

ISSN 0077-4995

NATIONAL INSTITUTE OF GENETICS JAPAN

ANNUAL REPORT

No. 36

1985

Published by
THE NATIONAL INSTITUTE OF GENETICS
Misima, Sizuoka-ken, Japan
1986

Annual Report
of the
National Institute of Genetics

No. 36, 1985



Published by
The National Institute of Genetics, Japan
1986

CONTENTS

General statement	1
Staff	6
Council and Advisory Committee	9
Association for propagation of the knowledge of genetics	10
Projects of research for 1985	12
Research activities in 1985	17
I. Molecular Genetics	
Promoter selectivity of <i>Escherichia coli</i> RNA polymerase: localization of a region of the β subunit involved in promoter selection. GLASS, R. E., JONES, S. T., NENE, V., NOMURA, T., FUJITA, N. and ISHIHAMA, A.	17
Promoter selectivity of <i>Escherichia coli</i> RNA polymerase: recognition of a heat shock promoter. FUJITA, N. and ISHIHAMA, A... ..	18
Promoter selectivity of <i>Escherichia coli</i> RNA polymerase: regulatory role of fMet-tRNA ^{Met} . NOMURA, T., FUJITA, N. and ISHIHAMA, A.	18
Promoter selectivity of <i>Escherichia coli</i> RNA polymerase: effect of promoter location. NOMURA, T., FUJITA, N. and ISHIHAMA, A.	19
Promoter selectivity of <i>Escherichia coli</i> RNA polymerase: effect of DNA supercoiling. FUJITA, N., OSATO, K., KANO, Y., IMAMOTO, F. and ISHIHAMA, A.	20
Promoter selectivity of <i>Escherichia coli</i> RNA polymerase: correlation between promoter strength and DNA structure. TACHIBANA, H. and ISHIHAMA, A.	20
Analysis of the gene for the stringent starvation protein of <i>Escherichia coli</i> . A novel transcriptional control during the stringent response. SERIZAWA, H. and FUKUDA, R.	21
Genetic mapping of the <i>Escherichia coli</i> stringent starvation protein gene. FUKUDA, R., NISHIMURA, A. and SERIZAWA, H.	22
Mechanisms of transcription initiation by influenza virus-associated	

RNA polymerase. HONDA, A., MIZUMOTO, K. and ISHIHAMA, A.	23
<i>In vitro</i> transcription and replication of influenza virus RNA. TAKEUCHI, K., NAGATA, K. and ISHIHAMA, A.	23
<i>In vitro</i> splicing of influenza viral mRNA. NAGATA, K. and ISHIHAMA, A.	24
Small RNA and CCA-enzyme like activity associated with influenza virus. KATO, A., UEDA, S. and ISHIHAMA, A.	24
Analysis of temperature-sensitive mutants of influenza virus. Effect of <i>ts</i> lesions on the segment 8. HATADA, E., FUKUDA, R., SHIMIZU, K. and HASEGAWA, M.	25
Function of nuclear factor I. NAGATA, K. and ISHIMI, Y.	26
Cloning of the 31B-D region of <i>Drosophila melanogaster</i> chromosome. INOUE, Y.	27
Codon usage and tRNA content in unicellular and multicellular organisms. IKEMURA, T.	28
Codon usage and chromosomal banding in human genome. IKEMURA, T. and AOTA, S.-I.	28
Codon usage, tRNA content, and rate of synonymous substitution. IKEMURA, T.	29
Organization of mitochondrial DNA from the male sterile cytoplasm of rice. NAWA, S., SANO, Y. and FUJII, T.	30

II. Microbial Genetics

Mutants of <i>Escherichia coli</i> defective in penicillin-insensitive murein DD-endopeptidase. IIDA, K., HIROTA, Y. and SCHWARZ, U.	32
Binding of penicillin to thiol-penicillin-binding proteins 3 of <i>Escherichia coli</i> : identification of its active site. HOUBAHERIN, N., HARA, H., INOUE, M. and HIROTA, Y.	32
Dispensability of either penicillin-binding protein-1a or -1b involved in the essential process for cell elongation in <i>Escherichia coli</i> . KATO, J.-I., SUZUKI, H. and HIROTA, Y.	33
Expression activities of <i>Bacillus subtilis</i> α -amylase secretion vectors. YAMANE, K.	33

III. Immunogenetics

A lethal gene-deletion in the mouse H-2 class III region associated

with meiotic recombination. SHIROISHI, T., SAGAI, T., NATSUUME-SAKAI, S. and MORIWAKI, K.	35
Effect of H-2 complex and non-H-2 background on urethan-induced chromosomal aberrations in mice. MIYASHITA, N. and MORIWAKI, K.	36
Effect of H-2 complex on the development of carcinogen-induced pulmonary adenoma in mice. MIYASHITA, N. and MORIWAKI, K.	37
Serological survey of alphaprotein-1 in wild mice. HARADA, Y., TOMITA, T. and MORIWAKI, K.	38
Characterization of Ly-2.1 ^w antigenicity newly found in Asian mice. KURIHARA, Y. and MORIWAKI, K.	39

IV. *Developmental and Somatic Cell Genetics*

Effects of hormones on growth pattern of human diploid cells in culture. KURODA, Y.	40
Antimutagenic activity of vitamin C on mutations induced by EMS in cultured Chinese hamster cells. KURODA, Y.	41
Combined effects of EMS and MMS on mutation in Chinese hamster cells in culture. KURODA, Y., KOJIMA, H. and KONISHI, H.	41
Enhancing effect of Ara-C on mutations in cultured Chinese hamster cells treated with EMS. KURODA, Y., SHINKAWA-TACHI, K., MORIMOTO, K. and KOIZUMI, A.	42
Freezing of <i>Drosophila</i> embryos after treatment with some chemicals. KURODA, Y. and TAKADA, Y.	43
Differentiation of embryonic cells of <i>Drosophila melanogaster</i> studied with the electron microscope. SHIMADA, Y. and KURODA, Y.	44
Brown-embryo lethals in <i>Drosophila melanogaster</i> . MINATO, K. ..	45
The role of the cephalic furrow during the embryonic development of <i>Drosophila melanogaster</i> . MINATO, K.	46
Genetical studies on the parthenogenesis in <i>Bombyx mori</i> : a selection of highly parthenogenetic lines. MURAKAMI, A., OHTSUKI, Y. and KITAZAWA, T.	46
Genetical studies on the parthenogenesis in <i>Bombyx mori</i> : thermal activation and its significance. MURAKAMI, A.	48

Sex and strain differences in lifespan of the adult silkmoth, <i>Bombyx mori</i> . MURAKAMI, A., KURODA, Y. and FUKASE, Y.	49
Genetical studies on the hereditary <i>mosaic (mo)</i> strain of <i>Bombyx mori</i> : function of polar-body. MURAKAMI, A. and KOBAYASHI, M.	50
Sex reversal by heterosexual parabiosis in hydra. SUGIYAMA, T. and SUGIMOTO, N.	52
Zygotic effect of the <i>daughterless</i> gene in <i>Drosophila melanogaster</i> . INOUE, Y.	52
Ovary freezing for the maintenance of silkworm genes. KUSUDA, J. and ONIMARU, K.	54

V. Cytogenetics

Karyological survey of wild mice in Ogasawara Chichi Island. KURIHARA, Y., SAKAIZUMI, M., TSUCHIYA, K., HARADA, Y. and MORIWAKI, K.	55
A genetic model for X-Y dissociation and testis weight in intersubspecies hybrids between <i>M. m. domesticus</i> (BALB/c) and <i>M. m. molossinus</i> . IMAI, H. T., LEE, W. H., TJAN, K. N. and MORIWAKI, K.	56
Chromosome observations of Sarawak ants. TJAN, K. N., IMAI, H. T., KUBOTA, M., BROWN, W. L. Jr., GOTWALD, W. H. Jr., YONG, H.-S. and LEH, C.	57
Modes of species differentiation and karyotype alteration in ants and mammals. IMAI, H. T.	58
The exceptionally low chromosome number $n=2$ in an Australian bulldog ant, <i>Myrmecia piliventris</i> Smith (Hymenoptera: Formicidae). IMAI, H. T. and TAYLOR, R. W.	59

VI. Mutagenesis and Radiation Genetics

Evidence suggesting high sensitivity of bone marrow cells to radiation-induced cell killing in the "wasted" mouse. TEZUKA, H., INOUE, T. and KADA, T.	62
The effect of age on the frequency of spontaneous and mutagen-induced micronuclei in mouse bone marrow. TEZUKA, H., TAMAI, K., MURAKAMI, K. and KADA, T.	62
Cancer susceptibility of the "wasted" mouse. INOUE, T., TAKAHASHI, J., OOTSUYAMA, A., TEZUKA, H. and KADA, T.	63

Effects of low-level tritiated water ingestion and age on the number of sister chromatid exchanges in mouse bone marrow cells. TEZUKA, H., BENZ, R. D., CARSTEN, A. L. and IKUSHIMA, T.	64
Radiation induced chromosome aberrations in the radiation sensitive mutants of <i>Caenorhabditis elegans</i> . SADAIE, Y. and SADAIE, T.	64
Mechanisms of bio-antimutagenesis. KADA, T. and INOUE, T. ...	65
Dietary desmutagens. KADA, T.	66
Mutagenic activities of EMS on somatic and recessive mutations in the soybean test system. FUJII, T. and TANO, S.	66
Origin and genetic background of three substrains in inbred PW mouse strain. TUTIKAWA, K., HARADA, Y. and MORIWAKI, K.	68

VII. Population Genetics

The role of compensatory neutral mutations in molecular evolution. KIMURA, M.	70
Variances and covariances of identity coefficients of a multigene family. OHTA, T.	71
A model of duplicative transposition and gene conversion for repetitive DNA families. OHTA, T.	71
Reciprocal altruism and reciprocal alliance between relatives. AOKI, K.	72
Gene diversity in finite populations. TAKAHATA, N.	72
Gene genealogy and variance of interpopulational nucleotide differences. TAKAHATA, N. and NEI, M.	73
The average frequency of private alleles in a partially isolated population. SLATKIN, M. and TAKAHATA, N.	73
Extranuclear differentiation and gene flow in the finite island model. TAKAHATA, N. and PALUMBI, S. R.	74
Introgression of extranuclear genomes in finite populations: nucleocytoplasmic incompatibility. TAKAHATA, N.	75
Genic homozygosity in populations which experience periodic bottlenecks. MARUYAMA, T. and FUERST, P. A.	76
Number of alleles in a small population that was formed by a recent bottleneck. MARUYAMA, T. and FUERST, P. A.	76

Dynamics of homozygous genetic loads in connection with the invasion of transposons. MUKAI, T.	77
---	----

VIII. Evolutionary Genetics

Homology between P-transposable element of <i>Drosophila melanogaster</i> and bacterial transposase gene of Tn3. SATTA, Y., GOJOBORI, T., MARUYAMA, T., SAIGO, K. and CHIGUSA, S.I. ...	79
Tn3 resolvase-like sequence in <i>P</i> transposable element of <i>Drosophila melanogaster</i> . SATTA, Y., GOJOBORI, T., MARUYAMA, T. and CHIGUSA, S. I.	79
Evolutionary features of oncogenes. GOJOBORI, T.	80
Rates of evolution of the retroviral oncogene of Moloney murine sarcoma virus and its cellular homologues. GOJOBORI, T. and YOKOYAMA, S.	80
Complete nucleotide sequence of an infectious clone of human T-cell leukemia virus type II: an open reading frame for the protease gene. SHIMOTOHNO, K., TAKAHASHI, Y., SHIMIZU, N., GOJOBORI, T., GOLDE, D. W., CHEN, I. S. Y., MIWA, M. and SUGIMURA, T.	81
DNA polymorphism of AIDS virus. GOJOBORI, T., YOKOYAMA, S. and MARUYAMA, T.	82
Homology of kringle structures in urokinase and tissue-type plasminogen activator: the phylogeny with the related serine proteases. TAKAHASHI, K., GOJOBORI, T. and NAORA, H. ...	82
Chromosome regions affecting hybrid sterilities of <i>Drosophila simulans</i> / <i>D. mauritiana</i> and <i>D. simulans</i> / <i>D. sechellia</i> males. KIMURA, K., LEE, W. H., WATANABE, T. K. and HATSUMI, M.	83
Notes on two interspecific hybrids of the <i>Drosophila melanogaster</i> subgroup: <i>simulans</i> / <i>teissieri</i> and <i>teissieri</i> / <i>mauritiana</i> . LEE, W. H. and WATANABE, T. K.	84
Cytogenetical and biochemical characters of wild mice collected from Kunming area. MORIWAKI, K., IMAI, H., KURIHARA, Y., YONEKAWA, H., QIN, C.-G. and SHI, L.-M.	85
Chromosome C-banding patterns of Korean wild mice. MORIWAKI, K., SUZUKI, K., LEE, W. H. and CHO, W. S.	87
The relationship between an isozyme locus <i>Pox-1</i> and life-history	

traits in wild rice. MORISHIMA, H. and SANO, R.	89
Computer studies on the molecular evolution. MIYATA, T.	90
Application of mitochondrial DNA restriction fragment length polymorphism to genetic monitoring of laboratory mice. YONEKAWA, H., KATOH, H., SAGAI, T. and MORIWAKI, K. ...	92

IX. *Human Genetics*

Mitochondrial DNA polymorphism in Japanese. HORAI, S. and MATSUNAGA, E.	97
Analysis of mitochondrial DNA polymorphisms in Japanese mon- keys. HORAI, S., MATSUNAGA, E., HAYASAKA, K., SHOTAKE, T. and NOZAWA, K.	98
Characterization of 2,7-anhydro- <i>N</i> -acetylneuraminic acid in human wet cerumen. SUZUKI, M., SUZUKI, A., YAMAKAWA, T. and MATSUNAGA, E.	99

X. *Applied Genetics*

The Indica-Japonica differentiation of rice cultivars in Thailand and its neighbouring countries. SATO, Y.-I., CHITRAKON, S. and MORISHIMA, H.	100
Variation in grain shape of the Indica and Japonica rice cultivars. SATO, Y.-I.	100
Distribution of genes for F_1 weakness in Asian native rice cultivars. SATO, Y.-I. and MORISHIMA, H.	101
Effect of a semi-dwarfness gene on morphological characters in rice with different genetic backgrounds and environmental condi- tions. SATO, Y.-I. and MORISHIMA, H.	102
Genic analysis of isozyme genes in rice. SANO, R., ISHIKAWA, R., BARBIER, P. and MORISHIMA, H.	102
Electrophoretic characterization of rice endosperms of Indica and Japonica with four kinds of protein fractions. ENDO, T. ..	103
Conservation methods of crop populations with mixed selfing and outcrossing. YONEZAWA, K. and OKA, H.-I.	104
Evolutionary significance of regulatory changes in the <i>Wx</i> gene expression of rice. SANO, Y.	106
Genetic control of alcohol dehydrogenase and estimation of some population parameters in <i>Hopea odorata</i> Roxb. (Dipterocarp-	

viii	ANNUAL REPORT OF NATIONAL INSTITUTE OF GENETICS NO. 36	
	aceae). IHARA, M., GADRINAB, L. U., SIREGAR, U. J. and IYAMA, S.	107
	Publications from Genetic Resources Section, Genetic Stocks Re- search Center. IYAMA, S.	108
	Data base design of DNA base sequences. MIYAZAWA, S.	108
	Publication	110
	Abstract of diary for 1985	119
	Foreign visitors in 1985	122
	Author index	125

GENERAL STATEMENT

This report gives an outline of research activities carried out in our institute during one year from January to December 1985. The total expenditure in the 1985 fiscal year amounted to 946 million yen, about 52% of which was personnel expenses. In addition, grants-in-aid in the amount of 140 million yen were rendered to selected staff members by the Ministry of Education, Science and Culture. New positions for two associate professors for the DNA Research Center, and new laboratories for visiting professors for the Departments of Ontogenetics and Integrated Genetics were approved in April. However, according to the 6th curtailment plan of the Government two members were cut, so the total number of regular staff remained the same as in the preceding year, that is, 92 including 53 research staff.

I am glad to note that three of our colleagues and associates were honored in the past year. First, Prof. Tomoko Ohta was awarded the Japan Academy prize for her "Theoretical studies on population genetics at the molecular level." Following Prof. Motoo Kimura who received the same prize in 1968, she is the second staff member of our institute to receive this award. Since she joined us in 1969, she has been devoting herself to challenging a number of extremely hard problems in population genetics and has succeeded in solving them quantitatively. In particular, Ohta's theories on linkage disequilibrium, on the role of slightly deleterious mutations in molecular evolution and on the concerted evolution of multigene families, are well known in the world. The award of this time in summer attracted additional public attention as she was the first woman to receive the prize singly. In autumn she won the Prime Minister's official commendation in commemoration of the United Nation's Women's Decade because of her outstanding contribution to raising the status of women. Secondly, our old friend, Prof. James F. Crow of the University of Wisconsin (U.S.A.) was elected Foreign Honorary Member of the Japan Academy because of his distinguished services in the promotion of the development of genetics in Japan. Dr. Crow has visited our institute almost every year since 1957, sometimes staying for a month, and he still continues collaborative studies with the staff of our institute. Among them M. Kimura, T. Maruyama,

T. Mukai and K. Aoki, have studied under Dr. Crow in his laboratory in Madison. It is remarkable that the collaborative studies have continued for more than 30 years, resulting in many significant papers in the field of population genetics. Third, Dr. Yataro Tazima, the former director and honorary member of the institute, was decorated with the Second Order of the Sacred Treasure for his contribution to academic as well as industrial societies. Before joining the institute, Dr. Tazima succeeded in the breeding of a new strain of silkworm with a sex-limited marker produced by irradiation—a pioneer work with chromosome engineering—and, taking advantage of this strain he elucidated the mechanism of sex determination in the silkworm; for this work he was awarded, together with Dr. Haruo Hashimoto, the Japan Academy prize in 1954. In the contemporary silkworm industry there are 68 commercially designated strains, of which 33 originated from a sex-limited Sable marking strain developed by Dr. Tazima.

