This paper serves as an introduction to the following papers which were presented at a colloquium entitled "Genetics and the Origin of Species," organized by Francisco J. Ayala and Walter M. Fitch, held January 30–February 1, 1997, at the National Academy of Sciences Beckman Center in Irvine, CA.

Genetics and the origin of species: An introduction

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Theodosius Dobzhansky (1900–1975) was a key author of the Synthetic Theory of Evolution, also known as the Modern Synthesis of Evolutionary Theory, which embodies a complex array of biological knowledge centered around Darwin's theory of evolution by natural selection couched in genetic terms. The epithet "synthetic" primarily alludes to the artful combination of Darwin's natural selection with Mendelian genetics, but also to the incorporation of relevant knowledge from biological disciplines. In the 1920s and 1930s several theorists had developed mathematical accounts of natural selection as a genetic process. Dobzhansky's *Genetics and the Origin of Species*, published in 1937 (1), refashioned their formulations in language that biologists could understand, dressed the equations with natural history and experimental population genetics, and extended the synthesis to speciation and other cardinal problems omitted by the mathematicians.

The current Synthetic Theory has grown around that original synthesis. It is not just one single hypothesis (or theory) with its corroborating evidence, but a multidisciplinary body of knowledge bearing on biological evolution, an amalgam of well-established theories and working hypotheses, together with the observations and experiments that support accepted hypotheses (and falsify rejected ones), which jointly seek to explain the evolutionary process and its outcomes. These hypotheses, observations, and experiments often originate in disciplines such as genetics, embryology, zoology, botany, paleontology, and molecular biology. Currently, the "synthetic" epithet is often omitted and the compilation of relevant knowledge is simply known as the Theory of Evolution. This is still expanding, just like one of those "holding" business corporations that have grown around an original enterprise, but continue incorporating new profitable enterprises and discarding unprofitable ones.

Darwin to Dobzhansky

Darwin summarized the theory of evolution by natural selection in the *Origin of Species* (2) as follows:

"As many more individuals are produced than can possibly survive, there must in every case be a struggle for existence, either one individual with another of the same species, or with the individuals of distinct species, or with the physical conditions of life. . . . Can it, then, be thought improbable, seeing that variations useful to man have undoubtedly occurred, that other variations, useful in some way to each being in the great and complex battle of life, should sometimes occur in the course of thousands of generations? If such do occur, can we doubt (remembering that many more individuals are born than can possibly survive) that individuals having any advantage, however slight, over others, would

have the best chance of surviving and of procreating their kind? On the other hand, we may feel sure that any variation in the least degree injurious would be rigidly destroyed. This preservation of favorable variations and the rejection of injurious variations, I call Natural Selection."

Darwin's argument is that natural selection emerges as a necessary conclusion from two premises: (*i*) the assumption that hereditary variations useful to organisms occur, and (*ii*) the observation that more individuals are produced than can possibly survive. The most serious difficulty facing Darwin's evolutionary theory was the lack of an adequate theory of inheritance that would account for the preservation through the generations of the variations on which natural selection was supposed to act. Theories then current of "blending inheritance" proposed that offspring merely struck an average between the characteristics of their parents. As Darwin became aware, blending inheritance could not account for the conservation of variations, because differences among variant offspring would be halved each generation, rapidly reducing the original variation to the average of the preexisting characteristics.

The missing link in Darwin's argument was provided by Mendelian genetics. About the time the *Origin of Species* was published, the Augustinian monk Gregor Mendel was performing a long series of experiments with peas in the garden of his monastery in Brünn, Austria-Hungary (now Brno, Czech Republic). Mendel's paper, published in 1866, formulated the fundamental principles of a theory of heredity that accounts for biological inheritance through particulate factors (now called "genes") inherited one from each parent, which do not mix or blend but segregate in the formation of the sex cells, or gametes (3).

Mendel's discoveries, however, remained unknown to Darwin and, indeed, did not become generally known until 1900, when they were simultaneously rediscovered by several scientists. In the meantime, Darwinism in the latter part of the 19th century faced an alternative evolutionary theory known as neo-Lamarckism. This hypothesis shared with Lamarck's original theory the importance of use and disuse in the development and obliteration of organs, and it added the notion that the environment acts directly on organic structures, which explained their adaptation to the ways of life and environments of each organism. Adherents of this theory rejected natural selection as an explanation for adaptation to the environment.

