Theory and Reality of Evolutionary Biology

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I am honored and humbled to receive the prestigious Kyoto Prize for the basic sciences of biology. I have been studying population genetics and evolutionary biology for more than 50 years. Today I would like to talk about a brief history of my Iife and what I have done in my scientific career.

1. My Family and Childhood

I was born on January 2, 1931, in a small village of Miyazaki Prefecture called Naka, about 15 km north of the city of Miyazaki in Kyushu Island of Japan. (It now belongs to the city of Miyazaki administratively.) At the time when I was born, my family operated a fairly prosperous business of liquor (shochu) making. However, this family business soon became bankrupt mainly because of the Great Depression in the 1930s and partly because of the mismanagement of my grandfather, Genzo Nei. After this bankruptcy, my father, Tadashi Nei, started an independent business as a farmer using a small portion of rice fields retained for the family (Fig. 1). My father and mother, Masae Nei, were both hard-workers and gradually regained most of the land they lost at the time of bankruptcy.

They had two boys and six girls as their children, and I was the second child and the first son. However, my brother, who was the fourth child, died at the age of three because of dysentery. This happened in 1936 when there were no antibiotics. This was the first traumatic event in my life, and I never forget the moment of his death. Except for this event, our family had a normal life, which was common in the rural area of Japan at that time. We had plenty of food, and we played a lot with our friends, going fishing and bird trapping. However, education was not particularly emphasized. In elementary school, we used nationally standardized textbooks, and our parents bought virtually no other supplementary books or magazines. In 1937 when I entered in the elementary school, the Japan-China War (invasion of the Japanese army into China) broke out, and in 1941 World War II (The Pacific War) started. In this period we were taught to be loyal to the country and sacrifice our lives for the glory of the country. This tendency was strengthened as the war expanded worldwide. For this reason, most people endured poor life (Fig. 2) and supported the military machine.

Near the end of World War II, most Japanese cities were attacked by American bombers

and burned out. The city of Miyazaki was no exception, and we often saw the city being bombed and burned at night from our village. Unexpectedly, part of our rural village was also bombed in the morning of April 18 of 1945. This bombing destroyed many houses and killed more than fifty people, including four of my relatives. Some of my father's rice fields were also bombed, and big craters were formed. As a young boy I rushed to the bombed area and found that some people were already dead while others were severely wounded and crying for help. However, I couId do nothing for them because I had no medical knowledge. Our village was located at the Pacific side of Kyushu Island and was considered as the prime target of invasion of the American military force into the mainland Japan.

World War II suddenly ended on August 15, 1945. The Emperor of Japan announced by radio that the Japanese government surrendered unconditionally to the American and Allied forces. This caused a confusion and chaos in the minds of many Japanese, but no civil war occurred. For myself, I welcomed the end of the war because if the war continued for another month I would have been most probably killed. Near the end of World War II, most secondary schools were closed, and the students were forced to work in military factories or help female farmers whose husbands were drafted for the war. This meant that most classes in schools were cancelled. After the war, schools were opened, but there was not much enthusiasm for learning. Furthermore, General Douglas McArthur, who was the Supreme Commander of the Occupation of Japan, introduced a series of reforms for the educational system in Japan.

Despite this unusual war-time and social turmoil, we received a solid and standard education at least in elementary school. At this time I was a normal boy and had no intention to become a scientist. I was the only son in my family, and my father wanted me to become a farmer to succeed his business. For this reason, he never encouraged me to study hard in school. Yet, I excelled in most study subjects and received a certificate of excellence at the end of each school year.

I have been a man of curiosity from the time when I was young. Particularly, I wanted to know how various machines worked properly. I remember that one day I decomposed a large wall clock to find out how it worked but I could not reassemble it. I also sliced the tip of my forefinger in the process of figuring out the mechanism of a rope-making machine in our barn. The scar made on my finger at the time remained unhealed for three or four years. However, a more serious incident occurred when I was decomposing the ignition equipment for a war-time bomb. I had collected several ignition equipments that were scattered in various parts of our fields. To see the inside of the ignition, I tried to open it with a pair of pliers. The ignition then exploded. It was not a big explosion but serious enough to damage

the pupil of my left eye. My father was very upset and immediately took me to an eye doctor in the city of Miyazaki. The doctor's prognosis was that my eye would heal if I stayed in his clinic for about a month. So, I followed his advice, but the damage never healed, and my left eye is still blind.