There have been several active personnel changes in the last year. Prof. Yasuo Nakagome of the Laboratory of Human Genetics left in April, together with Dr. Masao Yamada and Yutaka Nakahori, for the newly established Children's Medical Research Center of the National Children's Hospital in Tokyo. While working with human cytogenetics for about 15 years in Mishima, Nakagome produced a number of solid achievements; for example, he identified several new syndromes of structural aberrations by using the banding technique, elucidated the distribution of break points with respect to the nature of chromosome bands, and reported the interesting phenomenon of the loss of centromeres with aging. Dr. Eiichi Soeda of the DNA Research Center was transferred in June to the Institute of Physical and Chemical Research. While working in the institute for 10 years, Soeda became the first scientist in the world to determine the sequence of all the bases of polyoma virus and clarified the structure and function of its genes. He also improved the method of shotgun DNA sequencing by simplifying and speeding up its procedures.

Dr. Hiroko Morishima was promoted to professor of the Laboratory of Agricultural Genetics (March), and Dr. Hirotami Imai to associate professor of the Laboratory of Cytogenetics (July). Dr. Toshimichi Ikemura (the Faculty of Science, Kyoto University) was appointed as associate professor for the newly established Recombinant DNA Section of the DNA Research Center (April), and Dr. Sanzo Miyazawa (the National Institutes of Health in U.S.A.) to associate professor for the DNA Data Analysis Section of

the Center (December). Dr. Kyosuke Nagata (Research fellow of the Memorial Sloan-Kettering Cancer Center, U.S.A.) joined us as a research staff member of the Laboratory of Molecular Genetics (February), and Dr. Hiroshi Shimizu (graduate student of the Faculty of Engineering, Tohoku University) as a research staff member of the Laboratory of Developmental Genetics (June).

As a result of personnel changes during the last two years, the staff of the Laboratory of Molecular Genetics has wholly shifted, and they have actively started studies on the regulatory mechanisms of gene expression under the leadership of Prof. Akira Ishihama. Prof. Takeo Maruyama and Dr. Takashi Gojobori are constructing a DNA data bank, periodically sending "Newsletter" to the interested workers in Japan to inform them of the progress of the works and of the availability of the data. In the next fiscal year the scheduled upgrading of the electronic computer in the institute, and the addition of Miyazawa will help speed up the preparatory work in this area.

The Genetic Stock Research Center supplies, as far as possible, upon request of competent researchers in and outside of Japan, specific strains of mouse, *Drosophila*, silkworm, *E. coli*, *B. subtilis*, etc. that are kept in the Center. The total number of cases supplied in 1984 amounted to 282 (63 sent abroad) and the total number of strains supplied was 1733 (371 sent abroad). Data-base information about a variety of experimental organisms preserved in universities and research laboratories in Japan is being systematized by Dr. Shinya Iyama; he compiled the "Drosophila Genetic Stock List in Japan (1985)", which is available for distribution to related researchers in this country.

On April 20, the institute held its yearly open house for the public. Some of the research activities of each laboratory were exhibited, and movie films were shown. Double cherry blossoms on the campus were at thier best and some 1500 visitors enjoyed them. On October 26, public lectures were given at the National Science Museum in Tokyo; the titles were "Regulatory mechanisms of gene expression" by Prof. A. Ishihama, and "Evolution of mitochondrial DNA" by Dr. Naoyuki Takahata. In spite of it being a Saturday afternoon about 120 eager people listened to the lectures, which were followed by rather specialized questions and lively discussions.

Two occasional meetings took place in the institute. One was a technical

training course for "Shotgun DNA sequencing with personal computer analysis" organized by Dr. E. Soeda on January 9-11, as one of the activities of a specific research group (chairman: Prof. Y. Takagi of Kyushu Univ.) supported by a grant-in-aid from the Ministry of Education, Science and Culture. Among the many applicants, 8 who urgently needed to master the techniques were chosen and trained intensively. The other meeting was the 25th meeting of the directors of national institutes for joint use held on May 2. Twenty-three institutes participated and discussed various problems in advancing research activities under the present severe state of administrative and financial constraints.

International personnel exchanges were very active in the past year. Members of our staff went abroad on 39 occasions for the purpose of presenting research results at various scientific meetings, exchanging information, and carrying out collaborative studies or field investigations; two of them stayed longer than 3 months. On the other hand, 52 scientists visited our institute from abroad, with whom information and views on recent studies were actively exchanged. Some distinguished guests, including Professor Dr. F. Vogel of Heidelberg, delivered stimulating lectures at the Biological Symposium. Those who stayed longer than 1 month for cooperative studies were: Dr. Paul A. Fuerst, Ohio State University, U.S.A.; Dr. Robert E. Glass, Nottingham University, U.K.; Dr. Pierre Boursot, Université Montpellier II, France; Miss Pascale Barbier, Université des Sciences et Techniques du Languedoc, Montpellier, France; Dr. Nicole Houba-Herlin, Liège University, Belgium; Dr. Lee Won Ho, Pusan National University, and Mr. Kim Bong-Kee, Dan Kook University, Korea: Mr. Irwansyah Loekman, National Atomic Energy Agency, and Miss Lilian Ungson Gadrinab, SEAMEO Regional Center for Tropical Biology, Indonesia; Dr. Ajay Kumar Jain, King George Medical College, India; Dr. Yi-de Huang, Shanghai Institute of Plant Physiology, the Chinese Academy of Sciences, and Dr. Yuan shen Qiu, Guangdong Microbiology Research Institute, People's Republic of China.

Two years have passed since the administrative category of our institute was switched over to one for joint use by universities. Under the new system, visiting professors for 5 laboratories have been appointed. We have accepted 30 collaborative programs, 8 workshops, 12 graduate students, 10 research fellows from private corporations, and 3 scholarships from industries. Yet, there remain a number of problems to be solved in order

to achieve the reality of reform. Among others, the followings are of the highest priority: completion of the DNA Research Center including the establishment of the DNA data bank, which should meet the high demand of scientists in our country, and the construction of a second main building to accommodate the new Research Center and various facilities for joint use, together with lodgings for visiting researchers. We are all eager to do our best to accomplish the missions of the new institute. We wish to have continued encouragement and support of all the persons concerned.

E. Matsumaga

STAFF

Director

MATSUNAGA, Ei, D. Med., D. Sc.

Members

1. *Department of Molecular Genetics*

KADA, Tsuneo, D. Sc., Head of the Department

Laboratory of Molecular Genetics

ISHIHAMA, Akira, D. Sc., Professor

FUKUDA, Ryuji, D. Med., Associate professor

FUJITA, Nobuyuki, D. Sc.

NAGATA, Kyosuke, D. Pharm.

Laboratory of Mutagenesis

KADA, Tsuneo, D. Sc., Professor

SADAIE, Yoshito, D. Sc., Associate professor

INOUE, Tadashi, D. Ag.

TEZUKA, Hideo

Laboratory of Nucleic Acid Chemistry (Guest Member)

MIURA, Kin-ichiro, D. Sc., Professor

YAMANE, Kunio, D. Sc., Associate professor

2. *Department of Cell Genetics*

HIROTA, Yukinori, D. Sc., Head of the Department

Laboratory of Cytogenetics

MORIWAKI, Kazuo, D. Sc., Professor

IMAI, Hirotami, D. Sc., Associate professor

YAMAMOTO, Masatoshi, Ph. D.

Laboratory of Microbial Genetics

HIROTA, Yukinori, D. Sc., Professor

YASUDA, Seiichi, D. Sc., Associate professor

NISHIMURA, Yukinobu, D. Sc.

HARA, Hiroshi, D. Sc.

Laboratory of Cytoplasmic Genetics (Guest member)

SUZUKI, Hideho, D. Sc., Associate professor

YONEKAWA, Hiromichi, D. Sc., Associate professor

3. Department of Ontogenetics

KURODA, Yukiaki, D. Sc., Head of the Department

Laboratory of Developmental Genetics

SUGIYAMA, Tsutomu, Ph. D., Professor

NAWA, Saburo, D. Sc., Professor

FUJISAWA, Toshitaka, Ph. D.

SHIMIZU, Hiroshi, D. Eng.

Laboratory of Phenogenetics

KURODA, Yukiaki, D. Sc., Professor

MURAKAMI, Akio, D. Ag., D. Sc., Associate professor

MINATO, Kiyoshi, M. Sc.

YAMADA, Masa-Aki, M. Sc.

Laboratory of Physiological Genetics (Guest member)

SHIMADA, Yutaka, D. Med., Professor

KIJIMA, Hiromasa, D. Sc., Associate professor

4. Department of Population Genetics

KIMURA, Motoo, Ph. D., D. Sc., Head of the Department

Laboratory of Population Genetics

KIMURA, Motoo, Ph. D., D. Sc., Professor

OHTA, Tomoko, Ph. D., D. Sc., Professor

TAKAHATA, Naoyuki, D. Sc.

AOKI, Kenichi, Ph. D.

Laboratory of Evolutionary Genetics

MARUYAMA, Takeo, Ph. D., D. Sc., Professor

WATANABE, Takao K., D. Sc., Associate professor

TUTIKAWA, Kiyosi, Associate Professor

GOJOBORI, Takashi, D. Sc.

Laboratory of Theoretical Genetics (Guest member)

MUKAI, Terumi, Ph. D., D. Sc., Professor

MIYATA, Takashi, D. Sc., Associate professor

5. Department of Integrated Genetics

MATSUNAGA, Ei., D. Med., D. Sc., Head of the Department

Laboratory of Human Genetics

HORAI, Satoshi, D. Med.

Laboratory of Agricultural Genetics

MORISHIMA-OKINO, Hiroko, D. Ag., Professor

ENDO, Toru, D. Ag., Associate professor

FUJISHIMA, Tohru, D. Ag.

SATO-HIRAOKA, Yoichiro, M. Ag.

Laboratory of Applied Genetics (Guest member)

YONEZAWA, Katsuei, D. Ag., Associate professor

6. Research Facilities

Genetic Stock Research Center

SUGIYAMA, Tsutomu, Ph. D., Head of the Center

FUJII, Taro, D. Ag., Associate professor

IYAMA, Shin-ya, D. Ag., Associate professor

KUSUDA, Jun, D. Ag.

INOUE, Yutaka, D. Sc.

SANO, Yoshio, D. Ag.

NISHIMURA, Akiko

SHIROISHI, Toshihiko, D. Sc.

DNA Research Center

MARUYAMA, Takeo, Ph. D., D. Sc., Head of the Center

IKEMURA, Toshimichi, D. Sc., Associate professor

MIYAZAWA, Sanzo, D. Sc., Associate professor

Experimental Farm

FUJII, Taro, D. Ag., Head of the Farm

MIYAZAWA, Akira

7. Technical Section

MARUYAMA, Takeo, Ph. D., D. Sc., Chief of the Section

8. Department of Administration

AKATSUKA, Takao, Head of the Department

TAWARA, Koichi, Chief of the General Affairs Section

MATSUMURA, Tateo, Chief of the Finance Section

COUNCIL

Chairman

YAMAMURA, Yuichi; Emeritus professor, Osaka University

Vice-chairman

MOROHOSHI, Seijiro; Emeritus professor, Tokyo University of Agriculture and Technology

Members (Alphabetical order)

EGAMI, Nobuo; Director, National Institute for Environmental Studies

IINO, Tetsuo; Professor, Faculty of Science, University of Tokyo

INOUE, Eiji; Emeritus professor, University of Tokyo

NAGAKURA, Saburo; President, Okazaki National Research Institutes

NAKAJIMA, Tetsuo; Professor, Tamagawa University

NATORI, Reiji; Emeritus professor, Jikei University School of Medicine

NOMURA, Tatsuji; Director, Central Institute for Experimental Animals

OKADA, Tokindo S.; Director-General, Institute for Basic Biology

ONOE, Hisao; Director, Institute of Economic Research, Kyoto University

OOSAWA, Fumio; Professor, Faculty of Engineering Science, Osaka University

OZEKI, Haruo; Professor, Faculty of Science, Kyoto University

SAITO, Hiuga; Director, Institute of Applied Microbiology, University of Tokyo

SAKAI, Fuminori; Director, Japan Society for the Promotion of Science

SASA, Manabu; President, Toyama Medical and Pharmaceutical University

TAZIMA, Yataro; Director, Institute of Silkworm Genetics and Breeding

WATANABE, Itaru; Professor, Kitazato University

ADVISORY COMMITTEE

Chairman

MATSUNAGA, Ei; Director

Vice-chairman

TANAKA, Ryuso; Dean, Faculty of Science, Hiroshima University

Members (Alphabetical order)

FUKUDA, Ichiro; Professor, Faculty of Liberal Arts and Sciences, Tokyo Women's University
MIURA, Kin-ichiro; Professor, Faculty of Engineering, University of Tokyo
MUKAI, Terumi, Professor, Faculty of Science, Kyushu University
OHBA, Shigeru; Professor, Faculty of Science, Tokyo Metropolitan University
OKADA, Masukichi; Professor, Institute of Biological Sciences, University of Tsukuba
OSAWA, Shozo; Professor, Faculty of Science, Nagoya University
SASAKI, Motomichi; Professor, Chromosome Research Unit, Faculty of Science, Hokkaido University
TSUNEWAKI, Koichiro; Professor, Faculty of Agriculture, Kyoto University
YAMADA, Yukio; Professor, Faculty of Agriculture, Kyoto University

HIROTA, Yukinori; Professor, Laboratory of Microbial Genetics
ISHIHAMA, Akira; Professor, Laboratory of Molecular Genetics
KADA, Tsuneo; Professor, Laboratory of Mutagenesis
KIMURA, Motoo; Professor, Laboratory of Population Genetics
KURODA, Yukiaki; Professor, Laboratory of Phenogenetics
MARUYAMA, Takeo; Professor, Laboratory of Evolutionary Genetics
MORISHIMA-OKINO, Hiroko; Professor, Laboratory of Agricultural Genetics
MORIWAKI, Kazuo; Professor, Laboratory of Cytogenetics
SUGIYAMA, Tsutomu; Professor, Laboratory of Developmental Genetics

ASSOCIATION FOR PROPAGATION OF THE KNOWLEDGE OF GENETICS

MORIWAKI, Daigoro; President, Emeritus Professor of Tokyo Metropolitan University
KADA, Tsuneo; Managing Director, Professor of National Institute of Genetics
KURODA, Yukiaki; Managing Director, Professor of National Institute of Genetics
SINOTO, Yosito; Manager

WADA, Bungo; Manager, Emeritus Professor of University of Tokyo
TAZIMA, Yataro, Manager
OSHIMA, Chozo; Manager
YOSIDA, Tosihide H.; Manager
MATSUNAGA, Ei; Manager, Director of National Institute of Genetics

PROJECTS OF RESEARCH FOR 1985

1. DEPARTMENT OF MOLECULAR GENETICS

Laboratory of Molecular genetics

Studies on regulatory mechanisms of gene expression in *E. coli* (ISHIHAMA, FUKUDA and FUJITA)

Studies on molecular mechanisms of transcription and replication of animal viruses (ISHIHAMA, FUKUDA and NAGATA)

Laboratory of Mutagenesis

Molecular mechanisms of radiation- and chemical-induced mutations (KADA, SADAIE, INOUE and TEZUKA)

Environmental mutagens, desmutagens and antimutagens (KADA)

Biochemical factors involved in cellular repair of genetic damage and induced mutagenesis (INOUE and KADA)

Genetics of *Bacillus subtilis* (SADAIE and KADA)

Molecular mechanisms of unicellular differentiation in *Bacillus subtilis* (SADAIE)

Laboratory of Nucleic Acid Chemistry

Studies on the relationship between nucleic acid conformations and biological activities (MIURA)

Expression of *B. subtilis* genes (YAMANE)

Expression of the foreign genes in *B. subtilis* cell (YAMANE)

2. DEPARTMENT OF CELL GENETICS

Laboratory of Cytogenetics

Studies on species differentiation of mouse from cyto- and molecular genetic view points (MORIWAKI)

Cyto- and immunogenetical mechanisms for regulating tumor development in the laboratory and wild mice (MORIWAKI)

Immunogenetical studies on the mouse MHC (SHIROISHI* and MORIWAKI)
Development of new mouse strains from wild populations (MORIWAKI and SHIROISHI*)

Theoretical bases for chromosomal evolution in mammals and ants (IMAI)
Cytogenetical analysis of meiotic mechanisms in mice (IMAI and MORIWAKI)
Cytogenetical studies on *Drosophila* (YAMAMOTO)

Laboratory of Microbial Genetics

DNA replication in *E. coli* (YASUDA and HIROTA)
Cellular division in *E. coli* (HIROTA, NISHIMURA, and HARA)
Penicillin-binding proteins in *E. coli* (HIROTA, HARA and NISHIMURA)

Laboratory of Cytoplasmic Genetics

Peptidoglycan biosynthesis in *E. coli* (SUZUKI and HIROTA)
Studies on cytoplasmic genes during subspecies differentiation of house mouse *Mus musculus* (YONEKAWA)

3. DEPARTMENT OF ONTOGENETICS

Laboratory of Developmental Genetics

Genetic analysis of developmental mechanisms in hydra (SUGIYAMA and FUJISAWA)
Studies on transformation and cell differentiation in higher organisms (NAWA and YAMADA)
Mitochondrial DNA organization in male-sterile cytoplasms of rice (NAWA, SANO and FUJII)

Laboratory of Phenogenetics

Genetic studies on insect cells in tissue culture (KURODA and MINATO)
Developmental genetic studies on animal cells in tissue culture (KURODA)
Genetics of somatic mammalian cells in culture (KURODA)
Genetic studies on abnormal fertilization in the silkworm (MURAKAMI)
Mutagenesis in germ cells of the silkworm (MURAKAMI)

* Genetic Stock Research Center

Studies on the male-specific killing activity of SRO in *Drosophila* (YAMADA)

Laboratory of Physiological Genetics

Electron microscopic studies on differentiation of animal cells (SHIMADA and KURODA)

Nerve network formation in *Hydra* (KIJIMA)

Genetic analysis of nervous system development in hydra (KIJIMA and SUGIYAMA)

4. DEPARTMENT OF POPULATION GENETICS

Laboratory of Population Genetics

Theoretical studies of population genetics (KIMURA, OHTA, TAKAHATA and AOKI)