The rediscovery in 1900 of Mendel's theory of heredity led to an emphasis on the role of heredity in evolution. In the Netherlands, Hugo de Vries (4) proposed a new theory of evolution known as mutationism, which essentially did away

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Abbreviation: MHC, major histocompatibility complex.
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with natural selection as a major evolutionary process. According to de Vries (joined by other geneticists such as William Bateson in England), there are two kinds of variation in organisms. One is the "ordinary" variation observed among individuals of a species, which is of no lasting consequence in evolution because, according to de Vries, it could not "lead to a transgression of the species border even under conditions of the most stringent and continued selection." The other consists of the changes brought about by mutations, spontaneous alterations of genes that yield large modifications of the organism and give rise to new species. According to de Vries, a new species originates suddenly, produced by the existing one without any visible preparation and without transition.

Mutationism was opposed by many naturalists, and in particular by the so-called biometricians, led by Briton Karl Pearson, who defended Darwinian natural selection as the major cause of evolution through the cumulative effects of small, continuous, individual variations (which the biometricians assumed passed from one generation to the next without being subject to Mendel's laws of inheritance).

The controversy between mutationists (also referred to at the time as Mendelians) and biometricians approached a resolution in the 1920s and 1930s through the theoretical work of several geneticists (5). These geneticists used mathematical arguments to show, first, that continuous variation (in such characteristics as size, number of eggs laid, and the like) could be explained by Mendel's laws; and second, that natural selection acting cumulatively on small variations could yield major evolutionary changes in form and function. Distinguished members of this group of theoretical geneticists were R. A. Fisher and J. B. S. Haldane in Britain and Sewall Wright in the United States (6–8). Their work contributed to the downfall of mutationism and, most importantly, provided a theoretical framework for the integration of genetics into Darwin's theory of natural selection. Yet their work had a limited impact on contemporary biologists because it was formulated in a mathematical language that most biologists could not understand; because it was almost exclusively theoretical, with little empirical corroboration; and because it was limited in scope, largely omitting many issues, such as speciation, that were of great importance to evolutionists.

Dobzhansky's *Genetics and the Origin of Species* advanced a reasonably comprehensive account of the evolutionary process in genetic terms, laced with experimental evidence supporting the theoretical arguments. It had an enormous impact on naturalists and experimental biologists, who rapidly embraced the new understanding of the evolutionary process as one of genetic change in populations. Interest in evolutionary studies was greatly stimulated, and contributions to the theory soon began to follow, extending the synthesis of genetics and natural selection to a variety of biological fields.

The main writers who, together with Dobzhansky, may be considered the architects of the synthetic theory were the zoologists Ernst Mayr (9) and Julian Huxley (10), the paleontologist George G. Simpson (11), and the botanist George Ledyard Stebbins (12). [The National Academy of Sciences held in January 1994 a colloquium (13) to commemorate the 50th anniversary of the publication of Simpson's seminal book, *Tempo and Mode in Evolution* (11).] These researchers contributed to a burst of evolutionary studies in the traditional biological disciplines and in some emerging ones—notably population genetics and, later, evolutionary ecology. By 1950 acceptance of Darwin's theory of evolution by natural selection was universal among biologists, and the synthetic theory had become widely adopted.

The line of thought of *Genetics and the Origin of Species* is surprisingly modern—in part, no doubt, because it established the pattern that successive evolutionary investigations and treatises largely would follow. Dobzhansky writes in the preface: "The problem of evolution may be approached in two

different ways. First, the sequence of the evolutionary events as they have actually taken place in the past history of various organisms may be traced. Second, the mechanisms that bring about evolutionary changes may be studied. . . . The present book is dedicated to a discussion of the mechanisms of species formation in terms of the known facts and theories of genetics." The book starts with a consideration of organic diversity and discontinuity. Successively, it deals with mutation as the origin of hereditary variation, the role of chromosomal rearrangements, variation in natural populations, natural selection, the origin of species by polyploidy, the origin of species through gradual development of reproductive isolation, physiological and genetic differences between species, and the concept of species as natural units. The book's organization was largely preserved in the second (1941) and third (1951) editions, and in *Genetics of the Evolutionary Process* (14), published in 1970, a book that Dobzhansky thought of as the fourth edition of the earlier one, but had changed too much for publication under the same title.

Dobzhansky sought to extend the evolutionary synthesis to mankind in numerous articles and several books, most notably *Mankind Evolving* (15), published in 1962, a book that many judge to be as important as *Genetics and the Origin of Species*. Dobzhansky was a leading experimentalist and prolific writer, who published several books and nearly 600 papers dealing with leading questions in population and evolutionary genetics, as well as with philosophical problems and humanistic issues. The experimental organisms of most of his research were *Drosophila* fruitflies.