This incidence occurred when I was fifteen years old and changed my life in various ways. First, I lost my sense of measuring spatial distance, so that I could no longer play ball games with my friends. Second, this generated some kind of inferiority complex for me and made me less sociable, though most people did not notice my eye injury. However, this incidence also had a positive effect on my life. While I was in the clinic, I started to read various books, which were just about being published after the war. For this purpose, the loss of my left eye sight did not matter. I also read several textbooks, which were used in the pre-war secondary schools. Fortunately, I had one cousin who went to a pre-war high school and a university, and he had kept all the textbooks he used. Looking through these books, I came to realize how ignorant I was about mathematics, physics, chemistry, etc. I was particularly poor in English, because English was the language of our enemy and was not taught during the war time. I then decided to learn these subjects by myself by reading books. If I had followed the advice of my parents to become a farmer, this would not have been necessary. However, my curiosity to know various things had an overriding power.

I then worked hard to learn the subjects I missed during the war, even reducing my sleeping hours. This was the hardest time in my entire life, but this hard work eventually paid off. When I graduated from our high school, I was the top student in my class. When I took the aptitude test similar to the American ACT test for college entrance, I was one of the few highest ranking students in the entire Miyazaki Prefecture. Because of these achievements, two teachers of the school visited my home to persuade my father to let me go to college. He then approved it on the condition that I should study agriculture in the University of Miyazaki so that I could commute from home. My habit of learning various things by myself developed during this period, and it has not changed for my entire life. I have also developed my tendency not to trust what I hear unless I confirm it by reading books or original papers.

2. Student Life in Universities

The University of Miyazaki was a new university established in 1949 and operated by the new university system established by the McArthur regime. We had to take a liberal arts education in the first two years. This system was good for me, because I was interested in

basic sciences such as mathematics, physics, and chemistry. In high school, I liked mathematics and physics. In college we learned an elementary quantum chemistry, which I found fascinating. Another class that excited me was a biology course, in which we learned Thomas Morgan's chromosome theory of genetics. Conducting literature search, I then came to know that there is a field called population genetics, in which mathematics is used extensively. I then started to read the papers written by population geneticists S. Wright, J. B. S. Haldane, and R. A. Fisher in the library. For this reason, I decided to study genetics for my B. S. degree. However, our genetics professor was a cytogeneticist and was not interested in population genetics (Fig. 3). Nevertheless, I managed to write a paper on theoretical population genetics in English and publish it in a Japanese journal in 1953.

At that time there were very few geneticists who were acquainted with population genetics theory in Japan. I therefore had to study the subject by myself by reading original papers. (Actually, Motoo Kimura at the National Institute of Genetics was actively studying this subject, but I did not know it.) Population genetics is for studying the genetic change of populations by examining gene frequency changes, and therefore it was considered to be useful for understanding evolution, animal and plant breeding, and genetic diseases. Because I was in the college of agriculture, I wanted to make population genetics more useful for plant breeding.

Partly for this reason, I went to the graduate school of Kyoto University, which was a prestigious university in Japan and had good research facilities. In this university too, however, there were no professors who specialized in population genetics, but one professor (Katsumi Syakudo) was a Mendelian geneticist and was analyzing the genetic control of quantitative characters. There was also a famous professor named Hitoshi Kihara, who initiated a classical genome analysis with wheat species. He also had produced seedless watermelon by using the property of triploidy. In this university as well as in the University of Miyazaki, I had a full-time scholarship, so I did not have to worry about financial problems.

3. Population Genetics of Quantitative Characters

As mentioned above, Professor Syakudo was working on quantitative genetics, but his methodology was old. I then wanted to develop a better method. Around this time, Kenneth Mather in England published a book called "Biometrical Genetics." In this book a new way of analyzing the genetic basis of quantitative characters was presented. In this method one could decompose the total variance of a quantitative character into the additive genetic

variance (V_A), dominance variance (V_D), and environmental variance (V_E). I soon extended this method for the analysis of covariance between different characters. I therefore decided to estimate the variance and covariance components of several characters of rice plants by doing experiments for three or four generations. Because rice was an annual plant, this experiment required three or four years of work with the help from a few research assistants. In this experiment, I could estimate the components of genetic variances and covariances for characters such as plant height, flowering time, etc., but the agreement between the theoretical expectations and experimental results was not particularly high. I concluded that these results were due to environmental changes in different years. During this period, I also developed a statistical method for testing heritability of quantitative characters. I therefore combined these results and wrote a Ph.D. thesis (for Doctor of Agriculture), and this thesis was approved by the university in 1959. Just before I received my Ph.D. degree, I was appointed as an assistant professor of the Laboratory of Plant Breeding in Kyoto University.