Studies on molecular evolution from the standpoint of population genetics (KIMURA, TAKAHATA and OHTA)

Theoretical studies on the evolution of multigene family (OHTA)

Theoretical studies on variation and evolution of extranuclear DNA (TAKAHATA)

Theoretical studies on the evolution of altruism (KIMURA and AOKI)

Population genetical studies on gene-culture coevolution (AOKI)

Laboratory of Evolutionary Genetics

Theory of population genetics and evolution (MARUYAMA)

Studies on molecular evolution (GOJOBORI)

Evolutionary genetics of *Drosophila* (WATANABE)

Radiation genetics in mice (TUTIKAWA)

Laboratory of Theoretical Genetics

Theoretical and experimental studies of transposons in population of *Drosophila* (MUKAI)

Computer studies on the molecular evolution (MIYATA)

5. DEPARTMENT OF INTEGRATED GENETICS

Laboratory of Human Genetics

Studies on DNA polymorphisms in human populations (HORAI and MATSUNAGA)

Genetic studies on retinoblastoma (MATSUNAGA and HORAI)

Mitochondrial DNA restriction analysis of non-human primates (HORAI, MATSUNAGA, HAYASAKA, SHOTAKE and NOZAWA)

Laboratory of Agricultural Genetics

Evolutionary studies in wild and cultivated rice species (MORISHIMA and SATO)

Ecological genetic studies in weed species (MORISHIMA)

Genetic studies on rice reserve proteins (ENDO)

Behavioral genetic studies in animals (FUJISHIMA)

Laboratory of Applied Genetics

Methodology for the conservation of genetic resource populations (YONEZAWA)

6. RESEARCH FACILITIES

Genetic Stock Research Center

Studies and conservation of germplasm resources in rice and wheat species (FUJII and SANO)

Specificity of mutagen tolerance in higher plants (FUJII)

Exploitation of genetic ability of nitrogen fixation in Gramineae (FUJII, SANO and IYAMA)

Studies on genetic differentiation in rice (SANO)

Theoretical studies on breeding techniques (IYAMA)

Genetic studies of trees in natural forest (IYAMA)

Studies on gene transfer in Gramineae (SANO and FUJII)

Documentation of genetic stocks in Japan (IYAMA)

Studies on the management system of genetic stocks information (IYAMA)

Studies on chromosomal polymorphism in *Drosophila* (INOUE)

Analysis of fibroin genes of silkworm and its relatives (KUSUDA)

Molecular studies on the origin of silkworm (KUSUDA)

Coordination of DNA synthesis and cell division in *E. coli* (NISHIMURA)

Synthetic ColEI plasmids carrying genes for cell division in *E. coli* (NISHIMURA)

Development of mouse embryo freezing system (SHIROISHI and MORIWAKI)

Molecular mechanism of high frequency recombination in mouse MHC region (SHIROISHI and MORIWAKI)

DNA Research Center

Studies on primary structure of DNA (SOEDA)

Regulatory mechanisms of gene transcription (ISHIHAMA)

DNA data analysis (MARUYAMA)

Studies on codon usage (IKEMURA)

Studies on RNA molecules of mouse brain (IKEMURA)

Database design for DNA base sequences (MIYAZAWA)

RESEARCH ACTIVITIES IN 1985

I. MOLECULAR GENETICS

Promoter Selectivity of *Escherichia coli* RNA Polymerase: Localization of a Region of the β Subunit Involved in Promoter Selection

Robert E. GLASS*, Steven T. JONES*, Vishvanath NENE**,
Teruaki NOMURA, Nobuyuki FUJITA and Akira ISHIHAMA

RNA polymerase of *E. coli* is complex in structure, consisting of at least four different subunits, α , β , β' and one of sigma subunits. The catalytic site for RNA synthesis is located on the β subunit. This subunit is also involved in the recognition of promoters (Nomura et al., *Mol. Gen. Genet.* **193**, 8–16). Glass and his colleagues have set up a genetic system to analyze structure-function relationships of the β subunit (Nene and Glass, *Mol. Gen. Genet.* **194**, 166–172). This system is based on the isolation of a strain collection synthesizing several hundred variants of RNA polymerase, each variant carrying a known amino acid substitution at a known site in the β subunit.

Analysis of the altered functions of these mutant RNA polymerase has allowed us to locate regions required for specific functions. For example, we identified relaxed RNA polymerases, which were insensitive to ppGpp both *in vivo* and *in vitro* (Glass et al., *FEBS Lett.* **153**, 307–310; *Mol. Gen. Genet.*, **203**, 265–268). This indicates that a small region of RNA polymerase β subunit is a target for ppGpp.

Among pseudo-revertants of the *rpoB* (β subunit gene) amber mutants carrying internal deletions, we found a mutant RNA polymerase with altered promoter selectivity (Glass et al., *Mol. Gen. Genet.*, **203**, 487–491). The mutant enzyme lacks 55 amino acid residues near to the C-terminus of β subunit and strains carrying this mutation exhibit slower growth in minimal

* Department of Biochemistry, Queen's Medical Centre, University of Nottingham, Nottingham, England.

** Department of Pathology, University of Cambridge, Cambridge, England.

medium. The results allow localization of a region in the β subunit involved in promoter selection.

Promoter Selectivity of *Escherichia coli* RNA Polymerase: Recognition of a Heat Shock Promoter

Nobuyuki FUJITA and Akira ISHIHAMA

The *htpR* gene, the positive regulatory gene of heat shock response in *E. coli*, codes for a sigma-like protein (σ^{32}) and is supposed to participate in the specific recognition of heat shock promoters by RNA polymerase. We have purified RNA polymerase associated with σ^{32} ($E\sigma^{32}$) to apparent homogeneity from heat-shocked *E. coli* cells, and analyzed its molecular and functional properties.

The novel holoenzyme $E\sigma^{32}$ correctly recognized a heat shock promoter involved in the *groE* gene and efficiently initiated transcription at the same site as that found *in vivo*. However, $E\sigma^{32}$ could never recognize the promoters which are usually transcribed by regular holoenzyme ($E\sigma^{70}$), including *lacUV5*, *nusA*, *trp*, *recA*, *rplJ* and *rpsA* promoters. On the other hand, $E\sigma^{70}$ could not recognize the *groE* promoter, showing that strict difference exists in the promoter selectivity between two molecular species of the RNA polymerase holoenzyme. Several lines of *in vitro* evidence indicated that the two sigma subunits were easily exchanged between the two holoenzyme species. This finding supports the view that the spectrum of gene expression in *E. coli* is under dynamic control by intracellular levels of individual sigma subunits.

Promoter Selectivity of *Escherichia coli* RNA Polymerase: Regulatory Role of fMet-tRNA_f^{Met}

Teruaki NOMURA, Nobuyuki FUJITA and Akira ISHIHAMA

In *Escherichia coli*, the rate of RNA synthesis is related to the level of intracellular amino acid pool. This observation raised a hypothesis that tRNAs are involved in the control of RNA synthesis, their activities being regulated by charging amino acids. On the basis of the observation that the inhibition of the formylation of Met-tRNA_f^{Met} reduced not only the rate of protein synthesis but also that of RNA synthesis, the hypothesis was modified

such that $\text{tRNA}_f^{\text{Met}}$ is a specific regulatory molecule which mediates the coupling of transcription to translation. Using the *in vitro* mixed transcription system (Kajitani and Ishihama, *Nucleic Acids Res.* **11**, 671–686; *ibid.* **11**, 3873–3889), we found that ppGpp, the nucleotide factor involved in stringent control, interacts with purified RNA polymerase holoenzyme and alters its promoter selectivity (Kajitani and Ishihama, *J. Biol. Chem.* **259**, 1951–1957). On this line, we examined the effect of charged and uncharged forms of individual tRNAs on the transcription of specific genes. The results showed that both charged and uncharged forms of specific tRNAs except $\text{tRNA}_f^{\text{Met}}$ inhibited RNA polymerase by competing with DNA templates although the inhibitory activity was different among tRNA species examined and promoters used.

Both $\text{tRNA}_f^{\text{Met}}$ and Met- $\text{tRNA}_f^{\text{Met}}$ were inhibitors of RNA polymerase. However, formylation of Met- $\text{tRNA}_f^{\text{Met}}$ not only relieved this inhibition but also stimulated the transcription from some promoters (Nomura et al., *Nucleic Acids Res.* **14**, 6857–6870). This result supports the notion that $\text{RNA}_f^{\text{Met}}$ plays a regulatory role in transcription as well as in translation.

Promoter Selectivity of *Escherichia coli* RNA Polymerase: Effect of Promoter Location

Teruaki NOMURA, Nobuyuki FUJITA and Akira ISHIHAMA

A single promoter of the *rnh* gene coding for RNase H and two promoters of the *dnaQ* gene coding for DNA polymerase III ϵ subunit are located in a small vicinity of the *E. coli* chromosome and direct transcription in opposite direction (Nomura et al., *J. Biol. Chem.* **260**, 7122–7125). This year, we focused our studies on possible interference among these closely located promoters. For this purpose, we employed the *in vitro* mixed transcription system and used either a truncated DNA template carrying the three promoters on a single and the same DNA fragment or a mixture of truncated templates, each carrying a single promoter. The results indicated that the rates of open complex formation (parameter II of “promoter strength”) for the three promoters were unaffected depending on whether they were located on the same DNA strand or separated into individual fragments but the level of open complex (parameter I of “promoter strength”) increased for the *rnh* promoter when it was separated from the strong *dnaQ* promoters (Nomura

et al., *Nucleic Acids Res.* **13**, 7647-7661). This suggests a promoter interference between the convergently transcribed genes.

Promoter Selectivity of *Escherichia coli* RNA Polymerase: Effect of DNA Supercoiling

Nobuyuki FUJITA, Katsuaki OSATO*, Yasumasa KANO*,
Fumio IMAMOTO* and Akira ISHIHAMA

In this laboratory, promoter strength has been determined for a number of *E. coli* promoters in an *in vitro* mixed transcription system, in which mixtures of linear DNA fragments, each carrying a specific promoter, were used as the templates. Several lines of evidence, however, suggest that the strength of some promoters is affected by formation of DNA supercoiling both *in vivo* and *in vitro*. To quantitatively determine the influence of DNA supercoiling, two forms of circular DNA templates, relaxed and negatively supercoiled, were prepared for tryptophan (*trp*) promoter, tryptophan-lactose hybrid (*tac*) promoter, and *tac* promoter with UV5 mutation (*tacUV5*). The levels of transcription *in vitro* from these circular templates were determined by hybridization with single-stranded DNA probes followed by S1 nuclease digestion and gel electrophoresis. To improve the assay, we measured transcript of the β -lactamase (*amp*) gene on plasmid vector as an internal standard during RNA synthesis and hybridization.

DNA supercoiling differently affected the strength of the three test promoters, especially the rate of open complex formation (parameter II). Transcription from *trp* and *tac* promoters was stimulated whereas that from *tacUV5* and *amp* promoters was not significantly affected. These findings suggest that the activity of the "-10" signal is affected by DNA supercoiling.

Promoter Selectivity of *Escherichia coli* RNA Polymerase: Correlation between Promoter Strength and DNA Structure

Hideki TACHIBANA* and Akira ISHIHAMA

Using the "*in vitro* mixed transcription" system, we have determined two

-
- * Laboratory of Molecular Genetics, Institute of Physical and Chemical Research, Wako, Saitama.
 - * Graduate School of Science and Technology, Kobe University, Kobe.

parameters of the promoter strength, *i.e.*, the level (parameter I) and the rate (parameter II) of open complex formation, for more than 20 promoters from our collection of *E. coli* promoters (Kajitani and Ishihama, *Nucleic Acids Res.* **11**, 671–686; *ibid.* **11**, 3873–3888; Nomura et al., *Nucleic Acids Res.* **13**, 7647–7661). As an attempt to reveal correlation between the promoter strength and the DNA structure, we estimated the “opening potential” of DNA segments around promoters using the known thermodynamic parameters, which are based on the stabilities of nearest neighbor base pair doublets against thermal melting. A positive correlation was found between the calculated opening potentials in the -9 to $+3$ region ($+1$ represents the position at which transcription is initiated) and the rate of open complex formation (parameter II) (Tachibana and Ishihama, *Nucleic Acids Res.* **13**, 9031–9042).

**Analysis of the Gene for the Stringent Starvation Protein
of *Escherichia coli*.—A Novel Transcriptional
Control during the Stringent Response**

Hiroaki SERIZAWA and Ryuji FUKUDA

Up to 10 polypeptides associated with RNA polymerase and which may have some functions modulating RNA polymerase during transcription have been identified. Stringent starvation protein (SSP) is one such polypeptide. It forms an equimolar complex with the RNA polymerase holoenzyme, and is synthesized occupying more than 50% of the total protein synthesis when cells are exposed to amino acid starvation. To study the physiological function of SSP, we cloned the gene for the protein (Fukuda, R., *et al.* *Mol. Gen. Genet.*, **201**, 151–157, 1985).

This year, we determined the sequence of 1616 bp, which contained the complete coding region, as well as the flanking regions, and obtained the following results. 1. SSP has 212 amino acid residues and a molecular weight of 24,304.56. 2. At 350 bp upstream from the initiation codon of SSP, we found a stem and loop structure of a typical rho-independent transcription termination signal. The signal is located about 150 bp downstream from the three termination codons, which are arranged in phase following a long coding sequence. It was thus considered that the transcription termination signal was that of the preceding gene. 3. A long coding sequence starts from 45 bp downstream from the termination codon of the SSP gene

with a SD sequence and AUG initiation codon. 4. No obvious transcription termination signal was identified in the 3' flanking region of the SSP gene.

These results suggest that the SSP gene is a member of a multicistronic operon, and the regulation of its synthesis is not simple.

As the next step, we carried out nuclease S1 mapping of the *in vivo* transcripts to identify the transcription start site(s). During the ordinary growth of cells, the transcript of the SSP gene starts from 158 bp upstream from the initiation codon. We could identify a Pribnow sequence but not a -35 sequence corresponding to the transcription initiation. In contrast, when the cells were exposed to amino acid starvation, the transcription was initiated from a starting point further upstream, about 185 bp from the initiation codon, and the amount of SSP transcripts greatly increased. We could not identify the ordinary promoter sequences around the transcription starting site. These results indicate the existence of two different promoters for the SSP operon. The upstream promoter controls the SSP transcription during the stringent response of the cells, while the downstream promoter is used during the ordinary growth of cells.

Genetic Mapping of the *Escherichia coli* Stringent Starvation Protein Gene

Ryuji FUKUDA, Akiko NISHIMURA* and Hiroaki SERIZAWA

To determine the map position of the SSP gene, the following approach was undertaken. 1. The SSP gene was cloned to the plasmid pBR322. The resulting recombinant plasmid carried the intact Amp^R gene. 2. The recombinant plasmid was transfected and multiplied at 30°C in a temperature-sensitive *polA* Hfr strain, where it could replicate autonomously at 30°C, but could not at the nonpermissive temperature of 42°C. 3. The transformants were grown at 42°C in the presence of ampicillin. Stable ampicillin-resistant cells were those cells in which the plasmid had integrated into the chromosome at the SSP gene by homologous recombination. 4. The chromosomal site of drug resistance (and thus the integration site of the plasmid carrying the SSP gene) was mapped by Hfr matings and P1 transduction. 5. Plasmid integration at the chromosomal SSP gene was confirmed by the change in the restriction pattern of the SSP gene as indicated

* Genetic stock center of this institute.

by the Southern hybridization method.

The gene for SSP was mapped to the region between *gltB* and *glnF* at min 69.5 on the *E. coli* chromosome. It was thus established that the SSP gene is a hitherto unknown gene.

We are now trying to determine whether SSP is essential for cell growth or not, employing a similar plasmid integration technique.

Mechanism of Transcription Initiation by Influenza Virus-Associated RNA Polymerase

Ayae HONDA, Kiyohisa MIZUMOTO* and Akira ISHIHAMA

RNA-dependent RNA polymerase associated with influenza virus plays an essential role in transcription and replication of the viral genome. For initiation of transcription, the RNA polymerase cleaves host cell mRNA at specific sites (Kawakami and Ishihama, *Nucleic Acids Res.* **11**, 3637–3649) and uses the resulting capped RNA fragments as primers. The capped RNA-dependent transcription initiation can be by-passed if high concentrations of oligonucleotides were added as primers. A systematic analysis of the effect of the sequence and length of oligonucleotides on their priming activities indicated that the transcription initiation took place within a small region near the 3' termini of viral RNA segments only when primers were hybridizable at their 3' termini with templates (Honda et al., *J. Biol. Chem.*, **261**, 5987–5991). This suggests that the RNA polymerase is associated near the 3' termini of viral RNAs. Analysis of the RNA polymerase-binding site(s) on viral RNA is in progress, using RNA polymerase-viral RNA complexes isolated by the CsTFA centrifugation method (Kato et al., *Virus Research* **3**, 115–127).

***In vitro* Transcription and Replication of Influenza Virus RNA**

Kaoru TAKEUCHI, Kyosuke NAGATA and Akira ISHIHAMA

We have developed an *in vitro* transcription and replication system using isolated nuclei prepared from influenza virus-infected cells. In this system, two species of positive-sense RNA transcripts, *i.e.* mRNA and cRNA (full-sized complementary RNA without poly(A) tail), were found to be synthesized when analyzed by RNA-RNA hybridization using negative-strand

* Institute of Medical Science, University of Tokyo, Tokyo.

RNA probes from viral RNA segment 8 and gel electrophoresis. Inhibitors of DNA-dependent RNA polymerases such as α -amanitin and actinomycin D did not inhibit the reaction. Dinucleotide ApG, which is the best primer in RNA synthesis by the virion-associated RNA polymerase (Honda et al., *J. Biol. Chem.*, **261**, 5987–5991), enhanced the extent of RNA transcription.

By fractionating and reconstituting the system, we plan to identify factors responsible for transcription and replication of influenza RNA.