A Man for All Seasons

Theodosius Dobzhansky was born on January 25, 1900, in Nemirov, a small town 200 km southeast of Kiev in the Ukraine. He was the only child of Sophia Voinarsky and Grigory Dobrzhansky (precise transliteration of the Russian family name includes the letter "r"), a teacher of high school mathematics. In 1910 the family moved to the outskirts of Kiev, where Dobzhansky lived through the tumultuous years of World War I and the Bolshevik revolution. In those times the family was often beset by various privations, including hunger.

In his unpublished autobiographical *Reminiscences* for the "Oral History Project" of Columbia University, Dobzhansky states that his decision to become a biologist was made about 1912. Through his early high school years, Dobzhansky became an avid butterfly collector. A school teacher gave him access to a microscope that Dobzhansky used particularly during the long winter months. In the winter of 1915–1916 he met Victor Luchnik, a 25-year-old college drop-out, who was a dedicated entomologist specializing in Coccinellidae beetles. Luchnik convinced Dobzhansky that butterfly collecting would not lead anywhere and that he should become a specialist. Dobzhansky chose to work with ladybird beetles, which would be the subject of his first scientific publication in 1918. (Reference to Dobzhansky's publications can be found in the extensive bibliography published by the National Academy of Sciences, ref. 16.)

Dobzhansky graduated in biology from the University of Kiev in 1921. Before his graduation, he was hired as an instructor in zoology at the Polytechnic Institute in Kiev. He taught there until 1924, when he became an assistant to Yuri Filipchenko, head of the new department of genetics at the University of Leningrad. Filipchenko was familiar with Thomas Hunt Morgan's work in the United States and had started a *Drosophila* laboratory, where Dobzhansky was encouraged to investigate the pleiotropic effects of genes.

In 1927, Dobzhansky obtained a fellowship from the International Education Board (Rockefeller Foundation) and arrived in New York on December 27 to work with Thomas Hunt Morgan at Columbia University. In the summer of 1928 he

followed Morgan to the California Institute of Technology, where Dobzhansky was appointed assistant professor of genetics in 1929, and professor of genetics in 1936. In 1940 he returned to New York as professor of zoology at Columbia University, where he remained until 1962, when he became professor at the Rockefeller Institute (renamed Rockefeller University in 1965) also in New York City. On July 1, 1970, Dobzhansky became professor emeritus at Rockefeller University; in September 1971, he moved to the Department of Genetics at the University of California, Davis, where he was adjunct professor until his death in 1975.

On August 8, 1924, Dobzhansky married Natalia (Natasha) Sivertzev, a geneticist in her own right, who was at the time working with the famous Russian biologist I. I. Schmalhausen in Kiev. Natasha was Dobzhansky's faithful companion and occasional scientific collaborator until her death from coronary thrombosis on February 22, 1969. The Dobzhanskys had only one child, Sophie, married until her recent death to Michael D. Coe, professor of anthropology at Yale University.

In a routine medical check-up on June 1, 1968, it was discovered that Dobzhansky suffered from chronic lymphatic leukemia, the least malignant form of leukemia. He was given a prognosis of "a few months to a few years" of life expectancy. Over the following 7 years, the progress of the leukemia was unexpectedly slow and, surprising to his physicians, it had little if any noticeable effect on his energy and work habits. However, the disease took a conspicuous turn for the worse in the summer of 1975. In mid-November Dobzhansky started to receive chemotherapy, but continued living at home and working at the laboratory. He was convinced that the end of his life was near and dreaded that he might become unable to work and to care for himself. This never came to pass. He died of heart failure on the morning of December 18, 1975. The previous day, he had been working in the laboratory.

Dobzhansky was an excellent teacher and distinguished educator of scientists. Throughout his academic career he had more than 30 graduate students and an even greater number of postdoctoral and visiting associates, many of them from foreign countries. Some of the most distinguished geneticists and evolutionists in the United States and abroad are his former students. Dobzhansky spent long periods of time in foreign academic institutions, and was largely responsible for the establishment or development of genetics and evolutionary biology in various countries, notably Brazil, Chile, and Egypt.

Dobzhansky gave generously of his time to other scientists, particularly to young ones and to students. But he resented time spent in committee activities, which he shunned as often as he reasonably could. Throughout his academic career, he avoided administrative posts, alleging, perhaps correctly, that he had neither temperament nor ability for management. Most certainly, he preferred to dedicate his working time to research and writing rather than to administration.

