HUMAN CHROMOSOMES

SAJIRO MAKINO

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To

the late Teacher Professors,

Dr. Kan OGUMA,

Dr. Yoshimaro TANAKA and

Dr. Taku KOMAI

who inspired, encouraged and sustained
my interest in chromosome cytology

and

in memory of

my father who passed away in my boyhood

Foreword

In a foreword to a book of this nature, it is customary to write about the importance of the book and the achievement of the author in his field. However in this foreword, I would like to give a brief historical survey of cytological studies on human chromosomes closely related to my own collaboration. It is because there are now few left of those early researchers who made pioneering contributions as early as 1920's and also because this may answer requests for my comments on recent findings in this field.

Under the influence of Dr. Oguma, I became interested in chromosomes of insects while I was studying at Hokkaido University which I attended from 1916 to 1920 as a student majoring in plant physiology.

From Dr. Oguma I learned about the tripartite sex-chromosomes of the mantis and the fact that even the correct number of human chromosomes was not known. It is Professor Kan Oguma under whom Dr. Makino, the author of this book, studied many years ago.

Interested in human chromosomes, I was always eager to obtain suitable material. Using a human ovary obtained from a hospital in Sapporo in 1920, I was able to observe nuclear divisions in *corpus luteum* fixed with Flemming's solution. But it was impossible to determine the number of chromosomes due to unsuccessful fixation.

My interest in this problem remained strong even though in 1920, I moved to the Biological Laboratory at Kyoto University.

A new opportunity came in 1921, when a surgical removal of healthy testicles was performed at the hospital of Kyoto University on a patient suffering from tuberculosis of the epididymis.

In those days Allen's modified Bouin's solution was widely used by many mammalian cytologists. However I used Carnoy's solution for pretreatment and then Flemming's solution for fixation. This fixing method was used for pollen mother cells of wheat which was my chief material for research. At once I proceeded to prepare microtome sections by the paraffin method and then stained with iron haematoxylin. This procedure was commonly used in chromosome studies.

What I observed under the microscope were mainly the chromosomes of spermatogonia. The chromosomes were clearly visible. They were arranged in a rosette form. Feeling confident that the material was suitable for microscopical investigations, I sent the whole fixed material to Professor Oguma in Sapporo. He felt also that the material was good enough and it was decided to write a joint paper. At once Dr. Oguma made his own slide preparations which he examined with great care. Our studies revealed that 2n is 47 and n is 23_{II} + XO.

The results were published in a preliminary paper written in Japanese with an English summary in Zoological Magazine, Tokyo, 34 in 1922. The conclusions of this paper were

in good agreement with those of DE WINIWARTER (1912, 1921), the Belgian authority on human chromosomes.

This work eventually led to correspondence between the two (DE WINIWARTER & OGUMA) and OGUMA went to DE WINIWARTER's Laboratory in 1923. While in Belgium he finished the manuscript for the full paper (OGUMA et KIHARA, 1923) in French, which was published in Archives de Biologie, 33.

The chromosomes of spermatogonia and spermatocytes were superbly drawn by OGUMA himself. In addition a number of fine photomicrographs, which were taken by myself, were included. While he was staying in DE WINIWARTER's Laboratory, he carried out active research on human chromosomes.

In the mean time, PAINTER (1921, 1923) and EVANS and SWEZY (1928, 1929) in the United States proposed 2n=48 (XX-XY) independently and gained many supporters. This theory is characterized by the presence of an XY-pair in the first metaphase in which Y is considerably smaller than X.

OGUMA's interpretation of the XY-pair can be summarized as follows: The X-chromosome has two constrictions similar to that of *Rattus* and other rodents. Accordingly it is composed of three segments, P, D_1 and D_2. It is the P-segment which was considered as a Y-chromosome in the first metaphase (OGUMA 1937). On the contrary, advocators of the XY theory believed that the small Y chromosome was overlooked in the equatorial plate of the spermatogonia.

Most people thought one of these two numbers (2n=47 and 2n=48) must be right. However, in 1956, TJIO and LEVAN startled the world with their new finding of 2n=46, which is now recognized as the correct number.