In this process I learned that the genetic analysis of quantitative characters in wild plant and animal populations is very difficult and that to understand the evolutionary changes of populations we must study the genes controlling the characters under investigation. This experience was important for me to develop meaningful statistical methods for the study of evolution in later times.

4. Visit to US Universities

After I finished my Ph.D. thesis, I obtained a Rockefeller Foundation Fellowship to visit various universities in the United States, partly because of the help of Professor Motoo Kimura, with whom I was already acquainted (Fig. 4). This fellowship allowed me to choose a major university of my choice and to do research. However, they also gave me travel funds to visit various research institutions. I first chose the University of California at Davis as my home university, because Professor Robert Allard there was active in applying population genetics for plant breeding. I therefore stayed at Davis for about 6 months from June, 1960. However, staying at Davis, I found that Allard was doing essentially the same thing as what I did in Kyoto University. I therefore decided to change my home university to North Carolina State University at Raleigh, where theoretical population genetics with multiple genetic loci was being studied actively. The fact that my friend, Ken-ichi Kojima (Fig. 5), at the time of Kyoto University was an associate professor there, was also attractive.

However, my stay at Davis was not a waste of time, because I could learn English there.

Before visiting the United States, I could write English papers relatively easily, and therefore I had thought that I could live there without much trouble. Reality was very different, and I quickly discovered that writing, speaking, and hearing English were very different. Because my English pronunciation was very poor and hearing ability was bad, I could not make reasonable conversation with Americans when I arrived at Davis. This was a big disappointment for me, and I had to study English conversation seriously. It took six months for me to learn basic English conversation. However, because I knew theoretical population genetics more than most American geneticists, I could have meaningful discussion on this subject. I therefore visited several universities in the mid west (Wisconsin, Minnesota, and Iowa) and saw famous population geneticists like Sewall Wright, James Crow, and J. L. Lush in the winter of 1960. The most impressive person I met on this trip was Sewall Wright, whom I found a sincere and very knowledgeable person.

In January, 1961, I moved to Raleigh from Davis. The North Carolina State University had several theoretical population geneticists including Ken-ichi Kojima and Clark Cockerham with whom I could interact. Here I could write one paper on the effect of linkage intensity and gene interaction on the efficiency of artificial selection. In the summer of 1961 an international symposium on “Statistical Genetics and Plant Breeding” was held in the North Carolina State University, and many famous geneticists attended the symposium from around the world. This gave me a good opportunity to see many of them. Furthermore, I could present my own paper in the symposium. In this symposium, I was impressed with the attendant’s effort to understand the genetic nature of quantitative characters using various statistical methods.

5. Transition from Applied Science to Basic Science

At the end of September, 1961, I returned to Kyoto University. My experience of doing research in the United States changed my view considerably. In the U.S. there was a frontier spirit, and scientists were trying to do something new. The communication of new ideas among scientists was also very good, and there were little barriers among different departments of the same or different universities. It was not uncommon that in departments of applied science some people were working on basic science problems. For example, the department of plant breeding at Cornell University produced two Nobel laureates (George Beadle and Barbara McLintock) in Physiology and Medicine. In Japanese Universities feudalism and job security were still quite important.

In the 1960s, the molecular study of evolution was initiated in some American

universities, and interesting results were being published. After I came back from the United States, my interest shifted toward the basic science of evolutionary biology from the applied study of plant breeding. In 1962, I changed my job from Kyoto University to the newly established National Institute of Radiological Sciences. This Institute had a department of genetics, of which the mission was to understand the molecular basis of mutation and study the spreading of mutant genes in the population. However, as long as we could contribute to the progress of basic science, we were allowed to study various problems of genetics and population genetics. In 1963 I married my wife, Nobuko Hara,(Fig. 6) and then we have been living together for 50 years. In 1964 our son, Keitaro Nei, was born (Fig. 7), and in 1967 our daughter, Maromi Nei, was born. Having this family gave me a joy of life (Fig. 8).

Around 1965 the population genetic theory of interacting genes was poorly understood, and I therefore started to study this subject and various other problems in the new Institute. In 1967, I discovered that any pair of genes with epistatic effects (gene interaction) tends to be tightly linked on the same chromosome or the tightly linked genes tend to be maintained in the evolutionary process. Initially there was not much data that could be used for testing the validity of this finding, although I published one paper supporting my idea in Nature. In the 1980s and 1990s, however, the study of gene arrangement in the genome showed that most of the genes which interact strongly with one another were closely linked. For example, HOX genes that control the formation of the animal body pattern are closely linked except in a few animal species. Similarly, the major histocompatibility complex (MHC) genes are almost always tightly linked. By contrast, loosely interacting genes such as olfactory receptor genes are scattered all over the genome.