Dobzhansky was a world traveler and an accomplished linguist able to speak fluently six languages and to read several more. He was a good naturalist and never lacked time for a hike in the California Sierras, the New England forests, or the Amazon jungles. He loved horseback riding but practiced no other sports. Dobzhansky's interests included the visual arts, music, history, Russian literature, cultural anthropology, philosophy, religion, and, of course, science. His artistic preferences were unsystematic and definitely traditional. His favorite composer was Beethoven followed by Bach and other baroques; he loved Italian operas, but had little appreciation for most twentieth century music and a definite distaste for atonalism. (Of electronic and computer-composed music, he said that it is fit only for computers to listen to it.) In art, Dobzhansky admired the Italian Renaissance painters as well as the Dutch and Spanish masters of the seventeenth century; he appreciated the French Impressionists but detested cubism and all subsequent styles and schools of modern art.

Dobzhansky's obvious personality traits were magnanimity and expansiveness. He recognized and generously praised the achievements of other scientists; he admired the intellect of his colleagues, even when admiration was alloyed with disagreement. He made many long-lasting friendships, usually started by professional interaction. Many of Dobzhansky's friends were scientists younger than himself, who either had worked in his laboratory as students, postdoctorals, or visitors, or had met him during his travels. He was conspicuously affectionate and loyal toward his friends; he expected affection and loyalty in return. Dobzhansky's exuberant personality was manifest not only in his friendships but also in his antipathies, which he was seldom able, or willing, to hide.

Dobzhansky was a religious man, although he apparently rejected fundamental beliefs of traditional religion, such as the existence of a personal God and of life beyond physical death. His religiosity was grounded on the conviction that there is meaning in the universe. He saw that meaning in the fact that evolution has produced the stupendous diversity of the living world and has progressed from primitive forms of life to mankind. Dobzhansky held that, in man, biological evolution has transcended itself into the realm of self-awareness and culture. He believed that somehow mankind would eventually evolve into higher levels of harmony and creativity. He was a metaphysical optimist.

Dobzhansky's prodigious scientific productivity was made possible by incredible energy and very disciplined work habits. His enormous success as the creator of new ideas and as a synthesizer was, at least in part, based on his broad knowledge, phenomenal memory, and an incisive mind able to see the relevance that a new discovery or a new theory might have with respect to other theories or problems. His success as an experimentalist depended on a wise blending of field and laboratory research; whenever possible he combined both in the study of a problem, often using laboratory studies to ascertain or to confirm the causal processes involved in the phenomena discovered in nature. He obtained the collaboration of mathematicians to design theoretical models for experimental testing and to analyze statistically his empirical observations. He was no inventor or gadgeteer, but he had an uncanny ability to exploit the possibilities of any suitable experimental apparatus or experimental method.

Dobzhansky received many honors and awards. He was president of several professional organizations, including the Genetics Society of America (1941), the American Society of Naturalists (1950), the Society for the Study of Evolution (1951), the American Society of Zoologists (1963), the American Teilhard de Chardin Association (1969), and the Behavior Genetics Association (1973). He was a member of the National Academy of Sciences, the American Academy of Arts and Sciences, the American Philosophical Society, and of many foreign academies, such as the Royal Society of London. He received more than 20 honorary degrees from universities in the United States and abroad. He received the Daniel Giraud Elliot Medal (1946) and the Kimber Genetics Award (1958) from the National Academy of Sciences and numerous other medals, including the National Medal of Science, which he received in January 1964 from President Lyndon Baines Johnson (16, 17).

The 16 papers that follow were presented at a colloquium sponsored by the National Academy of Sciences to celebrate the 60th anniversary of the publication of Dobzhansky's *Genetics and the Origin of Species*. These papers are organized into four successive sections: Genetic Variation and Its Origins, Adaptation and Natural Selection, Population Differentiation and Speciation, and Patterns of Evolution.

Genetic Variation and Its Origins

In 1937, when Dobzhansky published *Genetics and the Origin of Species* (1), the DNA structure was not yet discovered, nor

were there any grounds to anticipate the tremendous impact that molecular biology would have on evolutionary research. We now know how genes are organized and function, and we can ask primeval questions such as what the original organisms were like or how ur-genes were organized. Walter Gilbert advanced in 1987 "the exon theory of genes" (18; see also 19) contending that introns have been around since the progenote, the earliest genetic organism, as spacers between the early, simple genes, and were thereafter used to assemble the complex genes that would later evolve as coalitions of the primitive ones. This hypothesis has been challenged with the alternative proposal that introns came about late in evolution and had nothing to do with the arrangement and rearrangement of gene pieces.

