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Mutation-Driven Evolution

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Preface

I started my career with theoretical population genetics in the 1960s after a short period of field work experience concerning quantitative genetics. At that time evolutionary studies were conducted primarily by comparing phenotypic characters among individuals within and between species. These studies did not give clear insights into the cause and the effect of evolution, because the genetic basis of phenotypic characters was not well understood.

In theoretical population genetics, we could consider a set of alleles at one or a few loci and study the theoretical changes of genotype frequencies due to mutation, natural selection, and genetic drift. These studies gave only possible evolutionary changes of populations, but they were still much better than intuitive arguments. For this reason, a large body of mathematical theories of evolution was developed. These theories depended on many simplifying assumptions about the breeding system, population structure, selection coefficients, gene interaction, etc., and different assumptions about these factors often generated very different predictions of evolutionary changes. This resulted in many controversies which could not be resolved easily because of the difficulty of doing experimental studies. At that time, population genetics was dominated by neo-Darwinism with the idea of pervasive natural selection, and I was working within the framework of neo-Darwinism. Furthermore, because it was difficult to identify the homologous genes between different species, population genetics studies were primarily concerned with the gene frequency changes within species.

In the early 1960s a number of molecular biologists were working on the evolutionary changes of genes and proteins at the molecular level, and this approach of studying evolution was integrated with population genetics theory in the latter half of the 1960s and in the 1970s. This integration transformed the study of evolution profoundly. First, we could now identify homologous genes in different species and study long-term evolution of genes by comparing the nucleotide or amino acid sequences from different species. Second, molecular data on the evolutionary change of genes soon indicated the importance of mutation in evolution. Third, comparison of the DNA contents of different species suggested that gene or genome duplication occurred frequently in the process of evolution. Because gene or genome duplication is a form of mutation in the broad sense, I realized that mutation is the driving force of evolution. Yet, this view was regarded as a heresy at the time when neo-Darwinism dominated the field. In the meantime the neutral theory of molecular evolution was proposed to explain the evolution of genes and proteins. This theory clearly showed that the evolution of nucleotide sequences has occurred mainly by random fixation of neutral mutations. However, most neo-Darwinians did not pay much attention to this discovery, because they believed that neutral evolution has nothing to do with phenotypic evolution, in which most evolutionists are interested. In fact, even the proponents of the neutral theory of molecular evolution stated that phenotypic evolution occurs mostly by natural selection, as will be mentioned later.

By the early 1970s, I came to believe that the principle of phenotypic evolution must be the same as that of molecular evolution because both types of evolution are controlled by mutation at the DNA level. I briefly presented this idea in my 1975 book *Molecular Population Genetics and Evolution*. However, few people paid attention to this view. I elaborated this idea in several publications in the 1980s including my 1987 book *Molecular Evolutionary Genetics*, but the response was not great. The problem was that the molecular biology of morphogenesis was not well developed at that time and it was not easy to show the roles of mutation in phenotypic evolution convincingly.

In the past two or three decades, this situation has changed dramatically, and it is now possible to evaluate the roles of mutation and selection in phenotypic evolution at the molecular level. In the 1980s I became interested in understanding the evolution of the adaptive immune system of vertebrates by studying evolutionary changes of immunoglobulins, major histocompatibility complex genes, T-cell receptor genes, etc. In later years I also studied the evolution of genes controlling body segmentation (HOX genes), flowering in plants (MADS-box genes), sensory receptors, microRNAs, etc. in collaboration with graduate students and postdoctorals in my laboratory. These studies have been very helpful in clarifying my view that the driving force of evolution is mutation and natural selection is of secondary importance. This view is different from Hugo de Vries's mutation theory, and I previously called it the new mutation theory of evolution or neomutationism. In this book, it will be called the theory of mutation-driven evolution to convey the message that the importance of natural selection is duly appreciated in conjunction with the role of mutation.