In the 1960s the DNA content in the genome was studied in various organisms, and it was shown that DNA content is generally higher in complex organisms like mammals than in simple organisms like insects. This result suggested that the DNA content has increased by gene duplication, but it was not clear whether complex organisms really have more duplicate genes than simple organisms, because duplicate genes can be melted down by mutations. I therefore studied this problem by considering the rate of amino acid substitution and the rate of gene duplication. My results suggested that the genome of complex organisms such as mammals contains a large number of duplicate genes but a large fraction of duplicate genes were nonfunctional (pseudogenes). These results were published in Nature in 1969. When the genome sequences of several organisms were deciphered in the early 2000s, my prediction was again shown to be right.

6. Brown University, University of Texas at Houston, and Pennsylvania State

University

While doing this type of work, I realized that the molecular study of evolution would be essential in the future and that to do the molecular study I should move to the United States where the study for acquiring new molecular data was most active. Fortunately, Brown University at Providence, Rhode Island, offered me a job of associate professor with tenure in 1968. I therefore moved to Brown University in February, 1969. In this university I was quite successful in getting research grants and starting my own projects, and in 1971, I was promoted to a full professor. In 1971, however, I had another job offer from Professor Jack Schull of the University of Texas Health Science Center at Houston. This job was quite attractive to me, because the teaching requirement was light and I could concentrate on my research. In the summer of 1972, I moved to Houston.

In this university, Jack Schull was appointed as Director of the newly established Center for Demographic and Population Genetics, and the Center was composed of a section for studying medical genetics and a section for studying theoretical population genetics. Jack was in charge of establishing the first section, and I was responsible for developing the second group. Because the university provided good financial support, we could develop a reputable Center relatively quickly. At that time there were many new evolutionary problems because new types of molecular data were being published. I had two junior theoretical population geneticists, Wen-Hsing Li and Ranajit Chakraborty, and many graduate students and postdoctoral fellows. By 1985, our Center had become one of the most productive research centers of molecular population genetics in the world. I have summarized many of our achievements in my 1987 book “MolecuIar Evolutionary Genetics.” In 1983 I also established a new journal “Molecular Biology and Evolution” in collaboration with Walter Fitch.

In 1989, however, Professor Robert Selander at Pennsylvania State University asked me to move to his university as Director of the Institute of Molecular Evolutionary Genetics, which would be newly created. After several months of deliberation, I decided to accept the new challenging job and moved to Pennsylvania State University in 1990. At this university, there were a group of molecular evolutionists, and their graduate students and postdoctoral fellows were working actively. In this university I studied mainly statistical methods that are useful for analyzing data on molecular phylogenetics and phenotypic evolution. Our institute was quickly recognized as one of the research centers in the field.

My researches conducted in the three U.S. universities were continuous and interconnected. I therefore would like to present a brief summary of my studies on each

research topic in the following. During this period I was interested primarily in statistical methods that are realistic and useful for data analysis in the study of evolution at the molecular level.

7. Molecular Evolution

(a) Genetic distance between different populations

Around 1970 many investigators were studying the genetic variation within and between populations by using protein electrophoresis, and they were interested in using these data for studying the extent of genetic differences between populations. However, there was no proper statistical method for measuring the number of nucleotide or codon differences between populations. Electrophoresis is a crude method of detecting codon (amino acid) differences, and one day in 1969 I suddenly realized that the number of codon differences can be estimated if we use the theory of the Poisson process of amino acid substitution in molecular evolution. I then developed a statistical method of measuring the genetic distance that is proportional to the number of codon differences per locus. Therefore, if the rate of codon substitution is known, we can estimate the time of divergence between populations from the genetic distance value. Of course, for this purpose we have to use a large number of protein loci. Yet, this method provided a theoretical basis of the construction of phylogenetic trees of populations. The papers in which this theory was published and improved have been cited over ten thousand times. Later I extended this method to be applicable to DNA sequence data, and this work has also been cited by a large number of investigators.

(b) Protein polymorphism and neutral theory

In the 1960s and the 1970s there was a great controversy over the mechanism of protein evolution and polymorphism. This controversy started when Motoo Kimura, Jack King, and Tom Jukes proposed in the late 1960s that the amino acid substitution of proteins occurs mostly by random fixation of neutral mutations. The presentation of this neutral theory irritated many established evolutionists such as George Simpson and Ernst Mayr, who were panselectionists and believed that evolution occurred almost always by natural selection. Many population geneticists at that time were studying morphological characters and believed that evolution cannot occur without natural selection. However, because I had read the papers describing properties of amino acid substitution in protein by Freese, Yoshida, Zuckerkandl, and Margoliash, I did not have any problem in accepting the neutral theory.