Prior to this finding, several investigators reported that some cells had 2n=46. For instance PAINTER (1921) could not count more than 46 in some of his most beautiful mitotic figures. In 1953, I was in Berkeley to give a lecture entitled "Problems in human chromosomes" when Dr. MAKINO showed me a fine photomicrograph of 46 human chromosomes which were individually numbered by him. This photomicrograph had been obtained from Dr. HSU who used tissue cultures of the spleen of a human male. HSU's count was 48. At that time we thought that the discrepancy was caused by end-to-end attachment and overlapping of chromosomes, thus making 2n=48 or 47 appear to be 46. In fact, however, HSU's specimen must have shown the correct number.

It is rather rare that the correct number of chromosomes turns out to be less than the erroneous count, as the case for human chromosomes has shown.

Since 1882 when FLEMMING observed cell divisions in human cornea, it took 74 years to determine the number of human chromosomes. The success finally achieved in 1956 and later years is due to the progress in tissue culture techniques and the squash method. The latter eliminated the problems inherent in microtome sections which may often have chromosomes at the nuclear plate missing or broken off.

Incidentally, I proposed in 1930 a formula for the types of sex-chromosomes in plants and animals. I shall devote some space in this foreword to this formula which has received little attention thus far.

Based on the old data, mostly from WILSON's classical book entitled "The Cell in

Development and Heredity" (1925), sex-chromosomes of various species were formulated as follows:

Table showing the types of sex-chromosomes

Species	Formulae of sex-chromosomes in the first division	Formulae of sex-chromosomes in the second division
(1) *Protenor belfragi*	$6_{II} + XO$	$6 + X, 6 + O$
(2) *Lygaeus turcicus*	$6_{II} + XY$	$6 + X, 6 + Y$
(3) *Tenodera superstitiosa*	$12_{II} + X_1YX_2$	$12 + X_1 + X_2, 12 + Y$
(4) *Thyanta calceata*	$12_{II} + X_1X_2Y$	$12 + X_1 + X_2, 12 + Y$
(5) *Rumex acetosa*	$6_{II} + Y_1XY_2$	$6 + X, 6 + Y_1 + Y_2$
(6) *Oncopeltus fasciatus*	$7_{II} + X + Y$	$7 + XY$

(H. KIHARA 1930)

The simplest examples are XO- and XY-types (Nos. 1 and 2 in the table). However the number of X- and Y-chromosomes is variable in many species. In the mantis (No.3) a sex-chromosome complex consisting of two X- and one Y-chromosomes is found. They are arranged in the order of X_1YX_2. Two X's go to one pole, while one Y in the middle goes to another pole. In *Thyanta* (No.4), also two X- and one Y-chromosomes are present. But the arrangement is different from that of the mantis. The formula is X_1X_2Y.

The sex-chromosomes of *Rumex acetosa* (a dioecious plant) represent the first case, where two Y-chromosomes are present (No.5). In this case, X is located in the middle and two Y-chromosomes are attached at the both end of X forming a tripartite complex, Y_1XY_2.

In the examples given above X- and Y-chromosomes are associated in the first meiotic mataphase and disjoin in the anaphase. They divide longitudinally during the second division.

The last example (No.6) is different from all the other cases. X and Y do not pair in the first metaphase. They divide longitudinally in the first anaphase. However the X- and Y-chromosomes are associated in the second metaphase and disjoin in the anaphase. Accordingly two types of sperms are produced, namely 7+X and 7+Y.

These examples are selected from organisms of male digamety whose formula for the female and the male is XX-XY. Some authors use same symbols (X and Y) for female digamety. In this case the order of the symbols is reversed, namely XY-XX. However it seems to me clearer to use Z and W instead of X and Y. So the formula for the female and the male becomes ZW-ZZ. This system has been adopted by silkworm geneticists.

Now I would like to add a few words about the author and his book. Dr. MAKINO was trained under Dr. OGUMA for many years as an animal cytologist. He published many distinguished papers and monographs on animal chromosomes. But he was not involved in research on human chromosomes until later. However after the discovery of the correct number of human chromosomes, which opened a new era in human genetics, he began his active work in this area.

No other discovery gave such a strong impetus to the studies of human genetics. This can be compared to the rediscovery of Mendelian laws in 1900. It is truly amazing that so many cytologists and geneticists started to study human chromosomes.