During this period, I came to realize that evolutionary biology must be rebuilt upon the knowledge of molecular biology. Every biological process involved in metabolism and reproduction of organisms is governed by the function of DNAs and RNAs at the most fundamental level. Environmental effects on the formation of organisms can also be studied by using the knowledge of epigenetics. Natural selection and genetic drift are ultimately determined by the differential rates of birth and death of individuals, which are again the consequences of metabolism and reproduction. Population genetics and ecology are useful for visualizing the long-term change in populations and for understanding the consequences of population size change and competition and cooperation of organisms. However, the prediction of population genetics is always abstract, and it does not give any explanations about how a particular character such as mammalian sex determination or vertebrate brain has evolved. To answer these types of question, we must use the molecular biology approach. For this reason, I have become interested in explaining the evolution of specific characters and attempted to give molecular answers to some of the questions Charles Darwin and other investigators have posed in the past.

Evolution is a broad subject encompassing many areas of biology such as molecular biology, genetics, ecology, sociobiology, and paleontology. In this book, however, I will be concerned primarily with the mechanism of evolution with an emphasis on genetic and molecular aspects. I have decided to do this, because this is the backbone of evolutionary biology and it has been controversial ever since Darwin's publication of *Origin of Species*. I will discuss this problem with a historical perspective to understand various theories of evolution presented in the past 150 years and their relationships with the new theory of mutation-driven evolution. The historical perspective presented here may not necessarily be the same as that of currently popular books such as Mayr's *Growth of Biological Thoughts*, because I found some misconceptions in these books when I examined the original sources. I have tried to present the views of the original authors as much as possible.

However, the main purpose of this book is to present a comprehensive theory of mutation-driven evolution considering the latest information on molecular and phenotypic evolution. We are all aware that phenotypic characters show an enormous amount of variation both within and between species. All this variation is ultimately caused by differences in the structure and function of DNAs and RNAs, whether the characters are affected by environmental factors or not. Therefore, phenotypic evolution must be ultimately explained in terms of molecular biology. For this reason, I will consider the evolutionary change of molecules, genes, and genomes and then the molecular basis of phenotypic evolution. Of course, our knowledge of the molecular basis of phenotypic evolution is quite limited. We have little idea about how the human brain, the elephant's trunk, the body structures of whales, etc. have evolved. Nevertheless, we are beginning to understand the molecular basis of many complex phenotypic characters, and the future of the study of phenotypic evolution is bright. However, these problems have to be studied by using new technologies and new evolutionary concepts.

In this book, I have tried to cover these new developments in evolutionary biology and critically examine both old and recent findings to establish the general principles of evolution. In my view evolution is not the enhancement of fitness of individuals or populations, but it represents the increase (or decrease) of phenotypic complexity, which may be measured by the number of cell types or some other quantity. For this reason, my conception of evolutionary biology is different from the currently popular views. In this book, I hope I have explained the theory of mutation-driven evolution in a logical fashion.

This book is written as a monograph rather than a textbook. Therefore, the topics covered are not necessarily comprehensive, and it is assumed that the readers are acquainted with the basic knowledge of genetics and molecular biology. Nevertheless, I have presented essential aspects of these disciplines that are required for understanding my arguments. I have used many examples to illustrate the importance of mutation in evolution, and in this case I have often presented the studies which have been conducted in my laboratory. I have done this because I am familiar with them and therefore I can avoid serious errors. However, this book is based on the knowledge accumulated by numerous investigators over the past several decades though the sources are not always clearly mentioned.

In Chapter 1 a brief history of scientific studies of evolution is presented, starting with Charles Darwin's work on evolution and covering subsequent development of studies that have led to the idea of mutation-driven evolution. Chapters 2 and 3 are devoted to the development of neo-Darwinism and its significance and limitations for the study of evolution. Chapters 4 and 5 present evolutionary changes in genes and genomic structures and their relationships with phenotypic evolution. The molecular basis of phenotypic evolution is presented in Chapter 6 with an emphasis on the mechanism of gene expression in the development of phenotypic characters and the interaction between genes and environmental factors. In Chapter 7 various mechanisms of generating hybrid sterility and inviability are discussed in relation to the formation of new species at both genic and genomic level. Here a new view of speciation is presented. Chapter 8 is devoted to the evolution of several important phenotypic characters such as sex determination and insect caste systems. Chapter 9 presents the general concept of mutation-driven evolution and its significance in the study of evolution. The final chapter presents a general summary of this book and conclusions.

