevolution. Evolution 36: 474-498.

Charlesworth, B., and C. H. Langley. 1986. The evolution of self-regulated transposition of transposable elements. Genetics 112: 359-383.

Charlesworth, D., N. H. Barton, and B. Charlesworth. 2017. The sources of adaptive variation. Proc. Biol. Sci. 284: 20162864.

Charnov, E.L. 1982. The Theory of Sex Allocation. Princeton Univ. Press, Princeton, NJ

Charnov, E.L. 1993. Life History Invariants. Oxford Univ. Press, Oxford, UK.

Cohan, F. M. 1984. Can uniform selection retard random genetic divergence between isolated conspecific populations? Evolution 38: 495-504.

Crow, J. F. 1948. Alternative hypotheses of hybrid vigor. Genetics 33: 477-487.

Crow, J. F., and M. Kimura. 1970. An Introduction to Population Genetics Theory. Harper and Row, Publ., New York, NY.

Crowther, T. W., et al. 2015. Mapping tree density at a global scale. Nature 525: 201-205.

Danchin, É., A. Charmantier, F. A. Champagne, A. Mesoudi, B. Pujol, and S. Blanchet. 2011. Beyond DNA: integrating inclusive inheritance into an extended theory of evolution. Nat. Rev. Genet. 12: 475-486.

Darwin, C. 1859. On the Origin of Species by Means of Natural Selection. John Murray, London, UK.

Delsuc, F., H. Brinkmann, and H. Philippe. 2005. Phylogenomics and the reconstruction of the tree of life. Nat. Rev. Genet. 6: 361-375.

ENCODE Project Consortium. 2012. An integrated encyclopedia of DNA elements in the human genome. Nature 489: 57-74.

Falconer, D. S. 1965. Maternal effects and selection response. Proc. XIth Internat. Cong. Genetics 3: 763-774.

Fisher, R. A. 1918. The correlation between relatives on the supposition of Mendelian inheritance. Trans. Royal Soc. Edinburgh 52: 399-433.

Flemming, H. C., and S. Wuertz. 2019. Bacteria and archaea on Earth and their abundance in biofilms. Nat. Rev. Microbiol. 17: 247-260.

Force, A., M. Lynch, B. Pickett, A. Amores, Y.-L. Yan, and J. Postlethwait. 1999. Preservation of duplicate genes by complementary, degenerative mutations. Genetics 151: 1531-1545.

Frank, S. A. 2007. Maladaptation and the paradox of robustness in evolution. PLoS One 2: e1021.

Galhardo, R. S., P. J. Hastings, and S. M. Rosenberg. 2007. Mutation as a stress response and the regulation of evolvability. Crit. Rev. Biochem. Mol. Biol. 42: 399-435.

Gerhart, J., and M. Kirschner. 1997. Cells, Embryos and Evolution. Blackwell Science, Malden, MA.

Goldenfeld, N., and C. Woese. 2011. Life is physics: evolution as a collective phenomenon far from equilibrium. Annu. Rev. Condensed Matter Physics 2: 375-399.

Goldschmidt, R. 1940. The Material Basis of Evolution. Yale Univ. Press, New Haven, CT.

Gupta, M., N. G. Prasad, S. Dey, A. Joshi, and T. N. C. Vidya. 2017. Niche construction in evolutionary theory: the construction of an academic niche? J. Genet. 96: 491-504.

Hahn, M. W. 2008. Toward a selection theory of molecular evolution. Evolution 62: 255-265.

Haldane, J. B. S. 1964. A defense of beanbag genetics. Perspect. Biol. Med. 7: 343-359.

Hansen, T. F., and D. Houle. 2008. Measuring and comparing evolvability and constraint in multivariate characters. J. Evol. Biol. 21: 1201-1219.

Higgins, K., and M. Lynch. 2001. Metapopulation extinction due to mutation accumulation. Proc. Natl. Acad. Sci. USA 98: 2928-2933.

Hochberg, G. K. A., and J. W. Thornton. 2017. Reconstructing ancient proteins to understand the causes of structure and function. Annu. Rev. Biophys. 46: 247-269.

Hölldobler, B., and E. O. Wilson. 1990. The Ants. Harvard Univ. Press, Cambridge, MA.

Jablonka, E., and M. J. Lamb. 2005. Evolution in Four Dimensions: Genetic, Epigenetic, Behavioral, and Symbolic Variation in the History of Life. MIT Press, Cambridge, MA.