The accumulation of new knowledge has become enormous. The scope of chromosome studies widened greatly and the results of massive work gave new insights to teratology, criminology, pathology and other fields in less than 20 years. Thus, a comprehensive monograph like this is very much needed.

Dr. MAKINO has reviewed great many papers, about 1900, in this field and has written an excellent summary for this book. I think this book is very useful to all human geneticists and should be kept in libraries of medical schools as well as in biological institutions.

I pay high tribute to Dr. MAKINO for the completion of this book and congratulate him on his accomplishment.

Thanks are due to the author who has given me an opportunity to write a foreword to this monumental book.

May 28, 1974

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Member of Japan Academy
Member of National Academy of Sciences, U. S. A.

Foreword

It has been my privilege to know Professor Sajiro MAKINO for more than a decade, a period of time during which more advances were made in human cytogenetics than in all the years preceeding it. Professor MAKINO has devoted his scientific lifetime to the study of chromosomes in a multitude of animals and in man, his interest in human cytogenetics being responsible for much of the early contributions to our knowledge of the chromosome constitution of human cancer and leukemia and in a number of congenital and hereditary disorders. The impressive number and quality of his contributions to the literature of cytogenetics attest to his productivity and energy. To me an equally enviable accomplishment of Professor MAKINO has been the training of a succession of graduate students, some of whom have worked in my laboratory, who have become outstanding scientists and cytogeneticists in their own right and whose application of high standards in their scientific work undoubtedly reflects their years of exposure to Professor MAKINO's guidance.

It has been a pleasure for me to read parts of this work prior to its publication, and, except when absolutely necessary, I have refrained from applying rigorous and biased criteria for changing the English of the text, since I felt that retention of Professor MAKINO's beautifully descriptive language was more important than grammatic rigidity. The reader of this book will not only find a wealth of information about human cytogenetics, drawn from the work of Professor MAKINO and his many students and from the important contributions of Japanese cytogeneticists, but also an emphasis on the karyotypic findings in the Japanese population. This, to my knowledge, has not been described for any other national or geographic group and constitutes an important contribution to medical science. Professor MAKINO has collected, classified, and presented an enormous amount of information on human cytogenetics, painstakingly describing the background to the various conditions and studies and giving detailed information on every human condition in which chromosomal investigations have been performed. It takes a Herculean effort to accomplish this and, thus, reflects the ebullient energy of Professor MAKINO at an age when in most of us it is waning.

Over the years I've come to appreciate the qualities of Japan and its people, primarily through my association with a number of students from Professor MAKINO's laboratory* and from other laboratories in Japan, who have worked with me. My personal and professional contacts with Professor MAKINO, both in the U.S. and in Japan, have afforded me opportunities to appreciate his outstanding qualities as man and scientist.

September, 1974, Buffalo

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* The following scientists from Professor Makino's laboratory and the Chromosome Research Unit at Hokkaido University have worked with me: Takaaki ISHIHARA, Yasumoto KIKUCHI, Toshio SOFUNI, Kiyomi YAMADA, Hatao KATO, Nobuo TAKAGI, Tatsuro IKEUCHI, Takao AYA, Sei-ichi MATSUI, Yoshitaka OBARA, Isamu HAYATA, and Mitsuo OSHIMURA.

Foreword

Japan has a long tradition in the field of cytogenetics. Since the beginning of the century many Japanese scientists have been involved in the study of the hereditary mechanisms of the cell both in plant and animal species.

Among the pioneers in the plant field may be cited K. FUJII, H. KIHARA, H. MATSUURA, Y. SINOTO and B. WADA. In the study of animal cells a proeminent position is occupied by K. OGUMA and S. MAKINO.

MAKINO's first scientific contribution written in English dates from 1931. Until today he has had a vast scientific output which numbers 275 scientific papers.

Professor Dr. SAJIRO MAKINO may be with justice called the 'doyen' of animal cytogenetics in Japan. With his energy, insight, enthusiasm and generosity he created at the University of Hokkaido, at Sapporo, a congenial atmosphere of research that attracted many young scientists to his laboratory. He can boast of having more than 70 disciples who took their Ph. D. under his guidance.