I am deeply indebted to my colleagues and students who have collaborated with me during the last three decades. Some of them helped me in developing statistical methods that have been used in our data analysis, whereas others conducted time-consuming data analysis. I am particularly grateful to Takashi Gojobori, Austin Hughes, Tatsuya Ota, Koichiro Tamura, Sudhir Kumar, George Zhang, Alex Rooney, Yoshiyuki Suzuki, Helen Piontkivska, Jongmin Nam, Yoshihito Niimura, Nikolas Nikolaidis, Dimitra Chalkia, Zhenguo Lin, Masafumi Nozawa, and Sabyasachi Das. Hie Lim Kim, Zhenguo Zhang, and Sayaka Miura also helped me in preparing several figures used in this book. I also would like to express my gratitude to Jan Klein, Pekka Pamilo, Wojtek Makalowski, Tatsuya Ota, and Alex Rooney, who read the entire manuscript of the book and provided constructive comments. I am extremely grateful to Tina Kushner, who prepared the final manuscript with great care and helped me in organizing the References list.

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CHAPTER 1

Selectionism and Mutationism

1.1. Darwin's Theory of Evolution

In the mid-nineteenth century, Charles Darwin (1859) published a book called *The Origin of Species*, which is regarded as one of the greatest books ever written in the history of science. Through this book, he could convince the world that all living organisms are not independent creations but they were derived from a single common ancestor by descent with modification. He did this by assembling massive data on evolution from various fields of biology and geology and considering the mechanism of evolution materialistically. It is often said that he could accomplish this achievement because he discovered natural selection.

The full title of the original edition (1859) of Darwin's book was "*On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life*." It is interesting to see that he defined the then new terminology natural selection within the title of the book, apparently because he wanted to avoid any misunderstanding of the terminology. This definition implies that natural selection is a mechanism to save favorable individuals for the next generation and has nothing to do with creation of innovative characters or variations. In his time the mechanism of generation of heritable variations was not known, and apparently for this reason Darwin considered natural selection as the major force of evolution. However, he never implied that natural selection has creative power. In the sixth edition of *The Origin of Species* (Darwin 1872, p. 63), he stated: "*Several writers have misapprehended or objected to the term Natural Selection. Some have even imagined that natural selection induces variability, whereas it implies only the preservation of such variations as arise and are beneficial to the being under the conditions of life.*"

If we use the current terminology in biology, the essence of his theory of evolution may be summarized as follows. (1) Most natural populations contain a large amount of phenotypic variation on which natural selection may operate. (2) Phenotypic variation is continuous rather than discontinuous and includes heritable components. (3) Darwin did not know the cause of phenotypic variation but suggested that it is generated by use and disuse of characters, climatic changes, correlation of growth (modification of a character generated as a consequence of natural selection for another character), and chance effects (random changes). (4) Evolution occurs gradually by means of natural selection, which inevitably causes extinction of less improved forms of life. (5) Accumulation of the results of natural selection gradually increases interpopulational differences in morphological and physiological characters and eventually generates new species, genera, families, etc. (6) All organisms on earth were derived from a single proto-organism through a slow process of descent with modification. (7) The similar organisms currently observed in different parts of the world have been generated by recent migration. (8) The discontinuity of paleontological data in different geological periods does not indicate that evolution occurred discontinuously but that the fossil record is incomplete and there are many gaps in the record.

The above statements are a brief summary of Darwin's theory of evolution. In practice, he was not as straightforward as mentioned above. He was a humble and cautious scientist and tried to avoid any dogmatic statements. This made his book acceptable for a wide range of evolutionists, but it

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also made his statements often ambiguous. In fact, he did not always distinguish between the causes of variations and the results of natural selection. If one accepts any form of inheritance of acquired characters (Lamarck 1809) as he did, the difference between the cause of variation and the result of natural selection necessarily becomes unclear. This is because the Lamarckian doctrine generates new heritable variations which are similar to the results of natural selection and the variations may be reverted to original characters when the environmental condition changes back to the original status. This is also true with the effects of climatic changes. This situation becomes worse if one accepts the blending inheritance to which Darwin subscribed (see Section 1.2). Darwin (1859) occasionally referred to sports as a source of new variation, but he did not think this was an important factor.