Jarosz, D. F., and S. Lindquist. 2010. Hsp90 and environmental stress transform the adaptive value of natural genetic variation. Science 330: 1820-1824.

Jensen, J. D., B. A. Payseur, W. Stephan, C. F. Aquadro, M. Lynch, D. Charlesworth, and B. Charlesworth. 2019. The importance of the Neutral Theory in 1968 and 50 years on: a response

to Kern and Hahn 2018. Evolution 73: 111-114.

Jones, A. G., S. J. Arnold, and R. Bürger. 2003. Stability of the G-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. Evolution 57: 1747-1760.

Jones, A. G., S. J. Arnold, and R. Bürger. 2007. The mutation matrix and the evolution of evolvability. Evolution 61: 727-745.

Jones, A. G., R. Bürger, S. J. Arnold, P. A. Hohenlohe, and J. C. Uyeda. 2012. The effects of stochastic and episodic movement of the optimum on the evolution of the G-matrix and the response of the trait mean to selection. J. Evol. Biol. 25: 2210-2231.

Kallmeyer, J., R. Pockalny, R. R. Adhikari, D. C. Smith, and S. D'Hondt. 2012. Global distribution of microbial abundance and biomass in subseafloor sediment. Proc. Natl. Acad. Sci. USA 109: 16213-16216.

Kempthorne, O. 1954. The correlation between relatives in a random mating population. Proc. Royal Soc. Lond. B 143: 103-113.

Kern, A. D., and M. W. Hahn. 2018. The neutral theory in light of natural selection. Mol. Biol. Evol. 35: 1366-1371.

Kimura, M. 1962. On the probability of fixation of mutant genes in populations. Genetics 47: 713-719.

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge Univ. Press, Cambridge, UK.

Kimura, M., and T. Ohta. 1973. The age of a neutral mutant persisting in a finite population. Genetics 75: 199-212.

Kiontke, K., and D. H. Fitch. 2013. Nematodes. Curr. Biol. 23: R862-R864.

Kondrashov, A. S. 1988. Deleterious mutations and the evolution of sexual reproduction. Nature 336: 435-440.

Krebs, J. R., and N. B Davies. 1997. Behavioural Ecology: An Evolutionary Approach. Wiley, New York, NY.

Laland, K. N., T. Uller, M. W. Feldman, K. Sterelny, G. B. Müller, A. Moczek, E. Jablonka, and J. Odling-Smee. 2015. The extended evolutionary synthesis: its structure, assumptions and predictions. Proc. Biol. Sci. 282: 20151019.

Laland, K., et al. 2014. Does evolutionary theory need a rethink? Nature 514: 161-164.

Lande, R. 1976. Natural selection and random genetic drift in phenotypic evolution. Evolution 30: 314-334.

Lande, R. 1978. Evolutionary mechanisms of limb loss in tetrapods. Evolution 32: 73-92.

Landenmark, H. K., D. H. Forgan, and C. S. Cockell. 2015. An estimate of the total DNA in the biosphere. PLoS Biol. 13: e1002168.

Lässig, M. 2007. From biophysics to evolutionary genetics: statistical aspects of gene regulation. BMC Bioinformatics 8 Suppl 6: S7.

Lewontin, R. C. 1974. The Genetic Basis of Evolutionary Change. Columbia Univ. Press, New York, NY.

Locey, K. J., and J. T. Lennon. 2016. Scaling laws predict global microbial diversity. Proc. Natl. Acad. Sci. USA 113: 5970-5975.

Lynch, M. 1986. Random drift, uniform selection, and the degree of population differentiation. Evolution 40: 640-643.

Lynch, M. 1990. The rate of morphological evolution in mammals from the standpoint of the neutral expectation. Amer. Natur. 136: 727-741.

Lynch, M. 2007. The Origins of Genome Architecture. Sinauer Assocs., Inc., Sunderland, MA.

Lynch, M. 2010. Scaling expectations for the time to establishment of complex adaptations. Proc. Natl. Acad. Sci. USA 107: 16577-16582.

Lynch, M. 2013. Evolutionary diversification of the multimeric states of proteins. Proc. Natl. Acad. Sci. USA 110: E2821-E2828.

Lynch, M. 2011. The lower bound to the evolution of mutation rates. Genome Biol. Evol. 3: 1107-1118.