Walter Gilbert, S. J. de Souza, and M. Long in "Origin of Genes" (20) review the two theories, as well as an intermediate position proposing that introns arose at the beginning of multicellularity and played a major role during the Cambrian explosion in creating new genes by exon shuffling. The authors argue that if exon shuffling originated with the progenote, exons should consist of an integer number of codons and should be correlated with compact regions of polypeptides. The evidence that they now present, they say, strongly supports the case.

The ultimate source of genetic variation was thought to be, at the time of the publication of *Genetics and the Origin of Species*, gene mutation. Dobzhansky was soon to realize that chromosomal mutations could also play important roles in the evolution sweepstakes. The significance of the transposable elements, first discovered by Barbara McClintock in the 1940s, would become apparent only several decades later. Transposable elements, say Margaret G. Kidwell and Damon Lisch (21), are ubiquitous in many kinds of organisms and account for 10–15% of the *Drosophila*'s genome and more than 50% of maize's. Transposable elements provide, indeed, genetic variation on a scale and variety that could hardly have been imagined even a few years ago.

Kidwell and Lisch point out the manifold effects of transposable elements. In the genotype, they are involved in many gene mutations, are ubiquitous, and incessantly shift their numbers and locations. Transposable elements modify phenotypes as well, subtly in some cases, causing drastic alternations of development and organization in others. From an evolutionary perspective, transposable elements may be seen as parasites of genomes, but like with other parasites, organisms have often become coadapted with them and have even learned to subvert them for their own benefit.

The word "virus" does not appear in the index of any of the three editions of *Genetics and the Origin of Species*. By 1970, when *Genetics of the Evolutionary Process* (14) was published, viruses had become a favored organism of molecular genetics, and the term "viruses" is represented by six entries in the index, mostly referring to bacteriophages, but there is also a discussion of the myxomatosis virus, introduced in 1950 in Australia to control a rabbit population explosion. Two decades later, the accumulation of virus gene sequences combined with the development of new phylogenetic methodologies has brought viruses into the mainstream of molecular evolution. Important insights that have been gained concern evolutionary processes but also epidemiology, public health, and geographic patterns of human migrations.

Walter M. Fitch and colleagues (22) investigate the HA1 domain of the hemagglutinin gene from human influenza A viruses isolated throughout the world from 1984 to 1996. The gene is evolving at a rate of 5.7×10^{-3} substitutions per site per year, about one million times faster than cellular genes. In several positions of hemagglutinin a majority of the nucleotide substitutions are nonsynonymous—i.e., result in amino acid replacements—which strongly supports positive Darwinian selection rather than neutral evolution. The authors aver that

gene sequence phylogenies may manifest which isolates are most likely to cause future epidemics and might therefore be used for vaccine production.

Dobzhansky's interest in human genetic diversity was motivated by science but also by his enduring concern with the human predicament. He saw that the pervasiveness of genetic diversity was the foundation of human individuality but provided no grounds for any sort of discrimination. Equality—as in equality in law and equality of opportunity—"pertains to the rights and the sacredness of life of every human being" (ref. 23, p. 4). In *Mankind Evolving* (15, p. 18) he wrote that "Human evolution has two components, the biological or organic, and the cultural or superorganic. These components are neither mutually exclusive nor independent, but interrelated and interdependent. Human evolution cannot be understood as a purely biological process, nor can it be adequately described as a history of culture. It is the interaction of biology and culture. There exists a feedback between biological and cultural processes."

For more than three decades, L. L. Cavalli-Sforza has sought to elucidate the geographic origins and dispersal patterns of human populations by investigating gene frequency distributions. Genetic information has accumulated exponentially, encompassing protein-encoding genes, nuclear and mitochondrial, as well as microsatellite and other DNA sequences. "Genes, Peoples, and Languages" (24) emphasizes the African origin of modern humans, whence the other continents were colonized starting ≈100,000 years ago, first West Asia, then East Asia and Oceania, both probably through the coastal route of South Asia, and later Europe and America, both from East Asia and the latter certainly from the north, via the Bering land passage created in the ice ages. Cavalli-Sforza sees that the genetic conclusions are confirmed by trees of linguistic families, although these are temporally shallow.