Conceptually, his theory of evolution consists of two processes: (1) generation of new variations and (2) natural selection of favored variations. In this sense his view is similar to the modern concept of evolution. Darwin provided strong arguments for the second process (not evidence), but he could not give satisfactory explanations for the first evolutionary process. Therefore, he simply assumed that sufficient amounts of variations always exist within populations and did not worry about the cause too much. This treatment of the first evolutionary process was unsatisfactory to many biologists (e.g. Thomas Huxley and Francis Galton), and various new hypotheses were proposed later. In fact, most of the later controversies on evolution were concerned with the generation of new variations, which are now known as mutations, and their relationships with the second process of natural selection. Around 1910, it was often said that "*natural selection may explain the survival of the fittest, but it cannot explain the arrival of the fittest*" (de Vries 1912, p. 827). The same criticism was raised repeatedly in the past (Morgan 1903, 1932), and it has become louder recently (e.g. Ohno 1970; Nei 1987; Kirschner and Gerhart 2005; Stoltzfus 2006).

1.2. Criticisms of Darwin's Theory

Darwin's theory of natural selection was supported enthusiastically by some distinguished biologists such as August Weismann and Alfred Wallace, but there were many biologists or paleontologists who opposed gradual evolution by natural selection and proposed alternative theories. For example, Herbert Spencer and Ernst Heckel accepted the inheritance of acquired characters as an evolutionary force at least to the same extent as natural selection (Bowler 1983). In fact, Lamarckism was considered as an important alternative theory in the post-*Origin* era, and this view was maintained even after genetics was firmly established in the 1910s and 1920s. The final rejection of Lamarckism occurred only when Luria and Delbruck (1943) showed that bacterial resistance to bacteriophages is caused by spontaneous mutation rather than by induction through phage exposure. Later Lederberg and Lederberg (1952) conducted indirect (sib) selection experiments with the bacterium *Escherichia coli* and showed that drug resistance in bacteria evolves by pre-adaptive mutations without being exposed to any drugs. Here sib selection refers to selection in which the drug resistance of a colony is judged by the result of the assay of its sibling colony, so that the selected colony line is never exposed to the drug. Similar results were obtained with respect to DDT resistance in *Drosophila melanogaster* (Crow 1957). Other popular theories at that time were saltationism, in which new species or subspecies are suddenly produced in geological time, and orthogenesis, which claims that each organism is destined to follow a certain course of evolution by some internal force. However, since these views were based on old mystical concepts about unknown laws of inheritance or evolution, they gradually disappeared. Some authors emphasized the importance of geographical isolation for speciation, whereas others suggested that hybridization is an important factor for the origin of new species (see Mayr 1982; Bowler 1983). These views were clearly concerned with short-term evolution, and they were not real criticisms of Darwinism, which dealt with long-term evolution. With the rediscovery of Mendel's law of inheritance in 1900, it became clear that they are not fundamentally important for explaining evolution.

One of the most serious objections raised against Darwin's theory of evolution was concerned with his claim that natural selection operates on continuous or fluctuating variation and this is sufficient for generating new species. This claim was questioned by Thomas Huxley and Francis Galton, who were otherwise staunch supporters of Darwin's evolutionary theory. In the middle nineteenth century, many biologists including Charles Darwin believed in the theory of blending inheritance, in which the characters of offspring of a pair of male and female parents tend to be intermediate between those of the two parents. Fleeming Jenkin (1867), who was a professor of engineering at the University of Edinburgh, indicated that Darwin's theory based on this idea creates various problems about effectiveness of natural selection because blending inheritance is expected to reduce the extent of variation every generation. He indicated that in the theory of blending inheritance even discontinuous variations such as sports (large effect variants) cannot be established in the population and therefore no new species can be generated. The idea of blending inheritance was abandoned only after Mendel's laws of inheritance were rediscovered (Morgan 1925, pp. 139–140; Fisher 1930, pp. 1–7). Another comment made by the physicist Jenkin was that Darwin's theory of natural selection was too speculative and experimental verification was necessary. In his 1869 letter to his friend J. D. Hooker, Darwin stated that "*Fleeming Jenkin has given me much trouble but has been of more real use than any other essay or review*" (Mayr 1982, p. 512). For these reasons, Darwin weakened his claim of the efficacy of natural selection, particularly about discontinuous variations. (Ironically, the significance of Jenkin's paper in the development of evolutionary theory is still debated by historians and philosophers (e.g. Gayon 1998; Bulmer 2004), but this is out of the scope of this book.)