***In vitro* Splicing of Influenza Viral mRNA**

Kyosuke NAGATA and Akira ISHIHAMA

Influenza virus RNA segment 7 codes for at least two polypeptides, M1 and M2, translated from separate mRNA species. Segment 8 also codes for two nonstructural polypeptides, NS1 and NS2, translated from separate mRNA species. The M1 and NS1 mRNAs are unspliced primary transcripts of viral RNA segments 7 and 8, respectively, whereas the M2 and NS2 mRNAs are supposed to be formed from their unspliced precursors after splicing.

To elucidate the molecular mechanism of splicing, attempts were made to establish an *in vitro* system using nuclear extracts prepared from influenza virus-infected human cells. A precursor RNA substrate was prepared in an *in vitro* transcription reaction using influenza virus segment 8-specific cDNA template and SP6 RNA polymerase.

In influenza-infected cells, two species of positive-strand RNA, viral mRNAs and template RNAs for RNA replication, are formed. In spite of the fact that signals for splicing are present in both RNA species, only mRNAs are spliced. Analysis on the molecular basis of this discrimination is in progress.

Small RNA and CCA-Enzyme Like Activity Associated with Influenza Virus

Atsushi KATO, Susumu UEDA* and Akira ISHIHAMA

The genome of influenza virus consists of 8 segments of single-stranded RNA ranging from 890–2340 nucleotides in length. Defective interfering

* Nippon Institute for Biological Sciences, Tokyo.

(DI) particles of influenza virus contain DI RNAs of 440–690 nucleotides in length, which are derived from the RNA polymerase genes (segments 1–3). Besides we found novel species of small-sized RNAs, referred to as smRNAs, of 80–100 nucleotides in length. Several species of smRNA were purified from viral particles. Sequence analysis of isolated smRNAs and of cDNAs reverse-transcribed from smRNAs revealed that smRNAs are similar to tRNA in their structure. These smRNAs were good substrates for the cellular CCA enzyme, which is involved the modification of the 3' termini of tRNAs.

In addition, we found a virion-associated enzyme activity, by which CMP was added to smRNAs at their 3' termini. The enzyme partially purified from virions catalyzed the cytidylation of not only smRNAs but also cellular tRNAs. Possible roles of the smRNAs and the smRNA-cytidylating enzyme in transcription or replication of influenza RNA are being studied.

Analysis of Temperature-sensitive Mutants of Influenza Virus —Effect of *ts* Lesions on the Segment 8

Eriko HATADA, Ryuji FUKUDA, Kazufumi SHIMIZU
and Masakazu HASEGAWA

The RNA segment 8 of influenza virus encodes two proteins that are only found in infected cells but not in virions, and have been designated as NS1 and NS2 proteins. The NS2 mRNA is produced by the splicing of NS1 mRNA, but the function of these proteins is still unknown. In order to study the role of NS1 and NS2 proteins in virus multiplication, we analysed temperature-sensitive (*ts*) mutants derived from the A/Udorn/72 (H3N2) strain, which have defects in segment 8.

As previously reported, we determined the base sequence change in segment 8 for three of these mutants. Two of them, ICR1629 and SPC45, have a single point mutation only in the NS1 protein, while the third mutant, ICR516, has an amino acid change only in the NS2 protein. The synthesis of the M1 protein, which normally accumulates late in the course of infection, was greatly reduced in MDCK cells infected with both of these NS1 mutants at the nonpermissive temperature of 40°C as compared to 34°C. Further studies revealed the reduced synthesis of the NS1 protein at 40°C for both NS1 mutants. In addition, NS2 synthesis was greatly decreased in SPC45 but normal in ICR1629. NS1 protein synthesized at

40°C was unstable in SPC45, while it was stable in ICR1629. The reduced synthesis of the NS1 protein in SPC45 could not be explained solely by the instability of the *de novo* synthesized protein. No significant differences were seen in the synthesis of virus proteins for the NS2 mutant, ICR516.

Which steps in the virus multiplication is impaired in these mutant virus-infected cells? For analysis, we have established a system for measuring the quantities of mRNAs, cRNAs (the template for vRNA replication), and vRNAs of eight genome segments separately. To get RNA probes for quantitative hybridization in the system, 170–500 bp fragments of individual viral ds-cDNAs were recloned in the SP-6 vectors, and the DNA fragments were transcribed by SP-6 RNA polymerase. To detect mRNA and cRNA, the RNA probe was obtained by transcribing the SP-6 recombinant DNA, which gave the intact 5'-end sequence of vRNA. As mRNA deletes 16 nucleotides of the 3'-end sequence, the RNA hybrid formed with mRNA is shorter than the cRNA hybrid. We can thus discriminate cRNA from mRNA on polyacrylamide gel electrophoresis. Transcription of the opposite strand of the SP-6 DNAs gave probes to analyze vRNAs.

Function of Nuclear Factor I

Kyosuke NAGATA and Yukio ISHIMI*

The *in vitro* synthesis of full-length adenovirus DNA requires three viral coded proteins and two host-derived proteins. One of the latter proteins, designated nuclear factor I (NFI), has been purified as an essential factor involved in initiation of adenovirus DNA replication and shown to be a site-specific DNA binding protein (Nagata et al., *Proc. Natl. Acad. Sci. USA* **80**, 6177–6181). Isolation and analysis of viral as well as cellular DNA sequences bound by NFI indicates that the sequence motif, TGG(N)_{8–7} GCCAA, is a minimum requirement for the binding. An interesting feature is that DNase-hypersensitive sites are detected around putative NFI-binding sites in isolated nuclei. Such DNase-hypersensitive sites are often observed in the transcriptionally active chromatin and the origin of some replicons.

Using an *in vitro* reconstituted chromatin from purified histones, assembly protein, NFI and DNA containing the NFI binding sequence, we proved

* Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo.

that DNase-hypersensitive sites were generated in the neighborhood of a *bona fide* NFI-binding site. This suggests that NFI is one of the trans-acting factors that facilitate DNase-hypersensitive sites in chromatin. Cloning of DNA sequences flanking to chromosomal NFI-binding sites and studies on possible functions of NFI in transcription are in progress.

Cloning of the 31B-D Region of *Drosophila melanogaster* Chromosome

Yutaka INOUE

Some spontaneous mutations are caused by insertion of the transposable elements in *Drosophila*. The chromosome of one spontaneous mutant, *daughterless* (*da*), was examined by hybridizing with several transposon DNA. The *da* locus is supposed to be 31B-D of the second chromosome by the deficiency-mapping. The six kinds of plasmid DNA containing *Drosophila* transposons (*P*, *Copia*, *Gypsy*, *Hobo*, *Roo*, **412**) were labeled by ³H-nick translation and hybridized to the control and mutant polytene chromosomes. The *P* element and *Hobo* didn't hybridize with the *da* chromosome. The *Copia*, *Roo* and **412** hybridized in 10–30 sites in the genome including the chromocenter, but none of them were specific to the 31B-D region of the *da* chromosome. The Gypsy-insertion was found to be specific to the 31B-D region of the *da* mutant chromosome. There were also 4–6 sites of Gypsy-insertion including the centromere in the *da* chromosome. Then a genomic library from one *da* mutant strain was made by inserting DNA fragments generated by partial MboI endonuclease digestion into BamHI site of Lambda cloning vector EMBL4. From a screening of approximately 20,000 plaques, 40 clones were purified which hybridize to a plasmid containing Gypsy-DNA. To identify plaques carrying genomic sequences from the polytene chromosome region of 31B-D, the DNA was isolated from each clone for in situ hybridization to the Canton-S polytene chromosomes which have no Gypsy-insertion at 31B-D, and one clone hybridizing to 31B-D was identified. This cloned *Drosophila* DNA insert was about 11 kb in length. From Southern gel analysis with nick-translated Gypsy-DNA as the hybridization probe, an approximately 4 kb fragment was found to be the Gypsy-free genomic part.

Codon Usage and tRNA Content in Unicellular and Multicellular Organisms

Toshimichi IKEMURA

Choices of synonymous codons in unicellular organisms were extensively analyzed using a DNA Sequence Data Bank, and differences in synonymous codon usages between *Escherichia coli* and the yeast *Saccharomyces cerevisiae* were attributed to differences in the actual population of iso-accepting tRNAs. There is a strong positive correlation between codon usage and tRNA content in either organism, and the extent of this correlation relates to the protein production levels of individual genes. Codon-choice patterns of multicellular organisms were also studied, and a diversity in G+C% at the 3rd position of codons in vertebrate genes was revealed. The highest G+C% was 98% (for chicken histone H2A), and the lowest was 35% (for human blood Christmas factor IX); genes for the abundant cellular "house-keeping" proteins (e.g. actin, histone, tubulin) usually have a high G+C% at this position. It was also found that genes with a high G+C% at the 3rd position (e.g. more than 80% G+C) are usually flanked by a wide genome portion (far larger than the coding region, e.g., >10 kb) of high G+C% (60–70%), and genes of low G+C% at the 3rd position (less than 50%) are flanked by a wide portion of low G+C% (usually less than 45%). It is interesting that large genome portions surrounding protein genes which are far larger than the coding region are kept either G+C-rich or A+T-rich, in accord with the G+C content of the 3rd positions. This may be related to some gross genetic information on gene expression lying in the large DNA segment, and/or the regional chromosome structure. The variation of G+C% distribution throughout the vertebrate genome should be a causative factor in generating peculiar codon-choice patterns, either extremely G+C-rich or A+T-rich, at the codon 3rd position and, therefore, results in the observed diversity of G+C% at this position of vertebrate genes. For details, see Mol. Biol. Evol. 2 (1985): 13–34.

Codon Usage and Chromosomal Banding in Human Genome

Toshimichi IKEMURA and Shin-ichi AOTA

During the course of our studies to elucidate the biological significance

of the segmental distribution of G+C content throughout the vertebrate genomes, computer analyses using a DNA Data Bank showed that the A+T-rich segments (e.g. 40–50 G+C%) of human genomes are mainly associated with chromosomal G (Giemsa) banding and the G+C-rich segments (e.g. 50–65 G+C%) with R (Reverse) banding. On the basis of cytological studies, G bands have been believed to correspond to A+T-rich, late replicating DNA segments, and R bands to G+C-rich early-replicating ones. Our findings based on the computer analyses are consistent with this previous knowledge. Thus we think that chromosomal banding is a factor in generating peculiar codon-choice patterns, either G+C-rich or A+T-rich at the codon 3rd position, and therefore the diversity of G+C% at this position of human genes, as well as presumably of other vertebrates. For details, see, Nucl. Acids Res. 14 (1986): 6345–6355.

Codon Usage, tRNA Content, and Rate of Synonymous Substitution

Toshimichi IKEMURA

The non-random choices of synonymous codons in *Escherichia coli* and yeast genomes have been previously attributed to the availability of tRNA molecules within a cell. The tRNA quantification data of *Salmonella typhimurium* in the present work revealed that the same is also true for this enterobacteria. There is a strong positive correlation between codon usage and isoaccepting tRNA content in these organism, and the extent of this correlation relates to the protein production levels of individual genes. In other words, codon choices of these organisms are constrained by tRNA availability, and this constraint is more evident for highly expressed genes than for moderately or poorly expressed genes. Extensive examination of synonymous substitutions occurring among Enterobacteriaceae showed that the levels of synonymous substitution in the former group of genes are clearly lower than those of the latter group. We therefore concluded that the evolutionary constraint imposed by tRNA content on codon choice decelerated, rather than accelerated, the synonymous substitution rate of Enterobacteriaceae genes. For details, see T. Ikemura in "Population Genetics and Molecular Evolution" (Eds. T. Ohta and K. Aoki), pp 385–406, Japan Scientific Societies Press, Tokyo/Springer-Verlag, Berlin.

Organization of Mitochondrial DNA from the Male Sterile Cytoplasm of Rice

Saburo NAWA, Yoshio SANO and Taro FUJII

Rice mitochondria of the male sterile cytoplasm, (*cms-boro*)*rf₁rf₁*, contained two supercoiled circular DNAs, designated B-1 and B-2, whereas no such DNAs were detected in the normal fertile cytoplasm, (*n-boro*)*rf₁rf₁*, showing that the male sterility is associated with the presence of the plasmid-like DNAs.

Whole sequences of B-1 and B-2 were cloned in the plasmid PUC 12. The cloned B-1 contained one restriction site for *Ava*I, *Ava*II, *Eco*RI, *Hinc*II and *Stu*I, and more than two sites for *Hha*I and *Rsa*I, but none for *Bam*HI, *Bgl*I, *Bgl*II, *Dra*I, *Hind*III, *Nae*I, *Pst*I, *Pvu*II, *Sac*I, *Sal*I and *Xba*I. The cloned B-2 contained one restriction site for *Bgl*II and *Bst*EII, and more than two sites for *Fok*I, *Hinf*I, *Hpa*I, *Msp*I, *Rsa*I, *Sau*3A, *Sca*I, *Taq*I and *Xba*I, but none for *Bal*I, *Bam*HI, *Bgl*I, *Eco*RI, *Hae*III, *Hind*III, *Pst*I, *Pvu*II, *Sac*I, *Sma*I and *Stu*I.

The distribution of sequences homologous to B-1 or B-2 in nuclear or mitochondrial DNAs from the male sterile strain, the normal fertile strain and the two fertile revertants derived from the male sterile cytoplasm was examined by using these cloned B-1 and B-2 as probes. Preparations of total DNAs were digested with restriction enzymes, fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters. Hybridization was carried out by using biotin-labelled B-1 or B-2. The labelled B-1 hybridized to at least four of the *Hind*III restriction fragments of male sterile DNA, showing that the chromosome, either nuclear or mitochondrial, contained sequences homologous to the free plasmid-like DNA in various regions. DNA from the normal fertile strain contained three *Hind*III fragments homologous to B-1 which were identical with those found in the sterile DNA. However, one homologous fragment, which was most strongly labelled in the sterile DNA, was missing in the normal fertile DNA. The similar results were obtained when *Eco*RI, *Hinc*II and *Bam*HI restriction fragments were examined. DNAs prepared from the two fertile revertants, which have been induced from the male sterile strain with EMS treatment, produced the same restriction patterns as the normal DNA. Thus, the reversion to fertility in the male sterile cytoplasm resulted in the loss of the chromosomal sequences homologous to B-1 and B-2, as well as the loss of the free plasmid-

like DNAs. However, the strain, (*cms-boro*) Rf_1Rf_1 , restored to fertility by a nuclear restoring gene Rf_1 contained the free plasmid-like DNAs and the same chromosomal sequences homologous to B-1 or B-2 as the male sterile strain, (*cms-boro*) rf_1rf_1 . Therefore, the presence of the homologous sequences alone, either in the integrated forms or free forms, is not sufficient to cause male sterility.

II. MICROBIAL GENETICS

Mutants of *Escherichia coli* Defective in Penicillin-Insensitive Murein DD-Endopeptidase

Kyoko IDA, Yukinori HIROTA and Uli SCHWARZ

The mutants of *Escherichia coli* that are deficient in the penicillin-insensitive DD-endopeptidase have been isolated. The strain JE10874 (*mepA*) has about 10%–20% of the residual activity and another strain, JE10368 (*mepB*) has 40%–50% of the activity found in the wild-type, parental strain, PA3092. The penicillin-insensitive endopeptidase is a periplasmic enzyme. Genetic mapping studies show that the mutation *mepA* is located close to *aroC* (50 min) and the other mutation, *mepB*, is very close to *malE* (91 min) on the chromosome. These mutants grow normally under a wide range of growth conditions; other phenotypic properties of the mutants are very similar to those of the parent strain. A double mutant (*mepA mepB*), and a triple mutant (*mepA mepB dacB*), deficient in both penicillin-insensitive and penicillin-sensitive endopeptidases, were constructed. Again, these mutants grew normally. We conclude that either the very low level of residual enzyme activity in the mutants is enough for their survival or that the penicillin-insensitive endopeptidase is not essential for survival under laboratory conditions. (For detail, see Mol. Gen. Genet. 1983, **189**, 215–221.)

Binding of Penicillin to Thiol-Penicillin-Binding Protein 3 of *Escherichia coli*: Identification of its Active Site

Nicole HOUBA-HÉRIN, Hiroshi HARA, Masayori INOUE
and Yukinori HIROTA

In order to determine the active site of penicillin-binding protein 3 of *Escherichia coli* (PBP3), the serine residue at position 307 was replaced with alanine, threonine or cysteine by oligonucleotide-directed site-specific mutageneses. Since a unique *Ban*II site exists at the position corresponding to serine-307, *Ban*II digestion of the plasmid DNA after mutagenesis resulted in significant enrichment of the mutant plasmids. For mutagenesis, the

gene coding for PBP3 (*ftsI*) was inserted into the expression cloning vector pIN-IIB. The hybrid protein produced was able to bind penicillin while mutant PBP3, in which serin-307 was replaced with either alanine or threonine, did not lead to any detectable binding. However, contrary to the report of Broome-Smith et al. (EMBO J. 1985, 4, 231-235) thiol-penicillin-binding protein 3, in which serin-307 was replaced with cysteine, was still able to bind penicillin. Replacement of serine-445 with an alanine residue had no effect on penicillin binding to PBP3. (For detail, see Mol. Gen. Genet. 1985, 201, 499-504.)

**Dispensability of either Penicillin-Binding Protein-1a or -1b
Involved in the Essential Process for Cell Elongation in
*Escherichia coli***

Jun-ichi KATO, Hideho SUZUKI and Yukinori HIROTA

A strain of *Escherichia coli* lacking the entire *ponB* gene (penicillin-binding protein *one B* gene) and a strain lacking the proximal part of the *ponA* gene (penicillin binding protein *one A* gene) were constructed by substitution with a drug resistance gene. These strains lost either penicillin-binding protein (PBP) -1b or -1a totally and their growth was apparently normal at 30°C and 42°C except that growth of the *ponB* deletion strain was poor on a nutrient agar plate containing no NaCl at 30°C and 42°C. Transductional experiments to introduce the *ponB* deletion into the *ponA* deletion strain, and *vice versa*, showed that the *ponA ponB* double deletion was lethal unless the deletion was functionally compensated, e.g., by the presence of a plasmid carrying either gene. Thus, either PBP-1b (*ponB*) or PBP-1a (*ponA*), but not both, is dispensable for cell viability, at least under ordinary culture conditions. Transduction experiments also suggested that the γ component of PBP-1b or the PBP-1b lacking the C-terminal portion encoded in the distal region to the *SphI* site on the *ponB* was sufficient for supporting growth of the *E. coli* cell. (For detail, see Mol. Gen. Genet. 1985, 200, 272-277.)