Therefore, I joined the neutralist camp and made some efforts to generate empirical evidence to support the theory.

To test the hypothesis of neutral evolution, however, we had to develop various statistical methods for analyzing data on protein polymorphisms. We first worked out the theoretical expectations of the distribution of single locus heterozygosity and compared the expected distributions with the observed ones obtained from many different organisms. The results from these studies showed that the expected and observed distributions agree quite well with each other though the statistical power was not very high (Fig. 9). Of course, this study was not sufficient to convince neo-Darwinians about the neutral theory, and the controversy is still continuing, as discussed in my 2013 book.

(c) Synonymous and nonsynonymous nucleotide substitution

Synonymous nucleotide substitutions in a gene do not change amino acids of the protein produced, and therefore they are considered to be more or less neutral. By contrast, nonsynonymous substitutions change amino acids so that they may change the relative fitness of the protein produced. Considering this situation, Takashi Gojobori and I invented a simple method for computing the number of synonymous (d_S) substitutions and the number of nonsynonymous substitutions (d_N) per nucleotide site and suggested that if w ($=d_N/d_S$) is significantly higher than 1, positive Darwinian selection may be operating in the gene. By contrast, $w = 1$ and $w < 1$ may represent the cases of neutral and negative selection, respectively. I also invented a statistical test of the difference between d_N and d_S. These methods are widely used for detecting positive selection at the present time. In fact, positive selection for major histocompatibility genes was discovered by this method, as will be mentioned below. However, I showed that this method should not be used for detecting selection at a single codon site.

8. Human Evolution

Humans are a highly heterogeneous group of individuals, and there are many different populations which are more or less isolated. For some time, human geneticists have been interested in knowing how these heterogeneous populations were formed. Luca Cavalli-Sforza and Anthony Edwards conducted an evolutionary study of human populations by using the allele frequencies for 5 blood group loci and reached the conclusion that modern humans originated somewhere near Afghanistan and spread through the world. I was

skeptical of this conclusion, because the number of genetic loci used was very small. I then started to collect allele frequency data for both protein and blood group loci. In 1974 we could use 35 protein loci and 21 blood group loci for computing genetic distances between Europeans, Orientals, and Africans.

This study suggested that the divergence time between Europeans and Orientals is about 55,000 years ago and Africans and Europeans or Asians diverged about 115,000 years ago (Fig. 10). Therefore, this dataset suggested that Africans were the first group of humans separated from others. This study was re-examined by using different genetic loci and many other populations, but the estimate of time of divergence between Africans and non-Africans has not changed very much. Furthermore, the fossil record of modern humans, which was about 200,000 years old, was later discovered in Africa. These data as well as Cann, Stoneking, and Wilson's (1987) mitochondrial gene data which supported Nei and Roychoudhury's results, later formed the out-of-Africa theory of human origins, which is now generally accepted. In addition, we later compiled data on microsatellite DNA loci and inferred the expansion of human populations on the world basis (Fig. 11).

9. Molecular Phylogenetics

(a) Neighbor joining method

When I presented my first phylogenetic tree for Drosophila species in 1971, the branch lengths of the tree were estimated by using a method called the unweighted pair-group method using arithmetic averages (UPGM). This method is based on the assumption that the rate of codon substitution is the same for all the species used. This type of method was often called phonetics. Around this time, this method was increasingly criticized by investigators who advocated the cladistics, in which the rate of codon substitution was allowed to vary with species and the branch length was estimated by using the parsimony method. The assumption of constant rate of codon substitution was certainly incorrect when a relatively small number of genetic loci were used. I therefore wanted to develop a method that would take care of the rate variation but without using parsimony methods. In collaboration with my student Yoshio Tateno, I first tried to modify Farris's distance-Wagner method by using the average pair-wise distances, but our computer simulation suggested that it does not work well.

I then started thinking of a method in which Fitch's least-squares method and the minimum evolution method are combined. At that time, Felsenstein (1985) published a paper on the bootstrap test of the reliability of a phylogenetic tree, of which the null hypothesis is a

star tree. Naruya Saitou and I then came up to the idea of constructing a phylogenetic tree by decomposing a star tree step by step. This method of tree construction is now called the neighbor joining method, and the tree constructed satisfies both the least-squares criterion and the minimum evolution criterion at each step of tree construction. However, this method was immediately criticized by the cladist group, who argued that this method is just an algorithm and there is no optimization process involved. Andrey Rzhetsky, Kei Takahashi, Sudhir Kumar, and I then examined various statistical properties of this method and provided a statistical justification.