To avoid the reduction of variation, there was a need for finding mechanisms that generate new variation. One such mechanism Darwin proposed was the hypothesis of pangenesis, in which hereditary substances called gemmules or pangenes were assumed to be shed by all parts of the organism and carried in the bloodstream or some other agencies to the germline cells with uneven contributions from different organs. When the contribution from a particular organ is large, the organ is manifested disproportionately in the next generation. In this way new variations are expressed in the next generation to offset the dissipation of variations by blending inheritance (Darwin 1868). This hypothesis was previously used by Lamarck (1809) to explain the mechanism of acquired characters. However, when Galton conducted blood transfusion experiments using different varieties of rabbits, no evidence of pangenesis was obtained (see Provine 1971). For the above reason, Huxley, Galton, and Bateson indicated that a new evolutionary theory based on discontinuous variations, which would not disappear by mixing with the original variations, should be developed.

At the end of the nineteenth century, the number of supporters of Darwin's theory of natural selection dwindled substantially, and the only major supporters were August Weismann, Alfred Wallace, Walter Weldon, and Karl Pearson. Around this time, Weldon and Pearson initiated a biometrical study of Darwinian evolution, but this study was severely criticized by William Bateson, who supported evolution by discontinuous variation. When Mendel's law of inheritance was rediscovered in 1900, Bateson took the law as support of his theory of discontinuous evolution and attacked the biometricians. This event contributed to an eclipse of Darwinism (Bowler 1983; Gayon 1998). Bateson is also known to have coined the word genetics.

1.3. Evolution by Discontinuous Variation

As mentioned above, Huxley and Galton did not believe that natural selection acting on continuous variation is sufficient to generate the large morphological and physiological differences between species. A number of evolutionists such as Bateson and de Vries therefore proposed the theory of evolution by discontinuous variation. They emphasized the importance of new discrete variations (mutations in the present terminology) in evolution. To support this idea, Bateson (1894) compiled and described various morphological oddities or discrete variations in the animal kingdom in his 598-page long book, *Materials for the Study of Variation*. These oddities included a bumblebee with legs attached to the antennae, butterflies with extra eyespots on the wings, frogs with extra vertebrae, a man with extra nipples, and many others (886 examples). He called the abnormality with one body part transformed into another a homeotic transformation. The purpose of this compilation was to show that there are many discontinuous variations in animal species and they could be the materials for generating new species. He believed that to understand the origin of variations and evolution one must study newly arisen variations rather than the variations that are observable in natural populations, with which Darwin was concerned. He also rejected Lamarckian inheritance. Unfortunately, it was later shown that most of the morphological oddities he compiled were not heritable and therefore they did not contribute to evolution. For this reason, his theory of evolution by discontinuous variation was not widely accepted. Interestingly, however, his homeotic transformation was later observed in many different groups of animals, and the recent genetic and molecular studies of formation of these abnormal animals led to the currently burgeoning field of developmental biology.

In the nineteenth century various forms of intraspecific variation were discussed in relation to evolution, but the discussion was always vague because Mendelian inheritance was not known and the concept of heritable and nonheritable variation had not been well established. As mentioned earlier, Darwin did not know how new variations occur. Therefore, his argument of evolution was concentrated on natural selection. Bateson was opposite to Darwin and studied mutational variants that were observable in natural populations. Unlike popular accounts, he was fully aware of the importance of natural selection for the establishment of new mutations in populations. He stated "*In the view of the phenomenon of variation here outlined, there is nothing which is any way opposed to the theory of the origin of species by means of natural selection or the preservation of favoured races in the struggle for life*" (Bateson 1894, pp. 80). He simply emphasized the importance of the study of discrete variations in evolution and was critical of panselectionism, which was popular at that time. Unfortunately, he did not provide any clear answer to the mechanism of occurrence of discrete variations. He stated "*Inquiry into the cause of variation is as yet, in my judgment, premature*." A few years later, de Vries (1901–1903, 1909, 1910) proposed that morphological and physiological characters are subject to random mutations and these mutations generate discontinuous variations. However, this was not a complete answer to Bateson's inquiry about the formation of mutant phenotypes. To answer his question, biologists had to wait for 100 years. Only in the last few decades, developmental and evolutionary biologists have begun to study this important problem. This issue will be discussed in Chapters 5–9.