MAKINO's contribution is very diversified. In his long career he has been able to renew himself using such different techniques as: living cell analysis, cinematography, autoradiography and spectrophotometric measurements of DNA. The range of species that he has investigated is equally impressive. His papers deal with chromosome studies in: insects, echinoderms, amphibians, fishes, reptiles, birds (such as the ostrich) and mammals (including the lion).

As early as 1932, in the days when animal cytology was a most unrewarding and primitive form of research he compiled the first list of chromosome numbers in vertebrates.

In the late '40s his work becomes more and more concentrated on mammals and he starts to be particularly interested in the chromosomes of tumor cells. The YOSHIDA sarcoma becomes the object of several studies and in 1951 he begins a series of papers entitled "Cytological Studies of tumors" which in 1971 reached the L contribution. His studies on cancer cover such diversified species as: the mouse, the rat, *Drosophila* (melanotic tumors), the dog and man.

Besides cancer he extensively studied many chromosome abnormalities in Man as well as the normal Japanese population. With an interest extending into ethnography MAKINO also analysed the chromosomes of the Ainu people. These are the original inhabitants of Japan now present in only a few hundreds in the northern island of Hokkaido.

If one asks what is the main contribution to cytogenetics of Sajiro MAKINO one may feel hesitant in face of a so vast body of cytological information. However, it seems to me to be fair to state that his most original work is the concept of stem line cells as progenitors of a neoplastic population (1952, 1957).

Having had an opportunity to look at the proofs of the manuscript of the book "Human

Chromosomes" during a recent lecture tour of Japan I must confess that the production of this volume reflects the vast knowledge in the field of human cytogenetics that MAKINO commands and is a product of his intellectual discipline and devotion to scientific endeavour.

It is a pleasure and a honour to have the opportunity to pay tribute to the *oeuvre* of S. MAKINO.

January 1st, 1975, Lund

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Preface

The foundation of this volume is formed by the outcome of my lectures delivered on random occasions during the past several years at many universities; and of special talks given before biological meetings and conferences, both domestic and international, at which I have attempted to outline the new and rapidly growing features in human cytogenetics. These were based largely on background information obtained, for the most part, by my colleagues and associates in collaboration with many clinicians working in various medical fields. It is during the last few decades that rapid and striking advances in cytogenetic methods, involving the use of tissue- and blood-cultures, colchicine and hypotonic solution pretreatment for the study of human chromosomes, have rendered possible their more precise and reliable analysis than afforded by older sectioning and staining procedures. Thus, this increase in technical competence has facilitated detailed reinvestigation of older knowledge, leading to a dramatic expansion of existing knowledge. Gross chromosomal abnormalities were, then, shown to be significantly implicated in the etiology of a variety of congenital disorders and developmental malformations in the human. Now, a series of important associations have been achieved between chromosomal changes and disease states. The causes of many human congenital anomalies, difficult to assess by surveys or statistical means alone, have been screened chromosomally in order to ascertain the relationship of the chromosome aberrations to the disease conditions. Critical information thus accumulated constituted a new approach to the etiological analysis of the causes of these disorders, and the data have proved of significance as criteria for the clinical diagnosis of a number of congenital syndromes and clinical anomalies. Beginning in 1959 and continuing to date, there is a bulk of information available in which cytogenetics has made appreciable contributions to the elucidation of the etiology and morphogenesis of normal and abnormal physical traits in human-beings, as well as to the analysis of the clinical consequences of chromosome abnormalities. Further, it has been shown that certain chromosome abnormalities are associated with abortion and infertility. Cytogenetics related to virology and radiology has also provided crucial information on the possible etiology of induced congenital and neoplastic disorders. At present, cytogenetic investigations of human subjects, from both clinical and pathological aspects, are often merely of scientific interest. But, just as often, they are of practical importance to clinicians as indispensable tools in the differential diagnosis of certain disorders, with the chromosomal findings serving as essential criteria. Cytogenetic progress has thus influenced many fields of clinical medicine and provided them with a considerable background of knowledge. Hence, the fruitful association of cytogenetics with clinical medicine has contributed to the rapid development of 'clinical cytogenetics', a new area of medical science.

Grown to large proportions, we have a good many important discoveries which have led to appreciation of a formerly unknown mechanisms in the causation of human diseases.