**Expression Activities of *Bacillus subtilis*
 α -Amylase Secretion Vectors**

Kunio YAMANE

α -Amylase is one of the major extracellular enzymes of *Bacillus subtilis*.

We cloned the α -amylase structural gene from an α -amylase hyperproducing strain *B. subtilis* NA64. Then we constructed a *B. subtilis* secretion vector using an *AluI*-DNA-fragment specifying the *B. subtilis* α -amylase promoter and signal-peptide-coding regions. When the *E. coli* β -lactamase structural gene was ligated downstream of the DNA fragment and the resultant chimeric plasmids were transferred into *B. subtilis*, the *E. coli* β -lactamase structural gene was expressed and the active β -lactamase synthesized was secreted into the culture medium from the *B. subtilis* cells.

In order to study the transcription initiation site of the secretion vector, we extracted mRNA from *B. subtilis* cells containing the chimeric plasmid at 6 and 12 h cultivation after inoculation. Then the site was determined by the method of the primer extension. The site was residue A at the -121 position from the translation initiator ATG and it was 3 bp downstream from the promoter region. Several artificial promoters were chemically synthesized and were inserted into the *B. subtilis* secretion vector to obtain the β -lactamase hyper-producing strains.

III. IMMUNOGENETICS

A Lethal Gene-Deletion in the Mouse H-2 Class III Region Associated with Meiotic Recombination

Toshihiko SHIROISHI, Tomoko SAGAI, Shunnosuke NATSUUME-SAKAI*
and Kazuo MORIWAKI

A haplotype aw18 is one of the H-2 recombinants generated from the B10.A/B10.MOL-SGR heterozygote. Based on the genetic constitution of the aw18 haplotype, the recombination point was mapped to the E_α -Slp interval in the H-2 class III region. Mating experiment revealed that this recombinant haplotype carries a single recessive lethal gene located in the E_α -Slp interval.

The H-2 class III region corresponding to the E_α -Slp interval includes genes encoding four complement components, such as C2, Factor B (Bf), Slp and C4. Recently, two steroid 21-hydroxylase genes, 21-OH.A and 21-OH.B, were also mapped in this region. To examine whether these genes are intact in the aw18 haplotype, southern blot analysis was carried out using DNA probes for 21-hydroxylase, C4 and Bf genes. When hybridized with a C4 probe, Hind III digestion of DNAs extracted from B10.A and B10.MOL-SGR, which are parental strains of the aw18 recombinant, yielded two fragments of 4 kb and 23 kb corresponding to C4 and Slp genes respectively. Likewise, the same DNAs digested with Bgl II yielded non polymorphic 8.8 kb fragment and polymorphic (9.8 kb in B10.A and 10.5 kb in B10.MOL-SGR) fragment when hybridized with 21-hydroxylase probe. In contrast, the aw18 homozygote exhibited only one fragment with either of these two probes, losing 4 kb C4 fragment and polymorphic 9.8 kb 21-hydroxylase gene fragment which is specific to the B10.A strain. The result suggested that gene deletion occurred in the C4 and one of the 21-hydroxylase genes. When hybridized with the Bf probe, digestion of the aw18 homozygote DNA with BamHI, EcoRI, Bgl II and HindIII showed non polymorphic fragments indistinguishable from those of the parental strains, indicating that there is no detectable gene deletion and gene rearrangement in the Bf gene of the aw18 recombinant.

* Department of Immunobiology, Cancer Research Institute, Kanazawa University.

The protein production of complement components encoded in the class III region in the aw18 haplotype was then tested by immunodiffusion for the C4 and Slp, and by tryptic peptide pattern analysis for Factor B, using plasma of the aw18/k heterozygote. The male produced Slp but neither male nor female produced C4 protein. This result supports idea that the aw18 haplotype has an intact Slp gene but not a C4 gene as expected from the southern blot analysis. The plasma of the same heterozygote showed production of Factor B of d and k haplotypes, indicating that the aw18 haplotype has an intact Bf gene derived from the B10.A strain.

All data obtained in this study clearly demonstrated that gene deletion occurred in the class III region being coupled with meiotic recombination between homologous chromosomes of B10.A and B10. MOL-SGR and caused recessive lethality due to this gene deletion.

Effect of H-2 Complex and Non-H-2 Background on Urethan-Induced Chromosomal Aberrations in Mice

Nobumoto MIYASHITA and Kazuo MORIWAKI

The incidence of *in vivo* urethan-induced chromosomal aberrations was examined in H-2 congenic strains of mice with B10 and A backgrounds. Chromosome analysis of bone marrow cells could divide seven lines of A.H-2 congenic strains into two groups; one with a higher frequency of chromosomal aberrations such as A/Wy (H-2^a), A/J (H-2^a), A.AL (H-2^{a1}) and A.TL (H-2^{t1}), and the other with a relatively lower frequency such as A.TH (H-2^{t2}), A.CA (H-2^f), A.BY (H-2^b) and A.SW (H-2^s). The same tendency was observed in the spleen cells. Among B10.H-2 congenic strains of mice, B10.A (H-2^a), B10.BR (H-2^k), B10.A(3R) (H-2¹⁸), B10.A(5R) (H-2¹⁵) and B10.S(9R) (H-2¹⁴) and B10.S(7R) (H-2^{t2}). F₁ hybrids between B10 (resistant) and B10.A (susceptible) were susceptible.

To determine the effect of non-H-2 genetic backgrounds on urethan-induced chromosomal aberrations, four pairs of strains which have the same H-2 haplotypes such as B10 vs. A.BY (H-2^b), B10.A vs. A/Wy (H-2^a), B10.S vs. A.SW (H-2^s), and B10.S(7R) vs. A.TH (H-2^{t2}) were compared. The strains with a B10 background exhibited the significantly higher frequencies of deletions and heavily damaged cells and the lower frequencies of exchanges than the strains with an A background.

These data suggested that at least two genes are involved in the regulation

of urethan-induced chromosomal aberrations in mice, one of which is mapped between S and D in the H-2 complex, and another not belonging to H-2 (Jpn. J. Cancer Res., 76, 1141-1145 (1985), Mutation Res., in press).

Effect of H-2 Complex on the Development of Carcinogen-Induced Pulmonary Adenomas in Mice

Nobumoto MIYASHITA and Kazuo MORIWAKI

The present study was performed to determine the locus in the H-2 complex which affects the development of pulmonary adenomas. For that purpose, a single s.c. injection of either urethan or 4-nitroquinoline 1-oxide (4-NQO) was achieved in 7 H-2 congenic strains with an A/Wy background and 12 strains with a B10 background.

In H-2 congenic strains with an A/Wy background, the number of adenoma foci per mouse was checked 5 months after the urethan treatment, and 7 months after the 4NQO treatment. The average number of adenoma foci per mouse was significantly higher in H-2 haplotypes a, tl and al, than in b, s, f and t2. These data showed that the gene (or genes) in the H-2K-H-2D interval determined the susceptibility to the development of the carcinogen-induced pulmonary adenomas. In the case of H-2 congenic strains with a B10 background, the average number of adenoma foci per mouse induced by urethan was also higher in H-2 haplotypes k, a and h2 than in b, d, f, s, r, i3, h4, i5 and t4. The number of foci in F₁ hybrids, between the susceptible and resistant strains, such as (A×A.BY)F₁ and (B10×B10.A)F₁ hybrids, was intermediate between those of the two parental strains. On the other hand, in (B10.A(4R)×B10.A(5R))F₁ mice, the number of foci was higher than those in the resistant parental strains both of which are recombinants of H-2^a and H-2^b.

These patterns in the response to carcinogens paralleled those obtained for immune response to lactate dehydrogenase-beta (LDH-B) and immunoglobulin gamma 2a (IgG2a) proteins. The difference in susceptibility to the development of pulmonary adenomas among H-2 congenic mice is likely to be due to the polymorphism of the I-E molecule, which is encoded by the Eα and Eβ loci in the H-2 complex. This possibility was also supported by the fact that the injection of anti-I-E^k monoclonal antibody could inhibit the growth of A/J derived transplantable lung tumor in vivo. These results suggest that the I-E^k molecule has a function of suppression of immune

surveillance system against the development of pulmonary adenomas.

Serological Survey of Alphaprotein-1 in Wild Mice

Yoshi-nobu HARADA, Takeshi TOMITA* and Kazuo MORIWAKI

Alphaprotein-1 (Aph-1) is an alloantigen which migrates immuno-electrophoretically to the α -region. Two allelic forms of this alloantigen, Aph-1A and Aph-1B were shown by reciprocal alloimmunizations between Mol-A strain (a strain derived from Japanese wild mice) and BALB/c strain of mice. The Aph-1A (BALB/c type) and Aph-1B (Mol-A type) are controlled by a codominant autosomal locus designated *Aph-1* (Harada et al., *Immunogenet.* **24**: 47-50, 1986). All of the common laboratory strains examined in the former study exhibited Aph-1A, whereas all derived from Japanese wild mice Aph-1B. This unique distribution of the alloantigens could probably be a reflection of evolutionary divergence occurring in mouse sub-species.

Table 1. Crossreaction of the wild mouse sera against anti-Aph-1B monospecific alloantiserum

Strain of Positive	No. examined	Strain of Negative	No. examined
M. Mol-Msm	2	M. DOM-PGN 2	2
M. Mol-Kgs	2	M. Dom-Blg	2
M. Mus-Blg 1	3	M. Cas-Tch 1	1
M. Mus-Blg 2	2	M. Cas-Bgr 1	2
M. BRV-MPL (BRV/2)	2*	M. Mus-Njl	2
M. Sub-Ias 2	2		
M. Sub-Ias 3	3		
M. Sub-Kjr 2	2		
M. Sub-Cht	3		
M. Sub-Shh 1	2*		
M. Sub-Shh 2	1		
M. Sub-Ac 1	2		
M. Sub-Jyg	2		
M. Sub-Bjn 2	2		
M. Sub-Bjn 3	2		
M. Sub-Tch	1		

* Weak reaction.

* Faculty of Agriculture, Nagoya University.

Sera from 21 wild derived strains were surveyed by gel precipitation with anti-Aph-1B monospecific alloantiserum (Table 1). Sera from 16 strains of mice including all of strains derived from Eastern Asia (Japan, Korea and China) crossreacted with the antiserum. Sera from two *M. m. domesticus* strains (M.DOM-PGN 2 and M. Dom-Blg) did not crossreact with it as expected.

Sera from M.BRV-MPL and M.Sub-Shh 1 crossreacted weaker than those of the other positive strains. The precipitin lines of these sera against anti-Aph-1B did not fused completely with that of control serum in Ouchterlony analysis. Mice of M.BRV-MPL and M.Sub-Shh 1 may have the third allele at the *Aph-1* locus.

Characterization of Ly-2.1^w Antigenicity Newly Found in Asian Mice

Yasuyuki KURIHARA and Kazuo MORIWAKI

Ly-2 antigen is expressed on thymocytes, suppresser T cells and cytotoxic T-cells, discriminating them from the other kind of lymphocytes. So far, two allelic forms of it, Ly-2.1 and Ly-2.2, have been known in laboratory strains of mice. Our previous study (Immunogenet. **22**: 211-218, 1985) demonstrated that thymocytes of *Mus musculus castaneus* and the related mice failed to react with Ly-2.2 and expressed very weak reaction to Ly-2.1-specific monoclonal antibody. One hybridoma clone, designated YK12-601, secreting monoclonal antibody specific to Ly-2.1^w antigen was established from spleen lymphocytes of C57BL/6 mice immunized by *castaneus* thymocytes. This monoclonal antibody strongly reacted with the Ly-2.1^w antigen, but weakly with Ly-2.1 antigen. The weak reaction for Ly-2.1 observed in the *castaneus* mice might have resulted from a minor structural change of the genuine Ly-2.1 antigen, suggesting that Ly-2.1^w could be the third allele.

Another monoclonal antibody, designated YK12-457 was isolated from the same immunization as YK12-601. This monoclonal antibody positively reacted with the thymocytes of twelve out of thirteen standard mouse strains including the recipient strain C57BL/6. Only exception was DM/Shi strain which was obtained from Aburabi Laboratories of Shionogi Pharmaceutical Co. (Dr. S. Makino). Thus, YK12-457 monoclonal antibody seems to be a kind of cytotoxic autoantibody for syngeneic thymocytes.

IV. DEVELOPMENTAL AND SOMATIC CELL GENETICS

Effects of Hormones on Growth Pattern of Human Diploid Cells in Culture

Yukiaki KURODA

Normal diploid fibroblasts obtained from human embryos die after about fifty population doublings in culture. This phenomena are useful for investigating the mechanism of aging by cultured human diploid cell as a model system. In the present study, the effect of hormones such as testosterone, insulin, gonadotropin and thyroxine on growth pattern of human diploid cells was examined in clonal cultures.

Normal diploid fibroblasts were obtained from a human abortive embryo of 10-week gestation. When cells at the sixth population doubling level were cultured as a clone from single cells, they showed a peculiar growth pattern. Some cells in the cell population grew exponentially, and other cells stopped to divide after several times of cell divisions. Some part of cells in population was maintained in an active state without any cell division during culture period of one week.

When testosterone, insulin or thyroxine was added to culture medium at concentrations of 0.05 to 0.5 $\mu\text{g/ml}$, it enhanced divisions for both dividing cells and non-dividing cells. Gonadotropin had stimulating effects only on dividing cells, resulting in shortening the cell cycle time, but they had no effect on non-dividing cells.

In our previous study, it has been found that growth factors and hormones could be divided into two groups: one stimulated all cells to divide in population, and other stimulated only dividing cells in population. The former substances such as epidermal growth factor (EGF) and fibroblast growth factor (FGF) had no effect on the increase in the number of population doublings of human fibroblasts. On the other hand, the latter substance such as hydrocortisone was effective in expanding the population doubling number. In this context, it was suggested that testosterone, insulin and thyroxine may have no effect on *in vitro* cell aging, while gonadotropin may have some effect on expanding the cell aging of human cells in culture.

Antimutagenic Activity of Vitamin C on Mutations Induced by EMS in Cultured Chinese Hamster Cells

Yukiaki KURODA

Vitamin C is abundantly contained in green tea, fruits, and vegetables. It is effective in preventing scurvy, and its antioxidant activity is useful for detoxification of some drugs and for protection against rancidity in food-stuffs. In the present experiment, it has been found that vitamin C had a marked effect in reducing 6-thioguanine (6TG) resistant mutations induced by ethyl methanesulfonate (EMS) in cultured Chinese hamster V79 cells.

Vitamin C alone had no detectable effect on cell survival at concentrations of less than 100 $\mu\text{g/ml}$. At a concentration of 300 $\mu\text{g/ml}$, the colony-forming activity of cells was completely inhibited. On the other hand, EMS had cytotoxic effect on survival of V79 cells. The value of LD_{50} of EMS was 541 $\mu\text{g/ml}$. In the presence of 100 $\mu\text{g/ml}$ vitamin C, the cytotoxic effect of EMS decreased markedly, giving the LD_{50} of more than 1,000 $\mu\text{g/ml}$.

Vitamin C also had a marked effect in reducing 6TG resistant mutations induced by EMS. Vitamin C alone had no detectable activity in inducing 6TG resistant mutations. EMS had a strong inducing activity of 6TG resistant mutations in the absence of vitamin C. At a concentration of 1,000 $\mu\text{g/ml}$, EMS induced 6TG resistant mutations at a frequency of 87.5×10^{-5} . In the presence of 100 $\mu\text{g/ml}$ vitamin C, the mutagenic activity of EMS reduced to about one third or one fourth. We are taking vitamin C from the diet at the rate of 20 to 500 $\mu\text{g/kg}$ body weight. Without extra vitamin C in the diet, the concentrations in human tissue are reported to range from 2 to 50 $\mu\text{g/100 g}$. Vitamin C at these concentrations may play a role in the prevention of mutagenic action of chemicals taken into the body from our environment. The inhibitory effects of vitamin C on the cytotoxic action and mutations induced by EMS in the present experiment are an interesting problem in understanding the mechanism of vitamin C.

Combined Effects of EMS and MMS on Mutation in Chinese Hamster Cells in Culture

Yukiaki KURODA, Hajime KOJIMA* and Hiroaki KONISHI*

Many chemicals present in our environment have been examined for their

* Biochemical Research Institute, Nippon Menard Cosmetic Co., Ltd., Ogaki, Gifu.

mutagenic activity by various test systems. Most of these tests were dealt with the effects of the single chemicals on organisms. In our environment, however, many chemicals were present together. It is necessary to examine the combined effects of different chemicals. In the present experiment, the combined effects of methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS) on 6-thioguanine (6TG) resistant mutations in Chinese hamster V79 cells were examined.

When EMS at a concentration of LD_{20} was combined with MMS at various concentrations and used for treatment of cells for 3 to 12 hours, some synergistic effects of both chemicals on the induction of 6TG resistant mutations were found, compared with the theoretical values calculated from the mutation frequencies obtained by single chemicals. However, when the cytotoxic effect of both chemicals was considered, the combined effects were not synergistic, but additional. When MMS at a concentration of LD_{20} was combined with EMS at various concentrations and used for treatment of cells, no definite results on combined effects of both chemicals were obtained in the induced mutation frequency, due to a strong lethal effect of MMS.

When cells were successively treated with EMS and MMS each for 3 hours, the induced mutation frequency varied, additional or synergistic, depending on the sequence of treatments with both chemicals and the concentrations and times of chemicals. When an interval of cultivation for 3 hours in normal medium was inserted between two treatments with EMS and MMS each for 3 hours, some repair phenomena in induced mutation frequency were observed. When cycloheximide, an inhibitor of protein synthesis, at a concentration of $10 \mu\text{g/ml}$, was added in the medium during the above interval, the induced mutation frequency was slightly reduced. Hydroxyurea at a concentration of $50 \mu\text{g/ml}$ had no effect on the induced mutation frequency. The study to examine the combined effects of both chemicals on chromosome aberrations is also progressed.