In the beginning of the twentieth century, de Vries proposed a theory of formation of new species (elementary species by his terminology) or varieties by means of mutations in his big book composed of two volumes, *The Mutation Theory* (de Vries 1901–1903). (He also rediscovered Mendel's law of inheritance.) He classified new variations into two types: (1) individual variations and (2) mutations. Individual variations correspond to Darwin's continuous variation, whereas mutations are discrete changes of phenotypic characters and may generate new elementary species by single steps of changes. He asserted that individual variations may be useful for producing new varieties or breeds within species but they never lead to new species and that to generate new species mutations are necessary. This assertion is based on the results of his extensive studies of hybridization and artificial selection in various plants and an evaluation of similar studies conducted by other investigators. A good example of the first part of his assertion is the existence of many different breeds of dogs which have been generated by artificial selection in the last 10 000 years. The extent of morphological variation among these breeds of dogs has become so extensive that many of the breeds would be regarded as different species if they were found in the wild without human intervention. In fact, de Vries stated that the two extreme ends of a character showing individual variation can be sometimes greater than the difference between two different species. However, because hybridization of these breeds produces healthy offspring, they are still regarded as members of the same species. The reason for this has not been well studied, but we are now beginning to understand it thanks to the recent progress of developmental biology, as will be discussed later.

To prove the second part of his assertion, de Vries compiled a large number of examples of elementary species in his book, *The Mutation Theory*.

Examples well known to us are cauliflowers derived from the cabbage *Brassica oleracia*, awnless oats, and strawberries without runners. These are elementary species recently generated by mutations and breed true when outcrossing is prevented. According to de Vries, however, these elementary species are not always purebred and may segregate a certain proportion of non-pure individuals in each generation.

The most famous examples of elementary species are those derived from the American evening primrose *Oenothera lamarckiana*, which he found in an abandoned potato field near Amsterdam. He first observed that there were conspicuously different forms of individuals growing in the wild populations of this species in the Netherlands. Conducting a breeding experiment for 14 years, he showed that *O. lamarckiana* continually produced small proportions of variant forms and that these variant forms either bred true or segregated into *O. lamarckiana* and the new types. Some of the new forms were morphologically quite different from *O. lamarckiana*, so that new (elementary) species names were assigned. Especially, one of the new elementary species, called *O. gigas*, was bigger and more vigorous than its parental species, *O. lamarckiana*. This form appeared only once among about 50 000 plants he examined in a 14-year period of study. Because his experimental results were very convincing, his mutation theory was welcomed by many biologists at the time of publication of his book (Allen 1969). However, de Vries's contention that new species arise by single mutational changes was later questioned. Davis (1912), Renner (1917) and Cleland (1923) showed that the strain of *O. lamarckiana* de Vries studied was apparently a permanent heterozygote for two chromosomal complexes and that most of de Vries's mutants were segregants from this unusual genetic form. This finding was a serious blow to de Vries's mutation theory (see Cleland 1972 for details), and some historians stated that de Vries's theory did not pass the test of time (Allen 1969) and was refuted resoundingly (Provine 1980, p. 55). Stebbins (1950, p. 102), a leading plant evolutionist in the twentieth century, also stated that "*de Vries' elementary species is either a figment of the imagination or a phenomenon peculiar to plants with self-pollination and an anomalous cytological condition*," despite the fact that de Vries was the first person to develop an evolutionary theory based on experimental studies.