Adaptation and Natural Selection

Starting in the late 1960s gel electrophoresis of soluble enzymes uncovered stores of genetic variation, much greater than had been suspected, in all sorts of animal and plant populations, as well as bacteria and other microorganisms. Whether this variation is adaptively important or just neutral noise became a matter of debate. The 1980s ushered in populational DNA sequencing. Much additional variation was discovered in the form of nucleotide differences between haplotypes. We now know that any two haplotypes of any gene differ on the average by several nucleotide substitutions, although most do not yield amino acid differences in the encoded protein. The neutral-selection controversy rages on.

Richard R. Hudson and collaborators (25) investigate the *Sod* gene (coding for the Cu,Zn superoxide dismutase) in *Drosophila melanogaster*, where an unusual polymorphism prevails. At the protein level two alleles, Fast and Slow, are discerned, with Slow absent in some populations but reaching frequencies ≈5–15% in many others. It turns out that all Slow alleles have identical DNA sequences (with trivial exceptions) even when they originate from different world continents. The Fast alleles fall into two categories: roughly half of them are identical, whether they come from Europe, Asia, or the Americas; the other half are heterogeneous, most of them distinguished from each other by several nucleotide differences. Adding to the puzzle is that the Fast alleles that are identical to each other are also identical to the Slow alleles except for the one nucleotide substitution that accounts for their different amino acid composition. Hudson and collaborators conclude that within the last few thousand years a previously rare allele has rapidly risen in frequency to the present levels. The process was driven by fairly strong natural selection.

Polymorphisms shared between species were investigated long and hard by Dobzhansky, mostly chromosomal rearrangements present in two closely related species, *Drosophila pseudoobscura* and *Drosophila persimilis*. DNA sequencing has uncovered numerous trans-specific polymorphisms, notably in the genes of the major histocompatibility complex (MHC) of mammals, where some shared alleles have persisted for millions of years. In plants of the family Solanaceae, alleles that are self-incompatible in fertilization have persisted across species barriers for 70 million years. *Drosophila* species also share DNA sequence polymorphisms that are several million years old.

Andrew G. Clark (26) develops mathematical models seeking to elucidate the causes of trans-specific shared polymorphisms. The shared self-incompatibility polymorphisms of plants and MHC alleles of humans and other primates are maintained by strong natural selection, because the protein products accrue a fitness advantage to the bearer of those alleles if they are different. The *Drosophila* polymorphisms, however, are recent enough that they might have persisted by neutral drift.

Three decades ago, Zuckerkandl and Pauling (27) conjectured that morphological evolution is largely caused by changes in the expression of genes, rather than in the amino acid sequence of the encoded polypeptides. Natalia A. Tamarina, Michael Z. Ludwig, and Rollin C. Richmond (28) explore the issue in two homologous genes in two species, *D. melanogaster* (*Est-6*) and *D. pseudoobscura* (*Est-5B*). The coding regions of these two genes share 80% of their nucleotide and amino acid sequences. The regions flanking the genes are, in contrast, so different that it is difficult to align their sequences to ascertain homology.

Tamarina and colleagues (28) make recourse to the magician's bag of tricks available to *Drosophila* geneticists. They pick up regulatory DNA segments from *D. pseudoobscura* and introduce them in the appropriate locations of *D. melanogaster*. The expression of the gene in the *D. melanogaster* transgenic flies becomes substantially altered. The expression of the two genes in normal flies follows similar patterns, yet the gene-regulating apparatus has become different in the two species.

It was not until the 1970s that demography was integrated into the theory of the dynamics of natural selection. Population genetics theory had until then treated all individuals in a population as effectively equivalent, without corroboration of longevity, age-dependent fecundity, and other life history parameters. The beginnings of an integration of the theories of population ecology and population genetics appeared in the 1970s, although this integration never engaged much attention from theorists or experimentalists, perhaps because of the many complexities involved. Dobzhansky and some of his students and collaborators made important experimental contributions to the problems (see refs. 29–35).

Wyatt W. Anderson and Takao K. Watanabe (36) analyze life history schedules of births and deaths to investigate the outcome of laboratory population experiments involving several chromosomal arrangements of *D. pseudoobscura* in various combinations. Coincidentally, it happens that all possible genetic outcomes occur: stable polymorphic equilibrium, unstable polymorphic equilibrium, and fixation for one of the alternatives. The authors conclude that, in these populations, both viability and fertility are important fitness components. Age-dependent female fecundity plays a particularly significant role in the outcome.