Enhancing Effect of Ara-C on Mutations in Cultured Chinese Hamster Cells Treated with EMS

Yukiaki KURODA, Kanako SHINKAWA-TACHI*
Kanehisa MORIMOTO* and Akira KOIZUMI*

In addition to the investigations on the combined effects of chemicals having

* Department of Public Health, Faculty of Medicine, University of Tokyo.

the same target bases in DNA, it is important to know the combined effects of more than two chemicals which are different in their action mechanism to DNA in the cells. In the present experiment, the effect of 1- β -D-arabino-furanosyl-cytosine (Ara-C), an inhibitor of DNA polymerase, on 6-thioguanine (6TG) resistant mutations in Chinese hamster V79 cells treated with ethyl methanesulfonate (EMS) was examined.

The treatment time of cells with both chemicals was 6 hours, in consideration of their cytotoxicity and the cell cycle time. The concentrations of chemicals were fixed for one chemical at that giving the LD₂₀ (EMS: 4×10^{-3} M, Ara-C: 2×10^{-6} M) and varied for the other.

EMS had a strong mutagenic effect on V79 cells. As for Ara-C, however, there has been a controversy in the previous reports as to whether it is mutagenic or not. In the present experiment, Ara-C had no detectable effect in inducing 6TG resistant mutations of V79 cells up to a concentration of 5×10^{-4} M.

The mutation frequencies of cells treated simultaneously or successively with EMS and Ara-C were higher than those obtained by treatment with EMS alone. These results indicate that Ara-C had an enhancing effect on mutations induced by EMS in Chinese hamster cells, although Ara-C alone was not mutagenic to the cells.

Freezing of *Drosophila* Embryos after Treatment with Some Chemicals

Yukiaki KURODA and Yuko TAKADA

In a series of experiments to freeze embryos of *Drosophila melanogaster* at -80°C , one of the most difficult problems was the fact that the vitelline membrane of embryos was resistant for freezing-protective agents to penetrate into embryos. It is necessary to permit the penetration of these agents such as glycerol or dimethyl sulfoxide, to avoid the formation of ice in the embryos at -80°C . The treatments of embryos with various chemicals were attempted to enhance the permeability of the vitelline membrane.

Eggs collected from adult flies of the Oregon-R strain of *D. melanogaster* were dechorionated by treatment with 3% sodium hypochloride solution for 10 minutes. Selected embryos at the appropriate stage of development were treated with pentaethyleneglycol-monododecyl ether (PEGE) at various concentrations for various times of incubation. When embryos were treated

with 20% PEGE for 30 minutes, neutral red, which was used for detecting the change in the permeability of the vitelline membrane, entered into embryos. These embryos were transferred to salt solution containing 10% glycerol and frozen at -80°C in an electric refrigerator. When they were defrosted and incubated at 25°C , they continued to develop and hatch to larvae at a low frequency.

Since PEGE had a toxic effect on embryos, some other non-toxic enzymes, which were effective in enhancing the permeability of the vitelline membrane, were tested. Embryos were treated with lipase, pancreatin, pectinase and trypsin at various concentrations. After treatments with these enzymes, their toxic effects on survival of embryos and the hatchability of treated embryos after freezing at -80°C were examined. When embryos were treated with concentrations as low as 0.01 to 0.1% of trypsin for 18 to 24 hours, their toxic effect was low with an increase in the hatchability of treated embryos after freezing at -80°C . During treatment for these long periods, however, embryos proceeded their development. More improved procedures for treatment of embryos with enzymes are now in progress.

Differentiation of Embryonic Cells of *Drosophila melanogaster* Studied with the Electron Microscope

Yutaka SHIMADA and Yukiaki KURODA

In the series of tissue culture studies of embryonic cells from *Drosophila melanogaster*, we have examined the fine structure of these cells cultured with and without ecdysterone.

When undifferentiated cells dissociated from post-gastrula embryos of the wild-type strain (Oregon-R of *D. melanogaster*) were cultured in medium K-17 (supplemented with 15% fetal calf serum and $0.1\text{ }\mu\text{g/ml}$ fetuin) without ecdysterone at 25°C , they differentiated into various embryonic or larval cells such as muscle, epithelial and nerve cells. In muscle cells, the formation of typical myofibrillar structure was observed; A, I, Z and M bands were clearly distinguished. In epithelial cells, interdigitations, gap junctions and desmosomes were formed between adjacent cells. Nerve cells extended many cytoplasmic processes, in which neurofilaments and neurotubules were found.

When these cells were incubated in the same medium with addition of

10 $\mu\text{g/ml}$ ecdysterone, two cell types could be distinguished at early stages of culture, i.e., dark and light cells. The former cells possessed a dark nucleus and a cytoplasm containing numerous endoplasmic reticula, mitochondria and ribosomes. The latter cells had a lightly stained cytoplasm with fewer endoplasmic reticula, mitochondria and ribosomes. After one week in culture, differentiation of muscle and nerve cells was evident. The former cells with the typical myofibrillar structures had adherent junctions between the same cell types. As in the nerve cells cultured without ecdysterone, the latter cells had neurotubules and neurofilaments in their processes. Tracheae with numerous bifurcations and infoldings were found in many cell types. Virus-like particles were observed in nuclei of many cells. After 2 weeks of culture, cells with vacuoles and degenerated endoplasmic reticula increased.

The results suggest that the fine structure of the cells cultured with and without ecdysterone may be useful for analyzing the more detailed characteristic action of the specific genes during normal development.

Brown-embryo Lethals in *Drosophila melanogaster*

Kiyoshi MINATO

Some embryos in *Drosophila melanogaster* have been known to die in brown embryos (BR) when eggs are irradiated with X-rays. This BR is thought to reflect dead embryos with some developed organogenesis.

The similar, but inherited syndrom was found and has been maintained stably for many generations in a sub-line of the Oregon-R strain in our laboratory. In this sub-line a high proportion (about 30%) of embryos died in BR. Furthermore, in some other strains maintained in our laboratory, the BR syndrome appeared possibly owing to sub-optimal culture conditions including bacterial infection of foods. In almost BR except one, however, dissapeared gradually from populations of flies according to the improvement of culture conditions. In only one strain, *fs(I)MAY-263*, BR has been maintained stably for many generations without dissapearing, in which about 30% of embryos died in brown embryos.

The external and internal morphology in brown embryos was observed microscopically in the above two lines showing the stable genetical syndrome of BR. As the results, the characteristics of BR in the two lines were very similar to each other. That is, some segmentation of the integument was

found, whereas the trachea, the gut and the head were not formed. Since most of yolk, however, had been consumed, the development of other organs may proceed considerably in brown embryos. Furthermore, a characteristic Malpighian tubule, which can not be seen externally in the normal embryos, was observed as the dark one in brown embryos. This darkeness of Malpighian tubule may be somehow associated with the phenomenon of brownish change of dead embryo.

The Role of the Cephalic Furrow during the Embryonic Development of *Drosophila melanogaster*

Kiyoshi MINATO

The oblique cleft of the cell layer is temporarily found laterally at about one-third distance from the anterior end of the embryo at the late-gastrula stage (3.5 hours at 25°C) of embryonic development of *Drosophila melanogaster*. As to the roles of this oblique cleft or "cephalic furrow" (CF), there are two concepts: (1) CF absorbs temporarily a stress derived from an intense increase of the cell layer: (2) CF is present as a boundary between the head and the body regions.

To know which of the two is the case, the mitotic frequency of cells in various regions was examined around the period of CF formation in histological preparations in the Oregon-R strain of *D. melanogaster*. It was found that mitosis was frequently seen not in the exterior but in the interior region of the CF. Although mitotic divisions in the insect embryogenesis is not generally followed by an increase in cell mass, the above finding may suggest some opposite situations to the concept of "absorvance of stress".

Genetic Studies on Parthenogenesis in *Bombyx mori*: A Selection of Highly Parthenogenetic Lines

Akio MURAKAMI, Yoshiki OHTSUKI* and Toshio KITAZAWA*

For several years, we have been attempting to isolate highly spontaneous or natural parthenogenetic lines in *Bombyx*. In the previous communication (this Report No. 35, 1985), we reported that a Chinese bivoltine strain, *Daizo*, showed a high rate of natural parthenogenesis. To investigate

* Breeding division, the National Sericultural Experiment Station, Tsukuba.

these results further, the natural parthenogenicity of the strain *Daizo* and two other strains, *J106* and *Cambodge*, which are often used for experiments on artificially-induced parthenogenesis, were investigated colouring serosa cells with purplish black as an indication of embryonic development. Unfertilized or non-developmental eggs remained light yellowish-white.

Results showed that the frequency of natural parthenogenesis in eggs laid from virgin moths of the strain *Daizo* (D) was 3.6%, and those of *J106* (J) and *Cambodge* (C) were 8.7 and 16.4%, respectively (bluish eggs or embryos seen through the egg-chorion in the latter strain were judged as the developed embryos). This contrasts with our previous conclusion that the *Daizo* strain has the highest frequency of natural parthenogenesis. Surprisingly, a remarkably high frequency of natural parthenogenesis was detected in eggs laid from some F_1 hybrids, $D \times C$, $C \times D$, and $C \times J$, ranging from 16.0 to 24.9%. In particular the F_1 hybrid of *J106* female and *Cambodge* male showed the highest frequency, 32.1% of all strains in this experiment. These findings show that the frequency of natural parthenogenesis varies from as lower 3.6% to as high as 32.1% depending on the stock or strain. The frequency of natural parthenogenesis appeared to be generally higher for the multivoltine strains and for crossed hybrids than for the bivoltine strains.

Generally, embryos developed from natural parthenogenesis died at the early embryonic diapause stage so that the frequency of hatching was always very low. Consequently, phenotypic analyses of natural parthenotes different from those of artificially-induced parthenotes appeared to be impossible. Either coloured or non-coloured egg or embryo from the F_1 virgin female moths in a cross, *J106* female and *Cambodge* male, was deprived of egg-chorion and then transferred one by one in drops of Grace's medium to make hanging-drop cultures, and cultivation was continued at 25°C without renewing the medium. Results showed that all coloured embryos grew the stage of bristle formation or of the late stage of organogenesis, and that most of the embryos from the non-coloured eggs also developed to the stage of bristle formation. A similar tendency was observed in eggs from the virgin moths of F_1 hybrid of the different crosses, *Cambodge* female and *J106* male, and *Daizo* female and *Cambodge* male. It, thus, can be tentatively concluded that eggs laid from virgin moths in *Bombyx mori* regardless of strains or stocks may have a high potentiality for natural parthenogenesis.

**Genetic Studies on Parthenogenesis in *Bombyx mori*:
Thermal Activation and Its Significance**

Akio MURAKAMI

In *Bombyx*, it is very rare for eggs to develop without the participation of sperm and these spontaneous parthenogenetic eggs usually do not survive to hatching. Theoretically, in parthenogenesis, a meiotic haploid oocyte duplicates before and/or during the first mitotic division, and the zygote has two sets of chromosomes derived from its mother. This may explain the lack of vigor in natural as well as induced parthenotes.

In this insect, artificial parthenogenesis can be easily induced by an appropriate treatment of unfertilized eggs or oocytes by various means. Among successful parthenogenetic methods, the thermal activation of ovarian eggs dissected from virgin moths (Astaurov, 1940) is the most common and a majority of the parthenotes survive to hatch. Some of them develop to adults with a normal reproductive function. It is of particular interest to note that the sex of these parthenotes is female. When the first meiotic division was blocked by exposure of ovarian eggs to a high temperature, the chromosome number was not reduced, diploidy (or ameiotic parthenogenesis) was maintained. Recently, Strunnikov and his colleagues (*e.g.*, 1983) devised another method combining activation of newly deposited unfertilized eggs with low and high temperatures. This method is characterized by the use of deposited unfertilized eggs. It is, therefore, of interest to compare hatchabilities (or viabilities) of artificially-induced and naturally-occurring parthenotes to understand the mechanism(s) of artificial activation of unfertilized eggs.

Hybrids between *C108* females and *Aojuku* males were used in the present experiment. For the combined activation, the newly deposited unfertilized eggs aged one to five days after natural laying were kept in a refrigerator at -11°C for 120–150 minutes and then immersed in warm water at 46°C for 12 minutes. After natural drying, eggs were treated similarly to normally-fertilized eggs. For thermal activation, ovarian eggs obtained from virgin moths were treated in the usual manner according to Astaurov (1940).

Hatchabilities of parthenotes obtained from combined activation varied from a few per cent up to several per cent depending on the treatment, compared to 30–50% for those obtained from the thermal activation. Hatchabilities of normally-fertilized eggs used as the control was over 95%.

Combined activation, although essentially analogous to natural parthenogenesis with respect to using deposited unfertilized eggs, produced parthenotes which survived to larvae with a sex ratio of 1:1. The difference in the sex-ratio between larvae obtained from these two activation methods is clearly due to the stage at which the eggs are treated: the deposited eggs had nearly completed the meiotic division forming four haploid chromosome sets or nuclei. In this method, diploidy may result from a fusion between two haploid nuclei (or meiotic parthenogenesis).

From these observations, it can be concluded that exposure to temperatures whether high or low is effective in inducing either meiotic or ameiotic parthenogenesis leading to the restoration of the diploid number of chromosomes. It is also very likely that high temperature treatment acts as spermatozoa do in fertilization in inducing cell divisions or differentiations.

Sex and Strain Differences in the Lifespan of the Adult Silkmoth, *Bombyx mori*

Akio MURAKAMI, Yukiaki KURODA and Yosoji FUKASE

In the silkworm, the entire lifespan takes about one year from egg to adult death after laying eggs. However, the greatest part of the lifespan is spent in the egg stage. From newly hatched larvae to the emergence of an adult takes only about 40 days when reared at the standard temperature of 25°C with a relative humidity of about 70–80%. Information on the adult lifespan is meagre, but preliminary observations indicate that it takes 1–3 weeks depending on the strain and sex. In spite of its brevity, the adult stage has advantages for studies on the lifespan and aging because during that time they feed on nothing, and their body is composed entirely of post-mitotic cells and so on (e.g., Osanai, 1985). This report describes one of a series of investigations on the genetics of longevity or lifespan in *Bombyx mori*.

Several silkworm strains, *C108*, *Aojuku*, *Daizo*, *Cambodge*, *od*, *pe:re* and some their F_1 hybrids, reared on mulberry leaves, were used in the present experiment. Newly emerged virgin moths were collected at about 10 a.m. every day. Each moth was kept separately on an egg-card covered with moth cover in a rearing room controlled at 25°C with a relative humidity of about 70–80% under dark conditions. Twice daily, observations were carried out, at 10 a.m. and 4 p.m.

Survival curves for each strain proved to be of a typical sigmoidal fashion regardless of the strain. The mean lifespan of each strain varied from 3–21 days. The lifespan of females was about 1.5 times longer than that of males for all strains tested except for the *Daizo*, a Chinese bivoltine stock. In the *Daizo*, the lifespan was so short, 3–5 days, that no marked sex-differences were observed. Virgin female moths of the *J106* laid over 90% of their eggs during the 4–5 days after the beginning of oviposition, while those of the *Daizo* laid over 90% of their eggs during the first 1–3 days and they begin oviposition, about 8 hr after eclosion from the pupae. The lifespan of the *J106* strain was found to be 15 days. The pupal stage took 10–14 days in the *J106* and *Daizo* strains. No significant differences in the duration of embryonic (about 10–14 days) and larval (about 21–25 days) stages were observed when using standard rearing conditions. This indicates that the lifespan in the *Daizo* is shorter than the *J106* only in regards to the adult stage. It appears that the *Daizo* strain is regarded as a mutant for a short adult lifespan in *Bombyx*.

The mean adult lifespan of the F_1 hybrid obtained from a cross between *Daizo* and *J106* was somewhat longer than that of the *J106*. In their back-cross, [*Daizo* $\times$ *J106*] $\times$ *Daizo*, two types for the adult lifespan corresponding to the *J106* and *Daizo* types with the ratio of 1:1 were observed. This observation indicates that the *Daizo* strain has a recessive genetic factor responsible for a short lifespan, whereas the *J106* has a dominant factor for a long one. This finding may rise a question as to whether or not the short lifespan factor in the *Daizo* strain is adult lethal. To get a final conclusion, further studies are required.

Genetic Studies on the Hereditary Mosaic (*mo*) Strain of *Bombyx mori*: Function of the Polar-body

Akio MURAKAMI and Masahiko KOBAYASHI*

In *Bombyx mori*, it is known that a hereditary mosaic (*mo*) gene responsible for the double-nucleus fertilization in an egg produces various types of mosaicisms (e.g., Goldschmidt and Katsuki, 1928, 1931). In the course of a neurogenetic investigation using a hereditary mosaic strain, we obtained 201 larvae, primarily egg-colour mosaics from a cross between the

* Faculty of Agriculture, University of Tokyo, Tokyo.

hereditary mosaic strain females heterozygous for two genes in egg-colour (*pe*) and translucent larva (*ok*) and males homozygous for these two recessive genes, *pe* and *ok*. These larvae were further classified into either nonmosaic or mosaic in regard to both sex and the autosomal traits, or non-mosaic for the sex and mosaics for the autosomal traits, and *vice versa*. As a result of classifications, there were 37 females, 28 males and 136 gynanders with a ratio of 1 : 1 : 2. This observation suggests that the fashion for meiotic divisions in *Bombyx* females supports the post-reductional types rather than the pre-reductional one.

If the pre-reductional division is the case, gynanders may arise through the two combinations of double-nucleus fertilization, either one of two primary polar-bodies and an ovum, or a primary polar-body and a secondary polar-body. While in the case of the post-reductional division type, both females and males may appear in addition to the gynanders. Two different combinations are also possible: an ovum and a secondary polar-body, and a pair of primary polar-bodies. It, thus, appears that the appearance of each female, male and gynander is caused by one of twelve random combinations between two nuclei among the four haploid nuclei formed by the meiotic divisions in oogenic cells. In other words, all four haploid nuclei from the primary oocyte have the same function in the *mo* strain and two of these four haploid nuclei have an equal function (or chance) to fuse with each sperm. In the wild strain, however, one of the meiotic products would differentiate into an oocyte pro-nucleus having a function to selectively receive and/or recognize a sperm in the course of the meiotic division, while the other three haploid nuclei would have no such function.