However, de Vries's interest was not in the instability of *O. lamarckiana* but the generation of new variants by mutation. Later studies have shown that his mutants were mostly due to chromosomal changes and included polyploids, aneuploids, translocations, inversions, and single gene mutations. Notably, *O. gigas* was a tetraploid (Lutz 1907; Gates 1908) and bred true. It is now well known that these chromosomal variants often form new species particularly in plants. Therefore, de Vries's claim was not really incorrect, as Wright (1977, pp. 411–412) emphasized. Dobzhansky (1951, pp. 287–294) also considered polyploidization as an important mechanism of generating new species. However, in the middle twentieth century speciation by polyploidization was thought to be rare, and this was certainly true in animals. For this reason, de Vries's mutation theory of evolution was discredited. In recent years, however, this view has changed dramatically as the genomic study of evolution progressed (Doyle et al. 2008; Velasco et al. 2010). It is now known that new species have arisen many times by polyploidization as a single step. This issue will be discussed later (Chapter 7).

de Vries was a visionary scientist and introduced experimental study of evolution for the first time. In the preface of his 1901 book (de Vries 1909), he stated: "*A knowledge of the laws of mutation must sooner or later lead to the possibility of inducing mutations at will and so of originating perfectly new characters in animals and plants. And just as the process of selection has enabled us to produce improved races, greater in value and in beauty, so a control of the mutative process will, it is hoped, place in our hands the power of originating permanently improved species of animals and plants.*" As every geneticist knows, this prediction was brought to its fruition in the latter half of the twentieth century.

At this stage, it should also be indicated that de Vries was fully aware of the necessity of natural selection for new mutant forms to be established in natural populations (de Vries 1909, p. 212). Because mutation is assumed to occur at random, disadvantageous mutations are obviously eliminated and only mutants that are competitively stronger than the original types can survive. He simply emphasized the importance of understanding the first process of evolution (generation of innovative characters) as envisioned by Darwin (1859). The fact that de Vries was not antagonistic to natural selection was also emphasized by Wright (1960) and Gayon (1998).

1.4. Mutationism

de Vries's mutation referred to any heritable change of phenotypic characters. With our current knowledge, this means any change of genetic materials including nucleotide substitutions, nucleotide insertions/deletions, gene duplications/deletions, gene transposition, changes in gene interactions, various types of chromosomal changes, and genome duplication. In de Vries's time, none of these detailed genetic changes was known, and only thing he could study was heritable changes of morphological or physiological characters.

As Mendelian genetics progressed, genes were identified as the unit of inheritance, and mutations generally implied heritable changes of genes. In the meantime chromosomal changes were discovered and shown to affect some morphological characters, but these changes were not always specific and therefore they were not studied on a regular basis. Polyploidization was also shown to generate new genetic variation, but its effect on phenotypic evolution was thought to be minor (Stebbins 1950).

For these reasons, mutations generally referred to changes in protein-coding genes rather than to chromosomal changes. This was particularly so in the work of Thomas Morgan (1916, 1925, 1932) and his school. During this gene-centric era, de Vries's idea that a new species can be generated by a single mutational event was ignored. Instead, a new school of thought advocating evolution by genic mutation became predominant, and a leader of this school was Thomas Morgan. Morgan's view is often called mutationism, somewhat inappropriately.

During the first 15 years after the rediscovery of Mendelism in 1900, there was leaping progress in the study of genetics. First, blending inheritance was disproved, and Mendelian inheritance of discrete characters was shown to apply to many characters in plants and animals. This indicated that genetic variability does not decay by bisexual reproduction but can be maintained in populations (Castle 1903; Hardy 1908; Weinberg 1908, 1963). Second, Johannsen's (1909) pureline theory showed that Darwin's continuous variation is composed of heritable and nonheritable variation and selection on the latter variation is ineffective. Third, Nilsson-Ehre (1909), East (1910), and Emerson and East (1913) showed that the inheritance of quantitative characters can be explained by the independent segregation of alleles at multiple loci. Weinberg (1910, 1984) and Fisher (1918) also showed that the correlation of quantitative characters between relatives as observed by the biometricians Galton, Weldon, and Pearson can be explained by Mendelian inheritance. These findings ended the heated controversy between the biometricians and the Mendelians. Fourth, Morgan, Sturtevant, Muller, and Bridges (1915) and Muller and Altenburg (1919) showed that new mutations arise spontaneously with a very low but measurable frequency and they are inherited as Mendelian characters. Most of the mutations were deleterious, but some of them appeared to be virtually neutral or slightly advantageous.