Lynch, M., M. Ackerman, J.-F. Gout, H. Long, W. Sung, W. K. Thomas, and P. L. Foster. 2016. Genetic drift, selection, and evolution of the mutation rate. Nature Rev. Genetics 17: 704-714.

Lynch, M., M. C. Field, H. Goodson, H. S. Malik, J. B. Pereira-Leal, D. S. Roos, A. Turkewitz, and S. Sazer. 2014. Evolutionary cell biology: two origins, one objective. Proc. Natl. Acad. Sci. USA 111: 16990-16994.

Lynch, M., and A. Force. 2000. Gene duplication and the origin of interspecific genomic incompatibility. Amer. Natur. 156: 590-605.

Lynch, M., and K. Hagner. 2014. Evolutionary meandering of intermolecular interactions along the drift barrier. Proc. Natl. Acad. Sci. USA 112: E30-E38.

Lynch, M., and J. B. Walsh. 1998. Genetics and Analysis of Quantitative Traits. Sinauer Assocs., Inc., Sunderland, MA.

Martincorena, I., A. S. Seshasayee, and N. M. Luscombe. 2012. Evidence of non-random mutation rates suggests an evolutionary risk management strategy. Nature 485: 95-98.

Maruyama, T., and M. Kimura. 1974. A note on the speed of gene frequency changes in reverse direction in a finite population. Evolution 28: 161-163.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press, Cambridge, MA.

Mayr, E. Where are we? Cold Spring Harbor Symp. Quant. Biol. 124: 124.

Mode, C. G., and H. F. Robinson. 1959. Pleiotropism and the genetic variance and covariance. Biometrics 15: 518-537.

Mora, C., D. P. Tittensor, S. Adl, A. G. Simpson, and B. Worm. 2011. How many species are there on Earth and in the ocean? PLoS Biol. 9: e1001127.

Otto, S. P., and N. H. Barton. 2001. Selection for recombination in small populations. Evolution 55: 1921-1931.

Pernice, M. C., et al. 2015. Global abundance of planktonic heterotrophic protists in the deep ocean. ISME J. 9: 782-792.

Pigliucci, M., and J. Kaplan. 2000. The fall and rise of Dr. Pangloss: adaptationism and the

spandrels paper 20 years later. Trends Ecol. Evol. 15: 66-70.

Pigliucci, M., and G. B. Müller. 2010. Evolution: the Extended Synthesis. MIT Press, Cambridge, MA.

Provine, W. B. 1971. The Origins of Theoretical Population Genetics. Univ. Chicago Press, Chicago, IL.

Roff, D. E. 1993. The Evolution of Life Histories. Springer-Verlag, New York, NY.

Schwartz, M. H., and T. Pan. 2017. Function and origin of mistranslation in distinct cellular contexts. Crit. Rev. Biochem. Mol. Biol. 52: 205-219.

Sella, G., and A. E. Hirsh. 2005. The application of statistical physics to evolutionary biology. Proc. Natl. Acad. Sci. USA 102: 9541-9546.

Sender, R., S. Fuchs, and R. Milo. 2016. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. 14: e1002533.

Shapiro, J. A. 2011. Evolution: A View from the 21st Century. FT Press Science, Upper Saddle River, NJ.

Stoltzfus, A. 2017. Why we don't want another "Synthesis". Biol. Direct 12: 23.

Suttle, C. A. 2005. Viruses in the sea. Nature 437: 356-361.

van den Hoogen, J., et al. 2019. Soil nematode abundance and functional group composition at a global scale. Nature 572: 194-198.

Wallace, A. R. 1870. Contributions to the Theory of Natural Selection. Macmillan and Company, New York, NY.

Walsh, J. B., and M. Lynch. 2018. Evolution and Selection of Quantitative Traits. Oxford Univ. Press, Oxford, UK.

Wang, S., T. Hassold, P. Hunt, M. A. White, D. Zickler, N. Kleckner, and L. Zhang. 2017. Inefficient crossover maturation underlies elevated aneuploidy in human female meiosis. Cell 168: 977-989.

Weissman, D. B., M. W. Feldman, and D. S. Fisher. 2010. The rate of fitness-valley crossing in sexual populations. Genetics 186: 1389-1410.

Welch, J. J. 2017. What's wrong with evolutionary biology? Biol. Philos. 32: 263-279.