It is well known that in the insect egg, there is no morphological differences among the four haploid nuclei formed from a primary oocyte, besides the nucleus arranged in the innermost of ooplasm functions as the oocyte pro-nucleus. The remaining three nuclei in ooplasm are not usually concerned in fertilization. In consequence, polar-bodies in the strict sense are not formed in the insect. However, each of the three haploid nuclei in females possessing a recessive gene *mo* may have an equal function to participate in syngamy with sperm. Moreover, these meiotic products seem to have a tendency to a high affinity for sperm. In any case, it is suggested that the *mo* gene may be deficient in the function for differentiating the oocyte pro-nucleus from the three other polar-body nuclei.

Sex Reversal by Heterosexual Parabiosis in Hydra

Tsutomu SUGIYAMA and Norio SUGIMOTO

Sex change from female to male occurs at a high rate in hydra when a female polyp is exposed to a male polyp by parabiosis for a limited period of time (1–3 days). In contrast, sex change in the opposite direction does not occur when a male polyp is parabiotically exposed to a female polyp (Tardent, 1968).

The mechanism involved in this masculinization process by heterosexual parabiosis was investigated. When a male strain which produces immotile sperm was used to masculinize female strains, the resultant masculinized animals produced only immotile sperm. Evidence was also obtained which indicated that the original female interstitial cells remained in large numbers in the masculinized animals but did not differentiate into gametic cells. These observations suggest that masculinization is achieved not by the hypothetical “masculinizing factor” (Wiese, 1953; Brien, 1963), but by the migration of the male interstitial cells from the male to the female tissue during parabiosis (Tardent, 1968). For details, see *Developmental Biology*, **110**, 413–421 (1985).

Zygotic Effect of the *daughterless* Gene in *Drosophila melanogaster*

Yutaka INOUE

The sex specific lethal effect of the *daughterless* (*da*: 2–40.3) gene is recessive, maternal and temperature-sensitive. The *da/da* homozygous females produce no daughters at 25°C and 29°C, but some daughters are rescued at the lower temperature of 18°C. The heterozygous females produce daughters at any temperature. To test the zygotic effect of *da*, the females heterozygous with the Curly-balancer chromosomes (*Cy/da*) were mated with *da/da* males for the four different *da* strains (#47, #46, JP83, #233), and the sex ratios (♀/♂) were examined in each type of progenies (*Cy/da* and *da/da*). The parental flies were placed at 18°C, 22°C and 29°C, respectively, for 4–5 days before letting them deposit eggs. The sex ratios of the *Cy/da* progenies were normal; 0.92 for #47, 0.94 for #64, 1.00 for JP83 and 1.00 for #233 on the average for the three temperatures. At the 18°C and 22°C conditions, although the #47 strain showed a slight significant difference

Table 1. The sex ratio (No. of females/No. of males) of *Cy/da* and *da/da* progenies from the *Cy/da* females mated with *da/da* males in the four strains at three temperature

Tempt.	#47			#64			JP83			#233		
	No. ^{a)}	<i>Cy/da</i>	<i>da/da</i>	No.	<i>Cy/da</i>	<i>da/da</i>	No.	<i>Cy/da</i>	<i>da/da</i>	No.	<i>Cy/da</i>	<i>da/da</i>
18°C	6417	1.07	0.87	3980	0.94	0.83	2299	0.97	0.94	4190	0.95	1.00
22°C	5813	0.86	0.95	4528	0.94	0.86	3436	1.09	0.93	5987	1.04	0.98
29°C	4962	0.84	0.73	2055	0.94	0.58	2301	0.93	0.46	5833	1.00	0.58

a) Number of flies counted.

* and ***: Significant at 0.05 and 0.001 level, respectively.

between *Cy/da* (1.07) and *da/da* (0.87), 7 cases out of 8 showed no significant differences between the sex ratios of *Cy/da* and *da/da* progenies. However, at a higher temperature of 29°C, all strains showed a lack of females in the *da/da* progenies; 0.73 for #47, 0.58 for #64, 0.46 for JP83 and 0.58 for #233. Compared with their *Cy/da* sibs, all four cases showed statistically significant differences, and three of them were highly significant ($p < 0.001$). This data suggests a zygotic deleterious effect in the *da/da* daughters at 29°C.

Ovary Freezing for the Maintenance of Silkworm Genes

Jun KUSUDA and Kimiharu ONIMARU

We previously reported that the silkworm ovaries preserved in liquid nitrogen produced mature eggs retaining the capacity for embryogenesis and hatching.

To apply this method for stocking mutant genes of silkworm, we attempted to freeze ovaries of Moricaud silkworm (P^M) which has a dark epidermis. The P^M was crossed with Cambodge (Camb) to enhance the ability of parthenogenesis. Ovaries taken from larvae of the F1 hybrid, P^M /Camb, were cooled at a rate of 1°C/min to -35°C in the presence of 1.5 M glycerol and preserved in liquid nitrogen. After 2 days, they were rapidly thawed (500°C/min) and the glycerol was removed. Forty three frozen thawed ovaries were transplanted into wild type female larvae. Some ovaries developed to generate mature eggs according to the state of the host development. When emerging as adults, the moths were dissected to obtain mature eggs with a chorion. Collected eggs (536) were immersed in a warm water bath (46°C) for 18 min to induce parthenogenetic development. This was followed by the treatment with HCl solution (specific gravity 1.075 at 25°C) to prevent diapause initiation. After about 10 days, 14 larvae hatched from the eggs, of which 10 developed to the 5th instar larvae. Among them, five had clear dark epiderms as the donor worms did, and the remaining five had normal epiderms like the hosts. Thus, ovary cells carrying P^M maker gene survived under sub-zero temperatures, and this technique could be applied for the maintenance of other mutant genes of silkworms.

V. CYTOGENETICS

Karyological Survey of Wild Mice in Ogasawara Chichi Island

Yasuyuki KURIHARA, Mitsuru SAKAIZUMI¹⁾,
Kimiyuki TSUCHIYA²⁾ Yoshinobu HARADA
and Kazuo MORIWAKI

In 1977, we found mice with a Robertsonian (Rb) translocation (9, 15) in the Ogasawara Chichi island located about 1000 km south of Tokyo (Genet. Res. Camb. **43**: 277-287, 1984). To know the present status of Rb-translocation in this island, the mice were collected in December, 1985 and March, 1986. Total number of the mice collected are 89 from five localities in the Chichi island as summarized below (distance and direction from "Kitafukurozawa" where the Rb-mice were previously collected, were shown in the parentheses);

Locality	No. mice collected	Date of collection
Mikazuki Yama (15 km, NW)	12 (m: 6, f: 6)	Dec., '85
Suzaki (1.5 km, NW)	10 (m: 5, f: 5)	Dec., '85
Sub-Tropical		
Agriculture Center (1 km, N)	20 (m: 10, f: 10)	Dec., '85
Kitafukurozawa	32 (m: 14, f: 18)	Mar., '86
Kominato (500 m, W)	15 (m: 8, f: 7)	Mar., '86

¹⁾ Tokyo Metropolitan Institute of Medical Science.

²⁾ Miyazaki Medical College.

Chromosome preparations were made from testis in male and tail skin culture in female. No chromosomal variant was observed in the mice tested. The mice with Rb (9.15) inhabited 9 years ago have probably been eliminated from the natural populations in Ogasawara Chichi island, suggesting a general genetic instability of Rb-variants in wild populations.

**A Genetic Model for X-Y Dissociation and Testis Weight
in Intersubspecies Hybrids between
M. m. molossinus (BALB/c) and
*M. m. molossinus***

Hirotami T. IMAI, Won Ho LEE¹⁾, Kiauw Nio TJAN²⁾
and Kazuo MORIWAKI

A linkage test of genetic factors controlling the terminal association of X-Y chromosomes and the testis weight was made by using Japanese wild mice (*Mus musculus molossinus*), BALB/c, and their hybrids (F_1 , N_{2-s}). When male mice were classified by the frequency of precocious dissociation of X and Y at metaphase I (X//Y) and the relative testis weight (RTW; Testis weight/body weight) as High X//Y having $X//Y \geq 50\%$, Low X//Y ($X//Y < 50\%$), large sized testis (Lt; $RTW > 3.0$), and small sized testis (St; $PTW < 3.0$), both *molossinus* and BALB/c were characterized by (Low X//Y, Lt) whereas their F_1 hybrids were (High X//Y, St). Four types of progeny (High X//Y, St), (High X//Y, Lt), (Low X//Y, St), (Low X//Y, Lt) were segregated at the 2nd backcross generation, though only (Low X//Y, Lt) males were obtained from (Low X//Y, Lt) sires. We assumed two genes *Sxa* (sex chromosome association) and *Twr* (testis weight regulator), adjoined in the common part of the X and Y chromosomes and controlled the end-to-end association of the sex chromosomes and the size of testis, respectively. The *Sxa* and *Twr* alleles of BALB/c were denoted as (*Sxa*^a, *Twr*^a) and those of *molossinus* were (*Sxa*^b, *Twr*^b), where the terminal association of the X and Y chromosomes dissociates precociously at metaphase I in heterozygotes for *Sxa* (i.e., *Sxa*^a/*Sxa*^b), and *Twr*^b is dominant to *Twr*^a. The genotype expected in (High X//Y, St) males was (*Sxa*^a, *Twr*^a)/(*Sxa*^b, *Twr*^b), and that of low X//Y males and their parental stocks was either (*Sxa*^a, *Twr*^a)/(*Sxa*^a, *Twr*^a) or (*Sxa*^b, *Twr*^b)/(*Sxa*^b, *Twr*^b). The segregation of (Low X//Y, Lt) males from (High X//Y, St) sires was interpreted simply as a single recombinant, and (High X//Y, Lt) and (Low X//Y, St) as double recombinant in the mating of BALB/c females $\times$ F_1 males.

¹⁾ Department of Biology, Pusan National University, Pusan 607, Korea.

²⁾ Department of Biology, Bandung Inst. of Technology, JL. Ganesa 10, Bandung, Indonesia.

Chromosome Observations of Sarawak Ants

Kiauw Nio TJAN¹⁾, Hirotami T. IMAI, Masao KUBOTA²⁾

William L. BROWN Jr.³⁾, William H. GOTWALD Jr.⁴⁾

Hoi-Sen YONG⁵⁾ and Charles LEH⁶⁾

This is a preliminary report on the chromosomes of Sarawak ants. Collection of ants and chromosome preparations were made at Bako National Park (Kuching, Sarawak) from June 9 to June 13, 1985. A total of 37 colonies including 5 subfamilies, 21 genera, and 29 species were examined. The colonies collected were labelled as HI85-(19-54, 67). The identification of species was made by Brown and Kubota, and the chromosome observations were made by Tjan. One set of dry specimens was deposited with the Sarawaku Museum.

Table 1. Chromosome numbers of Sarawak ants

Taxa (Colony number)	Chromosome number (n) 2n
PONERINAE	
<i>Mystrium camillae</i> (HI85-19)	32
<i>Proceratium</i> sp. (HI85-29)	48
<i>Cryptopone testacea</i> (HI85-41, 43)	(9) 18
<i>Leptogenys myops</i> (HI85-23, 25)	(24) 48
<i>Leptogenys</i> sp. (HI85-53)	54
<i>Pachycondyla rubra</i> (HI85-44)	20
<i>Diacamma</i> sp. (HI85-51)	58
<i>Odontomachus similinus</i> (HI85-27)	44
<i>Anochetus</i> sp. (HI85-50)	(17) 34
CERAPACHYINAE	
<i>Cerapachys</i> sp. (HI85-47)	50
MYRMICINAE	
<i>Crematogaster</i> sp. (HI85-45)	26

¹⁾ Department of Biology, Bandung Inst. of Technology, J.L. Ganesa 10, Bandung, Indonesia.

²⁾ Nakasone 13, Odawara, Kanagawa-ken, 250 Japan.

³⁾ Department of Entomology, Cornell University, Ithaca, New York, 14853, U.S.A.

⁴⁾ Department of Biology, Utica College of Syracuse University, Burrstone Road, Utica, New York 13502, U.S.A.

⁵⁾ Department of Zoology, University of Malaya, Kuala Lumpur 59100, Malaysia.

⁶⁾ Sarawak Museum, Kuching, Sarawak, East Malaysia.

Table 1. (Continued).

Taxa (Colony number)	Chromosome number (n) 2n
<i>Oligomyrmex</i> sp. 1 (HI85-22)	36
<i>Oligomyrmex</i> sp. 2 (HI85-38)	44
<i>Pheidologeton</i> sp. (HI85-35)	42
<i>Lophomyrmex</i> sp. (HI85-32)	38
<i>Tetramorium smithi</i> (HI85-31)	26
<i>Tetramorium</i> sp. 2 (HI85-26, 24)	20
<i>Tetramorium</i> sp. 3 (HI85-28)	24
<i>Acanthomyrmex</i> sp. (HI85-42, 67)	24
<i>Pheidole</i> sp. 1 (HI85-39, 40)	(10) 20
<i>Pheidole</i> sp. 2 (HI85-21)	18
<i>Pheidole</i> sp. 3 (HI85-49)	(9) 18
<i>Pheidole</i> sp. 4 (HI85-46)	18
DOLICHODERINAE	
<i>Iridomyrmex cordatus</i> (HI85-36, 37)	16
FORMICINAE	
<i>Anoplolepis longipes</i> (HI85-54)	(17)
<i>Acropyga</i> sp. (HI85-20, 52)	(15) 30
<i>Pseudolasius</i> sp. (HI85-34)	30
<i>Camponotus</i> sp. 1 (HI85-30)	40
<i>Camponotus</i> sp. 2 (HI85-48)	20

Modes of Species Differentiation and Karyotype Alteration in Ants and Mammals

Hirotsami T. IMAI

Two closely related topics for speciation and karyotype evolution of ants and mammals were investigated. First, I attempted to analyze the role of karyotype alteration in speciation by using a two dimensional graph (S- K_G graph). When the number of species and dissimilar karyotypes involved in a genus are denoted as S and K_G , the genus can be represented as a point (S, K_G) on the S- K_G graph. If karyotype alterations always initiate speciation as suggested by the stasipatric speciation model, and if speciation occurs without karyotype alteration, the genus will fall in the area $K_G/S \geq 1$ and $K_G/S \leq 1$, respectively. Among 420 mammalian genera examined, the number of genera showing $K_G/S > 1$, $= 1$, and < 1 was 21 (8.9%), 57 (24.3%), and 157 (66.8%), respectively. The same result was obtained

also in ants, i.e., $K_g/S > 1$, $= 1$, and < 1 was 2, 6, and 23. These observations strongly indicate that visible karyotype alterations (i.e., Robertsonian rearrangement, pericentric inversion, and tandem growth of constitutive heterochromatin) do not always contribute to speciation.

Next, I analyzed the karyotype evolution of ants and mammals quantitatively, and found a general tendency for karyotypes to evolve as a whole towards increasing chromosome numbers and/or arm numbers mainly by centric fission and pericentric inversion changing from acrocentric to meta-centric (p.i. (AM)). Finally, I proposed the minimum interaction hypothesis for interpreting such uni-directional alteration in ants and mammals. The minimum interaction hypothesis assumes that the karyotype evolution proceeds toward minimizing the genetic risks due to reciprocal translocations, and that an increase in chromosome number by centric fission may be one such mechanism utilized. The non-random distribution patterns of translocation polymorphisms and Robertsonian polymorphisms found in ants [the former was found only in ants with low chromosome numbers ($n < 12$) but the latter was characteristic to high-numbered species ($n > 12$)], was proposed as supporting evidence for the minimum interaction hypothesis.

**The Exceptionally Low Chromosome Number $n=2$ in
an Australian Bulldog Ant, *Myrmecia piliventris*
Smith (Hymenoptera: Formicidae)**

Hirotami T. IMAI and Robert W. TAYLOR*

The Australian bulldog ants of the species rich genus *Myrmecia* are among the most primitive of all extant Formicidae. *Myrmecia* is characterized by the exceptionally wide range of chromosome numbers among its species. Known number include $n=5, 6, 11, 16, 19, 25, 30, 33$ and 42 (Imai, Crozier and Taylor 1977, *Chromosoma* **59**: 341). Also, it is clear that morphologically very similar species may differ greatly in chromosome number. We report here two karyotypically very different but morphologically very similar species, either of which would previously have been identified as *Myrmecia piliventris* Smith on the conventional basis of worker morphological characteristics.

During recent studies in southeastern New South Wales and near Canberra

* CSIRO, Division of Entomology, GPO Box 1700, Canberra, A.C.T., 2601, Australia.

we observed the chromosomes of two *M. piliventris* colonies from Black Mountain, Canberra (35°17'S, 149°13'E), one of which (HI85-171) had $n=34$, while the other (HI85-172) had the very low number $n=2$, $2n=4$ (Fig. 1). These two colonies must surely represent separate species, yet the morphological features which distinguish their workers are of a nature conventionally recognized merely as intraspecific variation (see, for example, the discussion of *M. piliventris* by Brown (1953, *Bull. Mus. Comp. Zool.* 111: 20). This, of course, has important implications for future taxonomic work on the genus. The species with $n=2$ has been confidently identified by R. W. Taylor as *M. piliventris*.

The $2n=4$ karyotype comprises two pairs of submetacentrics ($2K=4ST$), of which both chromosomes have well developed C-bands at the proximal regions of their long arms. Homologues of chromosome 1 are homomorphic, but those of chromosome 2 show remarkable heteromeorphism in C-bands, and in the size of their short arms.

Three other colonies collected from Jerrabomberra Hill, near Queanbeyan (35°21'S, 149°14'E), N.S.W. (HI85-188) and at a much lower elevation near Nelligen Creek Bridge. W. of Batemans Bay (35°43'S, 150°10'E), N.S.W. (HI85-211 and -241) also had the same $n=2$ karyotype ($K=2ST$ and $2K=4ST$), suggesting that the species involved is both stable and fairly widely distributed.