Population Differentiation and Speciation

The concept of species is fundamental in evolutionary theory. The modern understanding of this concept can be traced to 1935 when Dobzhansky introduced what is now known as the "biological species concept" (37). Dobzhansky defined species as "that stage in the evolutionary process at which the once actually or potentially interbreeding array of forms becomes segregated in two or more separate arrays which are physiologically incapable of interbreeding" (37; also ref. 1, p. 312). Dobzhansky saw that the species is not only a category of classification, but in sexual organisms also a natural unit defined by the ability to interbreed or its absence. He called attention to the determining role played by reproduction "isolating mechanisms," a term that he created.

The biological species concept has recently been challenged on the grounds that it unduly neglects phylogeny. John C. Avise and Kurt Wollenberg (38) examine this criticism by bringing to bear recent gene coalescence theory with an analysis of multiple, gender-defined pathways in genealogical pedigrees. They conclude that the supposed sharp distinction between the biological species concept and the phylogenetic constructs favored by the critics is illusory. "Historical descent and reproductive ties," they write, "are related aspects of phylogeny, and jointly illuminate biotic discontinuity."

Among the reproductive isolating mechanisms identified by Dobzhansky (1) there was one, later called "gametic isolation," occurring when the "spermatozoa fail to reach the eggs, or to penetrate into the eggs; in higher plants, the pollen tube growth may be arrested if foreign pollen is placed on the stigma of the flower." Therese Markow (39) notes that the investigation of gametic isolation as an evolutionary mechanism has been unduly neglected. In the genus *Drosophila* alone, a huge diversification exists in the size and pattern of gametes and other internal reproductive traits affecting fertilization. For fertilization to occur, "sperm must successfully enter the female and be transported to the storage organs. . . [and] must stay alive with adequate motility until they are utilized by the female." Markow examines how these steps fail in different cases and draws a richly patterned quilt that one can see will likely be much extended as other organisms are investigated. The evolutionary possibilities by which these variegations may come about are virtually infinite.

Coniferous forests and oak woodlands along the North American Pacific Coast are inhabited by *Ensatina* terrestrial salamanders. Several species were thought to occur in California, but detailed morphological and coloration analysis led to the conclusion in 1949 that various forms were parts of only one polytypic species arranged in the form of a ring around the Central Valley of California (40). Dobzhansky (41) saw that virtually all stages in a speciation process could be identified along the ring, with complete reproductive isolation between the terminal populations meeting in the southern part of the valley. In Dobzhansky's view speciation was thwarted by ongoing gene flow via the intermediate populations around the ring.

Wake and colleagues demonstrated in the late 1980s (42–44) that gene flow could not hold the complex together: an analysis of protein variation in 19 populations along the ring disclosed great genetic differentiation among populations. David B. Wake (45) reviews mitochondrial DNA and other variation. The *Ensatina* population array is old, consisting of a number of geographically and genetically distinct components that have reached or approximate full species level. The evolutionary history elucidated is extremely complex, with repeated interludes of geographic separation and genetic interactions upon renewed contact.

Peter R. Grant and Rosemary Grant (46) see that Dobzhansky's *Genetics and the Origin of Species* is an appropriate starting point for investigating the speciation process and the underlying genetic changes. But in one respect, they note, Dobzhansky's book is disappointing because it says nothing about the genetics of birds, which are their consuming research interest. Birds are made to serve a good purpose for illustrating geographical patterns of morphological variation within species, adaptation to newly colonized habitats, rapid radiation in

archipelagos, and interspecies competition. The evolution of reproductive isolation is considered, but "the genetics of speciation are the genetics of other organisms, mainly *Drosophila*."

Peter and Rosemary Grant note differences between speciation in birds and speciation in *Drosophila*. It is significant that in birds the behavioral barriers that prevent mating evolve first, whereas post-mating isolation typically evolves much later, perhaps after gene exchange has all but ceased. Pre-mating isolation in birds may arise from nongenetic causes, often from factors such as song, which in many groups of birds is culturally inherited through an imprinting-like process. Of the factors involved in pre-mating isolation, such as plumage, morphology, and behavior, some are under single-gene control, but most are polygenetically determined.

Patterns of Evolution

The universal tree of life consists of three domains, or "empires," bacteria, archaea, and eukarya. The three multicellular kingdoms, animals, plants, and fungi, are just 3 of the 10–12 extant major branches of the eukaryote domain. Molecular evolutionary investigations in the last decade have elucidated the large genetic diversity encompassed by the set of all eukaryotes and, hence, the reduced proportion represented by the multicellular kingdoms. The existence and great genetic heterogeneity of the archaea have been discovered by molecular evolutionists also in the last few years, and so have been most of the species and higher taxonomic groups. The reconstruction of the universal tree and the assessment of the genetic diversity of each branch are buttressed by the hypothesis of the molecular clock of evolution, which has multifarious other applications in other evolutionary studies. How good is the molecular clock?