Whitman, W. B., D. C. Coleman, and W. J. Wiebe. 1998. Prokaryotes: the unseen majority. Proc. Natl. Acad. Sci. USA 95: 6578-6583.

Willham, R. L. 1963. The covariance between relatives for characters composed of components contributed by related individuals. Biometrics 19: 18-27.

Wilson, E. B. 1925. The Cell in Development and Inheritance. Macmillan Co., London, UK.

Wright, S. 1934a. An analysis of variability in number of digits in an inbred strain of guinea pigs. Genetics 19: 506-536.

Wright, S. 1934b. The results of crosses between inbred strains of guinea pigs, differing in number of digits. Genetics 19: 537-551.

Zhang, F., L. Xu, K. Zhang, E. Wang, and J. Wang. 2012. The potential and flux landscape theory of evolution. J. Chem. Phys. 137: 065102.

Zhang, J. 2018. Neutral theory and phenotypic evolution. Mol. Biol. Evol. 35: 13271331.

Figure 1.1. A broad overview of the Tree of Life, with the branch lengths being approximately equivalent to distance separated in time. As outlined in the following chapter, the exact structure of this tree is unlikely to be topologically correct in all aspects, and the full diversity of the internal phylogeny of major groups is not appropriately weighted. The central point is that the only two lineages in which complex multicellularity is present, metazoans and land plants, comprise only a small fraction of genetic diversity on Earth. From Delsuc et al. (2005).
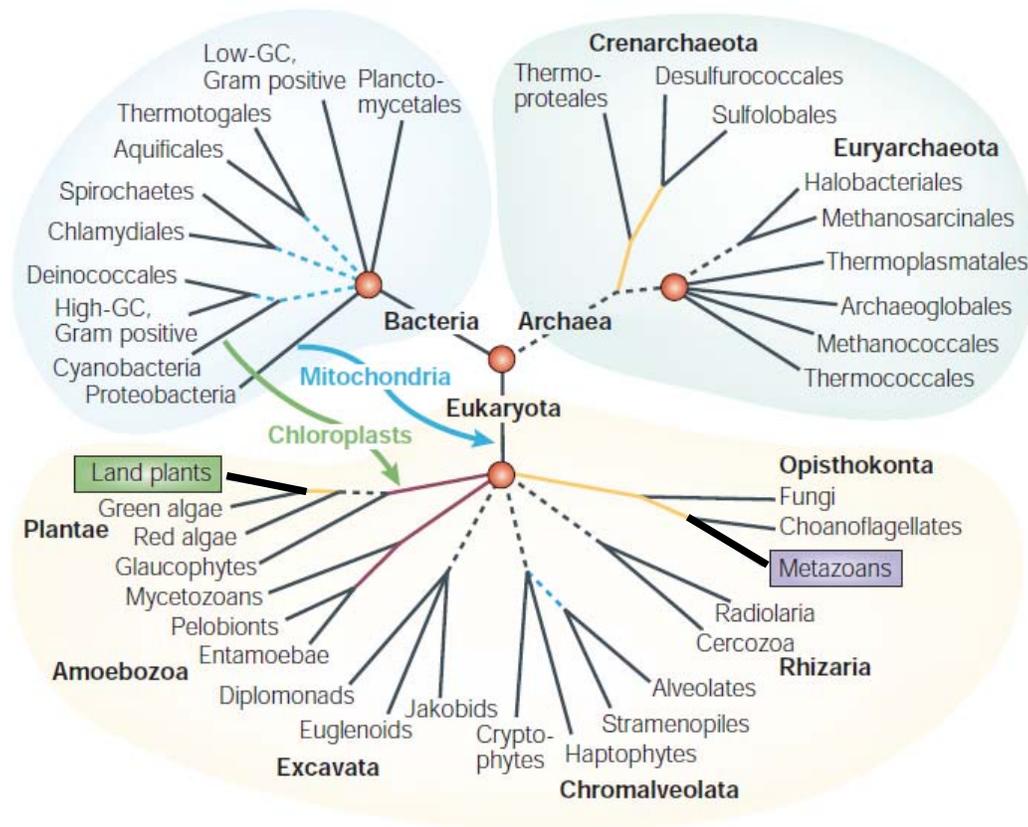
Figure 1.2. Summary of the major dimensions of the triad of environmental components influencing the tempo and mode of evolution.