This method produces phylogenetic trees very fast, and the trees generated are almost always biologically reasonable (Fig. 12). Therefore, it has now become the most frequently used method for constructing trees, and the original paper has been cited more than 34,000 times by now. However, this method still has some deficiencies, and we are trying to improve it.

(b) MEGA: Molecular Evolutionary Genetics Analysis

Around 1992, there were two major computer program packages for constructing phylogenetic trees. One was "PHYLIP" developed by J. Felsenstein, and the other was "PAUP" by D. L. Swofford. However, these packages included only traditional methods and did not include many new statistical methods. Furthermore, they were not user-friendly, and most users had to spend a considerable amount of time to understand the computational procedure. They were also only for constructing trees and did not include other statistical methods for studying molecular evolution. One day in 1992, Sudhir Kumar, Koichiro Tamura, and I had a conference about creating a new computer program package and decided to include many new computational methods developed in our laboratory as well as in others. In the production of this package, I took the responsibility for choosing the methods to be included and prepared the manual. However, the entire set of computer programs was written by Sudhir Kumar and Koichiro Tamura. The final product of MEGA was released in August, 1993 (Fig. 13). Although we did not officially advertise this program, it soon became popular, and the original package has been cited more than 3,700 times. MEGA has been updated several times, and currently MEGA5 is generally used. It is now the most frequently used software for constructing Phylogenetic trees.

10. Molecular Basis of Phenotypic Evolution

(a) Birth-and-death evolution

In the 1980s I became interested in the study of the molecular basis of phenotypic characters. Our approach was to investigate the evolutionary changes of genes controlling a specific phenotypic character rather than the evolutionary change of phenotypic characters themselves. The first group of genes we studied was the immunoglobulin genes that control the mammalian immunity against viruses, bacteria, and other parasites. Immunologists had already clarified the basic molecular structure of immunoglobulin genes (Ig), which are composed of various types of polypeptides, but their evolutionary change was not known. We first examined the evolutionary change of variable region genes of the Ig gene by comparing the human and mouse genomes. We soon discovered that the variable region genes are frequently subject to gene duplication and gene silencing (pseudonization) and that this gene family contains many pseudogenes. This result was against the then popular view that all multigene families are subject to concerted evolution and each gene family evolves as a group. Our results implied that each component gene is not an indispensable element but that many duplicate genes play similar functions and therefore some genes can be nonfunctional. Later we studied the evolutionary changes of many multigene families including such highly conserved genes as histone and ubiquitin genes, but almost all of them showed the birth-and-death evolution. This finding indicates that mutation (including gene duplication and gene death) plays an important role.

(b) MHC loci and positive Darwinian selection

However, there are some genetic loci which are subject to classical overdominant selection. A good example is a group of genes called the major histocompatibility complex (MHC) genes. It was known for many years that MHC loci are highly polymorphic, but the reason for this polymorphism was unclear and various hypotheses had been proposed. In 1988, Austin Hughes and I then suspected that the polymorphism is maintained by overdominant selection because two alleles at a locus in an individual cope with different sets of foreign antigens, and heterozygotes are expected to have a higher fitness. In fact, there are some experimental data to support this hypothesis. We then showed that the d_N/d_S ratio was significantly higher than 1 in most MHC loci. After we published these results with humans and mice, a large number of authors studied the same problem with many other vertebrate species and obtained a similar result. Therefore, MHC polymorphism is apparently maintained by overdominant selection. There are several other genes which are apparently under overdominant selection in vertebrates, and most of them are immunity-

related genes.

(c) Evolution of chemosensory receptors

In animals chemosensory receptors are used to find food, detect mates and offspring, recognize territories, and avoid danger. Animal genomes contain a large number of genes that allow these species to distinguish between millions of different odor and taste chemicals. Recent genomic studies have shown that the number of chemoreceptor genes is very large and vary enormously among different species and that each species contains a surprisingly large number of pseudogenes. To understand the mechanism of evolution of these unusual groups of genes, I conducted extensive statistical studies of olfactory (smelling element) receptors in collaboration with Yoshihito Niimura and Masafumi Nozawa. In this study we first identified the genomic location of all functional genes and pseudogenes in the genomes of several representative vertebrate organisms.