I would like to emphasize that it is indeed difficult to gather or even refer to *all* the cytogenetic features in man as gleaned from the vast literature on the subject. Furthermore, the ever-growing body of knowledge on the subject has rendered it difficult to make any generalizations regarding etiological problems in congenital and clinical diseases. The greater part of the contents of this volume covers the chromosome features of normal and affected states derived from the Japanese people, and which were collected through the enthusiastic efforts of my colleagues and coworkers with the extensive cooperation of many clinicians. In addition to clinical conditions, some chromosomal accounts, primarily derived from neoplastic, radiation-exposed and virus-infected subjects and conditions, are also presented in this volume. Cytogenetic approaches in these fields have become increasingly of value in basic research, both biological and medical, since gross chromosomal changes may be instrumental in the etiological analysis of many diseases. Chromosomal investigations are, thus, essential in both medical practice and in the biological implications in man.

My major aim in this work was to present on a large scale as possible original data obtained by my coworkers on a variety of subjects and fields, as well as those reported in the literature. I have thus endeavored to provide primarily a review of essential data from original papers, and some critical remarks were given where I considered them desirable. Any shortcomings in this book are more than compensated by recent *review articles* in which the ever-increasing knowledge on human cytogenetics has been catalogued and almost exclusively concerned with the relationship of chromosome abnormalities to problems of medical interest (Miller 1960; Harnden *et al.* 1960; Böök 1961; Ford 1961, 1962, 1964, 1966; Ferguson-Smith 1961; Bearn & German 1961; Miles 1961; Harnden & Jacobs 1961; Rappoport & Kaplan 1961; Sohval 1961; Hamerton 1961, 1969;, Lejeune & Turpin 1961; Robinson 1961; Hirschhorn & Cooper 1961; Hauschka 1961; Penrose 1961; Miller *et al.* 1961, 1972; Zellweger *et al.* 1961; Zellweger & Mikamo 1961; Hsu 1961; Fraccaro 1962; Carr 1962; Barr & Carr 1962; Hungerford 1962; Böök *et al.* 1963; Patau 1963, 1965; Chu 1964; Barr 1954, 1960, 1966; Turpin & Lejeune 1965; Sandbreg 1965, 1966, 1972; Sandberg & Hossfeld 1970, 1974; Atkin 1970; Sasaki & Makino 1967; Makino 1957, 1964, 1968, 1969, 1973, 1974; Levan 1956, 1969, 1973; Nichols 1969; Taylor 1969; German 1967, 1970; Mikkelsen 1971; Pearson 1972; *etc.*).

During the organization of the latter part of this volume, I was faced with the rapid and surprising developments of new staining methods for the study of human and other mammalian chromosomes involving quinacrine fluorescence and other banding techniques. The new methods have made possible the facile characterization and identification of individual chromosomes, which have led to precise morphological analysis of structurally altered chromosomes occurring in congenital and pathologic conditions. Current cytogenetic data provided by the new staining and banding techniques were compiled, as occasions arose, as far as possible.

It seems of significance to collect separately published research materials in a condensed form in one volume. Thus, cytogenetic data on normal as well as in pathological conditions derived from the Japanese population should be useful as a source or data book for succeeding studies and serve as a future clue for better understanding of the related features in Japanese and other races. I am hoping that, while it is not possible to refer to data from the whole world literature, this book represents a landmark, in that it summarizes much of the outstanding progress from past and current research activity

basic to some aspects of biological and medical problems. It is also intended, to encourage the interest in, or to complement the understanding of, the relationship and significance of chromosome abnormalities to certain congenital and clinical disorders, and as an approach to the still open question of the mechanism of cytogenetic disturbances related to clinical conditions. At the same time, on account of its theoretical and practical importance in human biology and medicine, this publication would be of some use as a working adjunct essential to students active in medical and biological researches, as well as to those who wish to acquaint themselves with the techniques and basic principle of animal cytogenetics, serving as a stimulus to speculation and further inquiry. Any suggestions or comments on this volume will be highly appreciated.