It has been known for some time that the time variance of molecular evolutionary events is larger than would be expected if the molecular clock were a stochastic clock, like the radioactive decay of isotopes. Francisco Ayala in "Vagaries of the Molecular Clock" (47) reviews two clocks, the genes *Gpdh* and *Sod*, investigated in his laboratory. *Gpdh* evolves in *Drosophila* very slowly, at a rate of 1.1×10^{-10} amino acid replacements per site per year. But the rate is much faster, $\approx 4.5 \times 10^{-10}$ in mammals, between Dipteran families, between animal phyla, or between plants, animals, and fungi. On the other hand, *Sod* evolves very fast in *Drosophila*, $\approx 16 \times 10^{-10}$, which is also the rate in mammals and between Dipteran families; but the rate becomes much slower, 5.3×10^{-10}, between animal phyla, and still slower, 3.3×10^{-10}, between plants, animals, and fungi. If one were to assume that *Gpdh* and *Sod* are good clocks and project the *Drosophila* rate to estimate the time of divergence of the three multicellular kingdoms, *Gpdh* would yield an estimate of 3,990 million years, *Sod* an estimate of 224 million years, both very much off the commonly accepted divergence time of $\approx 1,100$ million years. It is unlikely that many molecular clocks are as erratic as *Gpdh* or *Sod*, but molecular clocks should be applied with caution, particularly when remote extrapolations are made.

The hypothesis of the molecular clock was originally predicated on the assumption that the evolutionary replacement of one amino acid for another, or one nucleotide for another is most often of no adaptive consequence. If such assumption would obtain, the process of molecular evolution would be governed by a time-dependent stochastic process. The assumption of adaptive inconsequence seems safest in the case of synonymous nucleotide substitutions, which do not change the amino acids encoded by a gene. Jeffrey Powell and Etsuko Moriyama (48) explore a vexing problem, namely that organisms do not use alternative synonymous codons with the frequencies expected if synonymous substitutions were inconsequential. The deviations from random expectations are large

in *Drosophila* genes and they often persist through long periods of evolution.

Powell and Moriyama (48) exclude differential mutation rates as the cause of the codon bias. Rather, they conclude that natural selection is the cause. The determining factor is the relative abundance of the tRNAs that execute the translation of genes into proteins: genes that are expressed at high rates favor codons that match those tRNAs that are more abundant.

The genes in the nucleus of plants often occur as "families"—i.e., a gene encoding a particular polypeptide may exist in several copies of more or less remote evolutionary origin. Michael Clegg, Michael P. Cummings, and Mary L. Durbin investigate "The Evolution of Plant Nuclear Genes" (49) by focusing on three gene families, *rbc*S, *Chs*, and *Adh*. Additional copies are recruited at different rates in these families: new *Chs* and *rbc*S genes are recruited 20 times faster than *Adh* genes. The multiplication of gene copies and their divergence is particularly notable for *Chs* genes in the evolution of flowering plants.

The evolution of *Adh* in monocot plants is not consistent with the molecular clock hypothesis even for synonymous nucleotide substitutions. Clegg and colleagues conclude that natural selection plays a significant role in driving the evolutionary divergence of duplicated genes. They add that new alleles often arise by intragene recombination (49).

Multigene families occur in animals as well as in plants. Notable in humans and other mammals are genes associated with the immune system, such as the MHC genes and immunoglobulin (Ig) genes. Some multigene families, in animals as in plants, arise by concerted evolution, a process that generates new genes by interlocus recombination or gene conversion. Masatoshi Nei, Xun Gu and Tatyana Sitnikova (50) raise the question whether concerted evolution may account for the MHC and Ig families, as some authors have suggested. They note that member genes of these families are often more similar to homologous genes from different species than they are to other member genes within the same species. This would not be expected if concerted evolution were the main originating process of gene multiplication within a family. Phylogenetic analyses of several MHC and Ig multigene families display patterns inconsistent with the concerted evolution hypothesis. The evidence favors the conclusion that the creation of new genes by gene duplication has repeatedly occurred in the evolutionary history of organisms. Some duplicated genes persist in the diversified descendant species for a long time; others effectively disappear, either because they are deleted or have become nonfunctional by deleterious mutations.

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