The results we obtained were quite interesting. First, we discovered that in the human genome there are about 400 functional genes and 414 pseudogenes and they are scattered on all chromosomes except for chromosome 22 and the Y chromosome (Table 1). We then found that the cow genome has even more genes, 1,152 functional genes and 977 pseudogenes. Further studies indicated that land vertebrates generally have a larger number of genes than marine vertebrates but the number varies greatly among species. By contrast, a number of marine vertebrates such as zebrafish and lampreys have much smaller numbers of genes. This result suggests that a larger number of olfactory genes in land vertebrates than in marine vertebrates occurred probably because land vertebrates cope with air-born odorants as well as for watery odorants.

11. Theory of Mutation-Driven Evolution

At the present time most evolutionists believe in the neo-Darwinian theory of evolution, in which mutation is regarded to provide raw material for evolution but the real evolutionary change of phenotypic characters is caused by natural selection, which is initiated by environmental changes. This view was generated partly because most genic mutations observed around the 1920s were deleterious and did not appear to be useful for evolution. During the last 40 years, it has been shown that the evolutionary change of proteins occurs mainly by fixation of neutral mutations. However, phenotypic evolution is still believed to be caused by natural selection. In recent years the study of genomic sequences has shown that

genetic variation is caused by various genomic changes (gene duplication, segmental duplication, genome duplication, horizontal gene transfer, transposon, epigenetics) in addition to genic mutation. There are also many different ways of controlling gene expression, and phenotypic evolution occurs by changes of a large number of genes that interact with one another. During the past 40 years, I have been involved in the study of evolution of duplicate genes and multigene families as well as the evolution of phenotypic characters, as mentioned above. It is now clear that gene duplication causing copy number variation as well as the large number of gene families plays important roles in phenotypic evolution probably more than genic mutation. For these reasons I proposed that mutation is the driving force of evolution even in phenotypic characters and natural selection is of secondary importance. Recently, I have conducted an extensive literature survey and showed that as long as molecular data are available this new theory is generally applicable (Nei, Mutation-Driven Evolution, Oxford University Press, 2013). However, the applicability of this theory to the evolution of complex characters such as altruism and brain structure should be examined more carefully.

12. Future Studies

There are millions of different species inhabiting our planet, and they are classified into different kingdoms, phyla, classes, families, genera, and species. The morphological and physiological diversity of these organisms is enormous. How did this diversity evolve from a single proto-organism? Our answer to this question is quite limited at present. We do not know why and how animals and plants have evolved differently. Even the mechanism of differentiation of humans and chimpanzees is mysterious. In the future these questions will undoubtedly be studied at the molecular level. In these studies it would be very important to set up a hypothesis of mechanistic evolution and test the hypothesis by using all available data. In the past evolutionary biology was full of imaginative arguments, and it was often unclear whether we can accept the arguments or not. Availability of the recent molecular technique has transformed evolutionary biology into a scientific discipline with hypothesis testing. However, this type of research has just begun, and it will take enormous amounts of efforts and time to answer our questions. If one wants to develop a mathematical model of evolution, it is important to examine whether the assumptions made are realistic from the biological point of view.

In these studies it is important to doubt any scientific dogma and critically examine the dogma in a scientific manner. Because evolutionary biology is so diverse and complicated, a

single explanation or theory may not be able to explain all different evolutionary phenomena. We may have to consider different mechanisms for explaining different phenomena. However, the future of evolutionary biology is bright. If we use the molecular techniques developed in association with genomics and developmental biology, we will be able to solve various issues of evolution at least in a crude fashion.

With my father, ca. 1934　父と（1934年頃）

Fig. 1

Elementary school class photo, 2nd grade. (4th row, leftmost). At war time, no shoes were allowed for children except in winter.
小学2年生の時のクラス写真（4列目左端） 戦時中で冬期を除き生徒は裸足。

Fig. 2

Prof. Katayama and his lab, Miyazaki University, 1953 (leftmost).
宮崎大学の片山研究室にて（左端）(1953年)

Fig. 3

With Prof. M. Kimura (left) in Mishima, Japan, 1986
木村資生先生（左）と 静岡県三島市にて （1986年）

Fig. 4

Ken-ichi Kojima (left) and Therese Kelleher at North Carolina State University
小島健一君（左）とテレーズ・ケラハー ノースカロライナ州立大学にて

Fig. 5

Marriage ceremony was held at the shrine, Miyazaki Jingu, Miyazaki, 1963
結婚式。宮崎神宮にて（1963年）