Since February 12th, 1971, having retired in 1970 from a professorship at Hokkaido University and being excited by academic activities of specialists around me, I have spent some four years in preparing this book without having any particulary form of assistance. Though, to my regret, I experienced considerable insecurity and difficulty in verbal expression in a foreign language, I have greatly enjoyed the survey of the vast literature contributed by modern human cytogeneticists, which has led to the review of many brilliant discoveries.. I do not want to abandon chromosomal research work in the future, and I shall continue to pursue those studies, since I have worked with them over a period of 45 years; even under the very troublesome conditions which occurred during and after World War II. It seems natural for me to become involved in chromosome cytology. To spend one's life in scientific research is a grand pilgrimage, and is especially rewarding because of the privileges of academic freedom and independence.

July 3rd 1974, Sapporo

SAJIRO MAKINO

Acknowledgments

In general, a book can not be written or conceived by a single author, because the author compiles the knowledge and experience of those who have provided the data before and during his time. In this respect, it should be said that any author is largely indebted to his predecessors and his colleagues, who have allowed information to be included in the book. It is my pleasant duty to express my cordial appreciation for the courtesy shown by all those with whom I have had the pleasure of engaging in research, or who have directly or indirectly aided me in preparing this volume. However, the list of these collaborators would be too long to quote here, and most of their names were listed with grateful acknowledgement in my former papers published in Cytologia, Vol. 29, 1964 (p. 14), Jap. J. Human Genet., Vol. 12, 1967 (p. 84), and Cytologia, Vol. 33, 1968 (p. 370). Here, I wish to take this opportunity to express my deep gratitude to my late teacher Professor Dr. Kan OGUMA, who was one of the initiators of chromosome cytology in Japan and had stimulated me to deal with animal cytogenetics, for his expert guidance and eminent supervision. It is a great pleasure for me to offer my sincere appreciation to the late Dr. Yoshimaro TANAKA and the late Dr. Taku KOMAI who had lent important advice and much constructive criticism, particularly in the genetic field, with almost continuous encouragement and thoughtful help. I owe a special debt of gratitude to Dr. Hitoshi KIHARA, who has kindly offered the professional Foreword, for many valuable suggestions, and for careful supervision given to me as a leading authority. I am much indebted to Dr. Avery A. SANDBERG, who read various parts of this manuscript and gave stimulating discussions and constructive comments and for his generous Foreword. I should also like to thank Dr. Antonio LIMA-DE-FARIA for his thoughtful Foreword and personal discussions we have had together. Further, my warm acknowledgements must be extended to the following colleagues and contemporaries for many an act of friendly assistance shown in the course of this work and for their generosity in supplying many items of valuable information and material for this book: Drs. Akio A. AWA; Takao AYA; Hiroko FUJITA; Makoto HIGURASHI; Takaaki ISHIHARA; Tatsuro IKEUCHI; Tadashi KAJII; Hatao KATO; Yasumoto KIKUCHI; Yoshikazu KUROKI; Seichi MATSUI; Kazuya MIKAMO; Junichi MURAMOTO; Yasuo NAKAGOME; Yuh H. NAKANISHI; Hidetsune OISHI; Hideo OKUMURA; Koso OHAMA; Yasushi OHNUKI; Masao S. SASAKI; Toshio SOFUNI; Nobuo TAKAGI; Akira TONOMURA; Kiyomi YAMADA; Satoshi YANAGISAWA; Michihiro YOSHIDA; and Toshihide, H. YOSIDA. A particular obligation is owed to Dr. Motomichi SASAKI and Dr. Tetsuji KADOTANI, who helped me in many friendly ways with close contact in the course of this work. Also, my sincere gratitude should be extended to Dr. Taro TAKEMI, the President of the Japan Medical Association; Dr. Minoru SHIROTA, the Director of the Yakult Institute for Microbiological Research; Dr. Norio KONDO, Professor of Plant and Animal Breeding, the Research Institute of Evolutionary Biology; and Dr. Kura KUBOTA, Professor of Anatomy, the Tokyo Woman's Medical College; for their generous aid in various ways and for particular interest in my work. Finally, cordial thanks are offered to the staff of the publisher, Igaku Shoin Ltd., for an act of good many cooperation in publishing this volume. Also, I would like to express my appreciation to the publishers who granted permission to quate or reproduce the material and figures in this volume.

SAJIRO MAKINO

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