Morgan's theory of evolution (1916, 1932) was based on the above findings by geneticists. He separated the process of generating innovative characters and the preservation of these characters in evolution, as was done by Bateson and de Vries. In his view the first process is accomplished by random mutations that occur at each genetic locus whether the character is continuous or discontinuous. The second process of preservation of new mutations is achieved by natural selection or genetic drift. This view was conceptually somewhat different from Darwin's view of natural selection, which is supposed to act for the "preservation of favored races in the struggle for existence." Darwin's view was developed under the assumption that "favored and unfavored races" always exist in the population. Furthermore, because the genetic entities of favored or unfavored races were not known in Darwin's time, the outcome of the struggle for existence was always vague. Morgan was clearly in a better position than Darwin in the conceptual formulation of natural selection because of the new genetic knowledge. In his early days Morgan (1903) was critical of the efficacy of natural selection and found it somewhat teleological. In his 1916 and 1932 books, however, he presented a clear form of mutation-selection theory.

Some authors stated that Morgan was a typologist and did not have a population concept (e.g. Allen 1978; Mayr 1982). In his book *The Scientific Basis of Evolution* (Morgan 1932), however, he presents a rather incisive discussion of population and quantitative genetics. On page 132, he states: "*If the mutant is dominant ... and is bred to the wild type, the mutant character will appear again in half of the progeny, and if advantageous, i.e., if one that increases the chance of survival of the individual and the race, it will gradually spread through the race. If the new mutant is neither more advantageous than the old character, nor less so, it may or may not replace the old character, depending partly on chance; but if the same mutation recurs again and again, it will most probably replace the original character. If the new character is a disadvantageous one, it will soon be eliminated.*" This statement indicates that Morgan had a good grasp of the concept of population genetics.

Morgan's mutationism was quite popular in the first quarter of the twentieth century. Actually, this view was held by most evolutionary geneticists at that time, and Morgan was merely a spokesman for them (Wright 1960). Another leader of mutationism or the mutation-selection theory was Hermann Muller (1929), who was more proficient in mathematics than Morgan. This theory clearly showed that any evolutionary change of phenotypic characters should be studied by examining the mutational change of genes, and this idea has changed the concept of evolution forever though there are still some evolutionists who investigate only phenotypic changes. However, as the study of mutations and polymorphic alleles proceeded, a number of observations which were unfavorable to mutationism were noticed. First, most mutations observed in laboratories were deleterious and did not appear to contribute to evolution at all. Second, morphological characters were mostly quantitative, and their variation was thought to be a product of interaction of a large number of genes and environmental factors. Therefore, the relationship between mutation and selection was unclear. Third, even though some discrete characters showed seemingly Mendelian inheritance, the characters were often controlled by additional modifier genes. For example, the heredity of guinea pig coat color pattern could be explained by a number of loci, but there was a complicated interaction among different loci (Wright 1927).

To explain these observations, Morgan's mutationism appeared too simplistic. Although Morgan had a reasonably good grasp of the population concept as an experimentalist, he was not proficient in mathematics and was gradually left behind as the mathematical theory of population genetics advanced in the 1920s and 1930s. As mentioned in Section 1.5, this development initiated a new era of neo-Darwinism, and the rise of neo-Darwinism caused Morgan's mutationism to decline gradually. At present Morgan's view is often called "mutationism," but this is not really appropriate because he accepted natural selection as an agent of eliminating unfit genotypes, as Darwin did. However, because this terminology is widely accepted and Morgan certainly considered mutation as the primary force of evolution, I will use the word mutationism to represent Morgan's view in this book.

1.5. Neo-Darwinism

The term neo-Darwinism has been used to represent various forms of modified Darwinism since the late nineteenth century. At the present time, however, it usually refers to the evolutionary theory formulated by Fisher (1930), Wright (1931, 1932), and Haldane (1932). Evolution refers to the long-term genetic change of populations or species, so that it is difficult to do experimental studies. However, once Mendelian genetics was established, it was possible to predict the evolutionary changes of populations under simplified assumptions. Although this prediction was very crude, it was much better than intuitive speculations. After conducting extensive mathematical studies in the 1920s and 1930s, the three founders of population genetics, Fisher, Wright, and Haldane, reached the conclusion that natural selection is much more important than mutation. This was opposite to the view of mutationism. Around this time, however, various experimental evolutionists initiated studies of natural