Fig. 6

My Mother carrying my son, ca. 1975
母に抱かれる長男・啓太郎（1975年頃）
Fig. 7

Family trip to San Francisco, 1980
家族旅行。サンフランシスコにて（1980年）
Fig. 8

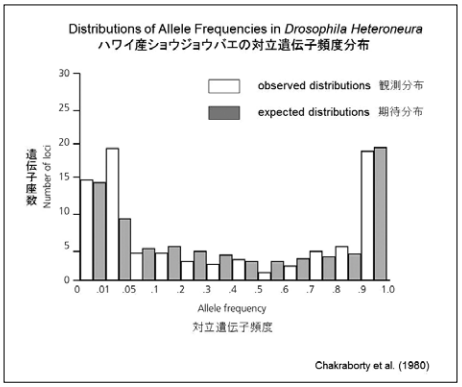


Fig. 9

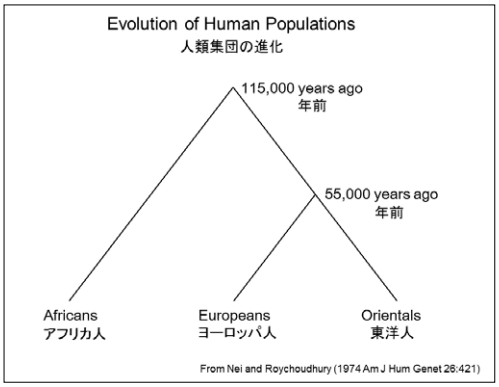


Fig. 10

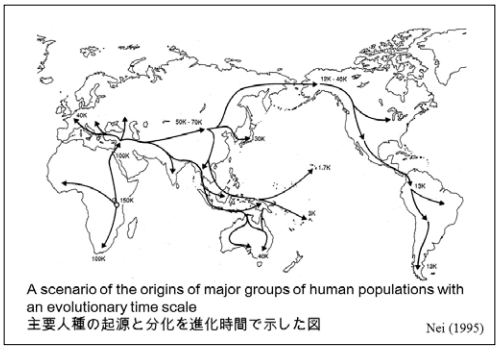


Fig. 11

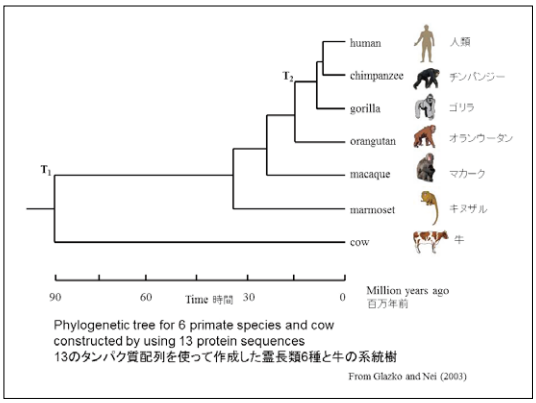
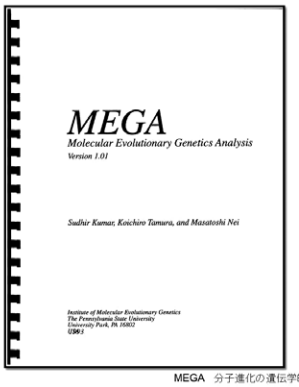


Fig. 12



MEGA　分子進化の遺伝学的解析用ソフト

Fig. 13

Numbers of functional genes and pseudogenes in the multigene families for chemosensory receptors and immunoglobulins.
化学感覚受容体と免疫グロブリンの多重遺伝子族内の機能遺伝子と偽遺伝子の数

	Olfactory	Pheromone	Taste	Immunoglobulin		
	OR	V1R	T2R	IgVH	IGVλ	IGVκ
Humans	388 (414)	5 (115)	25 (11)	46 (84)	33 (38)	34 (38)
Mouse	1063 (328)	187 (121)	35 (6)	89 (64)	3 (0)	80 (78)
Dog	822 (278)	8 (33)	16 (5)	81 (66)	43 (61)	16 (9)
Cow	1152 (977)	40 (45)	19 (15)	12 (5)	23 (9)	9 (13)
Opossum	1198 (294)	98 (30)	29 (5)	24 (7)	45 (27)	76 (48)
Platypus	348 (370)	270 (579)	5 (1)	43 (21)	14 (7)	9 (9)
Chicken	300 (133)	0 (0)	3 (0)	1 (48)	1 (24)	0 (0)
Xenopus	1024 (614)	21 (2)	52 (12)	38 (42)	8 (4)	45 (10)
Zebrafish	155 (21)	2 (0)	4 (0)	38 (9)	0 (0)	8 (5)
Lamprey	40 (27)	3 (7)	0 (0)	0 (0)	0 (0)	0 (0)
Amphioxus	34 (9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Nei, Niimura, and Nozawa (2008)

Table 1