The even more remarkable chromosome number $n=1$, $2n=2$ was reported recently for a species of the *Myrmecia pilosula* (Smith) group by Crosland and Crozier (1986, *Science* 231: 1278). This karyotype comprises one pair of submetacentrics ($2K=2SM$) with large C-banded blocks at their pericentromeric regions. Among the smaller species of *Myrmecia*, *M. piliventris* and *M. pilosula* are morphologically rather distinct from each other, but their karyotypes can be systematically related. We assume that the $n=1$ *pilosula* karyotype would have been derived from $n=2$ karyotype by reciprocal translocation (though not necessarily in a lineage including morphologically *piliventris*-like species). More information about the details of C-bands and the karyology of other related species is desirable. *M. pilosula*, *M. piliventris*, and their many chromosomally unstudied relatives, must stand as prime analysis of karyotype evolution and speciation in ants.

H. T. IMAI was supported in this investigation by the Scientific Visitors Program of the Division of Entomology, CSIRO. Appropriately labeled

voucher specimens are deposited in the Australian National Insect Collection, Canberra.

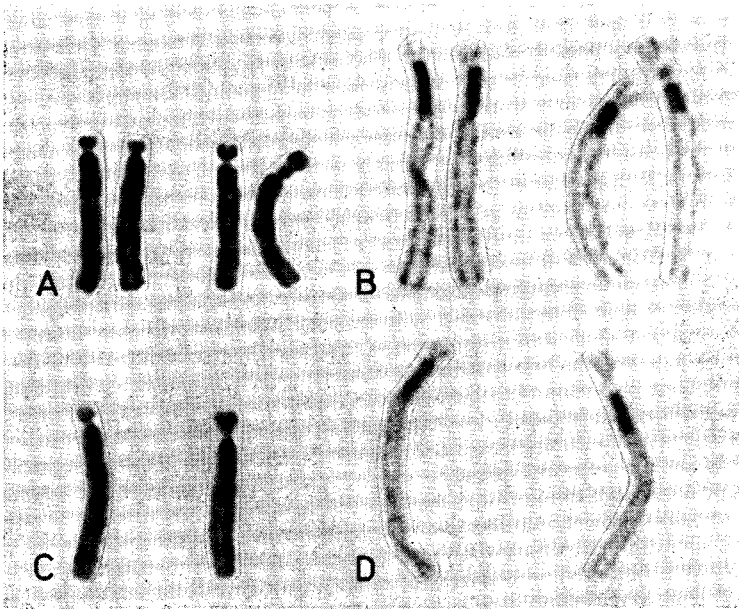


Fig. 1. Karyotypes of *Myrmecia piliventris*. A and B. $2n=4$ (worker). C and D. $n=2$ (male). A and C. Conventional staining by Guimosa. B and D. C-banded karyotypes.

VI. MUTAGENESIS AND RADIATION GENETICS

Evidence Suggesting High Sensitivity of Bone Marrow Cells to Radiation-Induced Cell Killing in the "Wasted" Mouse

Hideo TEZUKA, Tadashi INOUE and Tsuneo KADA

Bone marrow cells of 26 day-old wasted mice were found to be susceptible to gamma ray-induced chromosomal aberrations (Tezuka *et al.*, Mutation Res., **161**, 83). We examined the dose-response relationship between exposure to gamma irradiation and the induction of chromosomal aberrations in bone marrow cells (mainly proerythroblasts and erythroblasts), and the subsequent development of the aberrations to micronuclei in bone marrow polychromatic erythrocytes. The doses used were 0, 0.25, 0.5, 1 and 2 Gy. Normal appearing littermates served as controls. The frequency of cells with aberrations showed 2- to 4-fold increases in wasted mice over that in controls. The frequency of micronuclei, however, was 50-75% lower in wasted mice than in controls.

This indicates that most aberrant bone marrow cells die before nuclear expulsion in wasted mice. There may be a certain stage which is sensitive to cell killing during erythropoiesis. This is in contrast with results of *in vitro* experiments using wasted mouse-derived lung fibroblast cultures where the lack of radiosensitivity was shown (Inoue *et al.*, Cancer Res., **46**, 3979).

The Effect of Age on the Frequency of Spontaneous and Mutagen-Induced Micronuclei in Mouse Bone Marrow

Hideo TEZUKA, Koichi TAMAI¹⁾, Kazuo MURAKAMI²⁾
and Tsuneo KADA

In vivo mutagenicity tests in mammals are usually conducted on sexually mature young animals, 9 to 12 weeks old. Consequently, the effects of chemicals on very young animals are rarely observed. One way of over-

¹⁾ Health Science Research Institute, Yokohama.

²⁾ Sanwa Kagaku Kenkyusho, Co., Nagoya.

coming this is to score the frequency of micronuclei appearing in bone marrow polychromatic erythrocytes (BMPCE) of animals at various ages after birth. The appearance of such cells reflects chromosomal aberration induction in erythroblasts.

We examined BMPCE of male mice at 1, 3, 6, 9, 13 and 18 weeks of age. The mice were sacrificed 24 to 30 hours after treatment with mutagens. Unexposed animals of the same age served as controls. The frequency of micronuclei in controls remained constant at low frequencies. In mutagen-treated animals, the frequency of micronuclei was significantly higher than that of controls across all age groups. However, the effect of age on the frequency of such mutagen-induced micronuclei differed with the type of agent. A marked induction of micronuclei was observed in 1 week old mice treated either with a DNA crosslinking agent mitomycin C or with alkylating agents such as methylmethanesulfonate or cyclophosphamide. In older mice, the frequency of such micronuclei decreased with age. In contrast, the frequency of micronuclei in mice treated with vincristine sulfate, a spindle poison, was low in 1 to 6 week old mice, but increased markedly in 9 week old mice, and thereafter remained constant.

The results presented suggest the involvement of multiple cellular mechanisms, including DNA repair, in the induction of micronuclei. The enzyme activity involved in such mechanisms may be altered with developmental stages.

Cancer Susceptibility of the "Wasted" Mouse

Tadashi INOUE, Junko TAKAHASHI, Akira OOTSUYAMA¹⁾
Hideo TEZUKA and Tsuneo KADA

The mouse mutant "wasted" (*wst/wst*) is a putative animal model for Ataxia-Telangiectasia (A-T), a human genetic disease characterized by chromosomal instability, radiation-hypersensitivity and increased predisposition to cancer. Although the wasted mouse shares many characteristics with A-T, cancer susceptibility has not been examined because of the short life span of the mutant. To overcome this, we performed skin grafts from normal and wasted mice onto recipient normal mice. We then sequentially applied the cancer initiator dimethylbenzanthracene and the cancer promoter TPA to the grafted areas of the recipients. While several papillomas

¹⁾ National Cancer Center Research Institute.

developed on grafts from healthy controls, no tumors appeared on skin from mutant mice. This indicates that some functions required for cancer induction are deficient in the wasted mouse.

Effects of Low-Level Tritiated Water Ingestion and Age on the Number of Sister Chromatid Exchanges in Mouse Bone Marrow Cells

Hideo TEZUKA, Robert D. BENZ,¹⁾ Arland L. CARSTEN¹⁾
and Takaji IKUSHIMA²⁾

We quantitated the number of sister chromatid exchanges (SCEs) occurring in male BNL-H/S mice maintained on 3.0, 7.5, 15 or 30 $\mu\text{Ci/ml}$ tritiated water (HTO) for 1, 2, 4, 8, 12, 18 or 22 weeks. Identically housed mice kept on normal water served as controls. Following the predetermined exposure time, animals were infused with bromo-deoxyuridine and then sacrificed. Their bone marrow cells were removed, processed and the number of SCEs counted. In the control groups, the number of SCEs/cell decreased slightly, but significantly with age. The number of SCEs in animals exposed to the various levels of HTO was essentially identical and remained constant with age. The difference in the numbers of SCEs between control and exposed mice slowly increased with the duration of exposure. We speculate that this "anti-aging" effect of low level tritium irradiation may be caused by constant accumulation of trace DNA damage or by constant stimulation/induction of enzymes involved in the repair of radiation-induced damages.

Radiation Induced Chromosome Aberrations in the Radiation Sensitive Mutants of *Caenorhabditis elegans*

Yoshito SADAIE and Tamiko SADAIE

The fraction of aberrant metaphase chromosomes in gamma irradiated early embryos of *C. elegans* decreased substantially upon incubation of the gravid worms following exposure. The decrease was blocked by the *rad-2* mutation but not by the *rad-1* mutation of the same epistasis group which

¹⁾ Medical Department, Brookhaven National Laboratory, Upton, NY 11973, USA.

²⁾ Research Reactor Institute, Kyoto University, Osaka.

makes worms sensitive to radiation and chemicals. Eggs laid immediately after irradiation by the *rad-2* mutant showed low hatchability. However, those laid some time after exposure hatched normally. This suggests that chromosomal aberrations are not fatal in *C. elegans* which has holocentric chromosomes.

Mechanisms of Bio-Antimutagenesis

Tsuneo KADA and Tadashi INOUE

Agents suppressing cellular mutagenesis have been known for some time and their modes of action have been analyzed in the field of bacterial genetics. More recently, a number of mutagens have been detected in our environment and their genotoxicities recognized. Therefore, it is necessary to have knowledge about antimutagens and their modes of action in order to assess the genotoxic nature of our environment.

The word antimutagen has an old origin and has been adopted for factors which reduce the rates of spontaneous and induced mutagenesis by different modes of action. We proposed a distinction among categories of antimutagens. The "desmutagens" relate to chemical or biochemical modification of mutagens outside the cells. On the other hand, factors, interfering with cellular functions producing genetically stable informative genes from primary damages in DNA, should be distinguished from desmutagens and named "bio-antimutagens".

Our studies on mechanisms of bio-antimutagenesis were recently reviewed (T. Kada, T. Inoue, T. Ohta and Y. Shirasu: in Antimutagenesis and Anticarcinogenesis: Mechanisms, Ed. D. M. Shankel et al., Plenum Press, New York, pp. 181-198, 1986). The followings are the major mechanisms so far studied *in vitro* in the bacterial systems.

- a) Increase in the fidelity of DNA replication (epigallo-catechin-gallate, EGCg, a green tea factor).
- b) Repair promotion of DNA damage (cinnamaldehyde, cobaltous chloride, tannic acid, etc.). Involvement of recombination repair involving *recA* protein as well as UV excision repair are shown.
- c) Inhibition of error-prone (SOS) repair (5-fluoro-uracil and cordycepin (3'-dA)).

Dietary Desmutagens

Tsuneo KADA

The word "desmutagen" was proposed to indicate those agents which inactivate mutagens by reacting directly with them (Kada et al., 1981). In addition, many investigators are aware that factors exist which reduce the yield of mutations by indirect means, such as inhibition in the formation of mutagens from precursors or by inhibition of metabolic activation of mutagens. These agents should be also classified as "desmutagens".

In earlier studies, we found that mutagens are produced by a reaction between sorbic acid and nitrite and that the mutagenic activities were irreversibly lost by treatment with extracts obtained from different kinds of vegetables. Crude extracts prepared from a number of vegetables suppressed, *in vitro*, the mutagenicities of pyrolysate mutagens formed in proteinous food through heating. Analysis of cabbage desmutagens showed that a hemo-protein possessing peroxidase activity would enzymatically inactivate Trp-P-1 or Trp-P-2 (Inoue et al., 1981). Later studies showed that heat-resistant desmutagenic factors exist in vegetables. Studies on burdock revealed that high molecular ingredients could adsorb mutagens, resulting in an irreversible loss of mutagenic activity (Morita et al., 1984).

More recently, we found that isolated fibers of many vegetables adsorb pyrolysate mutagens such as Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, IQ, etc. (Kada et al., 1984). It seems that the bindings are very firm and that mutagens can be recovered partially only under special conditions such as grinding with special organic solvents.

When mutagens are working as initiators of cancer, many desmutagens can be considered "anticarcinogens" whose modes of actions must involve the above suggested models of desmutagenesis outside and inside the animal and human body. Obviously the *in vivo* events may be much more complex.

Mutagenic Activities of EMS on Somatic and Recessive Mutations in the Soybean Test System

Taro FUJII and Shigemitsu TANO*

Somatic mutation is an event occurring in differentiated somatic cells as

* Faculty of Agriculture, University of Tokyo.

a result of mutagenic treatments or spontaneous mutation. Recessive mutation which can be recognized in the M_2 generation is true genetic damage since this event is inherited in the following generations by transmission through germ cells. However, somatic mutation events and the induction of genetic damage have positive correlations since the events take place randomly among the cell population in the tissue, and some of the cell(s) involving the gene mutation will succeed to the germ line through differentiation. To study this problem, somatic mutations induced by ethyl methane-sulfonate (EMS) in M_1 plants and the segregation of chlorophyll deficient mutants in the M_2 population were investigated with soybean plants (strain T-219). Localization and distribution of the drug in the seed tissue were traced using ^{14}C -labeled EMS.

Treatment with 0.05% EMS induced about 16 spots per leaf together with more than 30 spots in other leaves of the heterozygotes. Most of the leaves at the 0.1 and 0.2% treatment plots showed abnormal growth, and had 30 or more spots, which were not scored accurately. Yellow green spots appearing on the leaves of the dominant homozygotes were also examined. More than 20 spots per leaf were observed at the 0.1% treatment plot, and a large number of spots was observed with the highest EMS concentration treatment.

Segregation of the dominant and recessive homozygotes and heterozygotes from the heterozygotic M_1 plants showed a nearly 1:2:1 ratio in all treatment plots. The results indicated no selective killing effect of the mutagen for the two types of germ cells having Y_{11} or y_{11} genes, indicating the dominant and recessive genes may respond equally to EMS. Some of the M_2 families had segregated mutants, for instance, at the 0.2% EMS treatment plot, 10 (6.3%) of the 146 M_2 plants, derived from 51 dominant homozygotes and 95 heterozygotes, had segregated mutant seedlings. Dose vs. mutation frequency relationships were not similar for somatic and recessive mutations, there was an almost linear relationship for the former and a leveling off at a high EMS concentration for the latter, respectively. In the M_2 generation, chlorophyll mutation frequencies per μg of EMS were estimated to be 0.14, 0.61 and 0.41% at 0.05, 0.1 and 0.2% treatments, respectively. The present results indicate that the scoring of somatic mutations can aid in the assessment of the extent of recessive mutational events. However, more detailed studies are required to obtain the conversion factor from somatic mutation to recessive mutation because the events may vary

with the kinds of mutagens and test systems.

Origin and Genetic Background of Three Substrains in Inbred PW Mouse Strain

Kiyosi TUTIKAWA, Yoshimasa HARADA and Kazuo MORIWAKI

In order to develop the experimental basis for a standard protocol of the mouse spot test using available strains of mice in Japan, the work group in which members from 10 laboratories collaborate, have started to study with ethylnitrosourea by using the cross between any non-agouti strain and PW/a substrain. In this report the origin and genetic background of three PW substrains will be described briefly.

One of the authors (K.T.) received one adult male and two pairs of young mice in the same litter which derived from the cross involving strains NB,

Table 1. Alleles presented in PW/a, PW/b and PW/c substrains of mice

Locus	Chromosome	Alleles present		
		PW/a	PW/b	PW/c
Idh-1	1	a	a	a
Pep-3	1	b	b	b
Akp-1	1	b	b	b
Hc	2	l	l	l
a	2	a	a	a
Car-2	3	b	b	b
b	4	b	b	b
Gpd-1	4	b	b	a
Pgm-1	5	b	b	b
Ldr-1	6	b	b	b
Gpi-1	7	a	a	a
p	7	p	p	p
c	7	c ^{eh}	c ^{eh}	c ^{eh}
Hbb	7	s	s	s
Es-1	8	b	b	b
Es-2	8	b	b	b
d	9	d	d	d
se	9	se	se	se
Mod-1	9	b	b	b
Trf	9	b	b	b
Es-3	11	a	a	a

LU and AC from Dr. M. C. Green at Jackson Laboratory, Bar Harbor, in October 1956. The progeny were transferred to National Institute of Genetics, Mishima, in 1957. After six alleles fixed to *aa*, *bb*, *c^{ch}p/c^{ch}p*, *d se/d se*, sib-mating started with two pairs from different litter in 1961. Thereafter two substrains 'a' and 'c' inbred under full-sib mating regime independently each other for 88 and 77 generations. Substrain 'b' separated from 'a' at 66 generation and maintained further twenty generations. Three substrains of the PW strain were thus formed, designated PW/a, PW/b and PW/c.

As shown in Table 1, three substrains share the same alleles at twenty loci tested. Only one allele of *Gpd-1* (on chromosome 4) differ between PW/c and the other two substrains. In addition, all substrains show the identical chromosome C-band patterns consisting of all positively stained chromosomes. The same H-2 haplotype (*H-2^b*) carrying *H-2K.33* and *H-2D.2* is present in three substrains.

VII. POPULATION GENETICS

The Role of Compensatory Neutral Mutations in Molecular Evolution

MOTOO KIMURA

A pair of mutations at different loci (or sites) which are singly deleterious but restore normal fitness in combination may be called compensatory neutral mutations. Population dynamics concerning evolutionary substitutions of such mutants were developed by making use of the diffusion equation method. Based on this theory and, also, by the help of Monte Carlo simulation experiments, a remarkable phenomenon was disclosed that the double mutants can easily become fixed in the population by random drift under continued mutation pressure if the loci are tightly linked, even when the single mutants are definitely deleterious. More specifically, I consider two loci with alleles A and A' in the first locus, and alleles B and B' in the second locus, and assign relative fitnesses 1, $1-s'$, $1-s'$ and 1 respectively to the four gene combinations AB , $A'B$, AB' and $A'B'$, where s' is the selection coefficient against the single mutants ($s' > 0$). Let v be the mutation rate per locus per generation and assume that mutation occurs irreversibly from A to A' at the first locus, and from B to B' at the second locus, where A and B are wild type genes, and A' and B' are their mutant alleles. In a diploid population of effective size N_e (or a haploid population of $2N_e$ breeding individuals), it was shown that the average time ($\bar{T}$) until joint fixation of the double mutant ($A'B'$) starting from the state in which the population consists exclusively of the wild type genes (AB) is not excessively long even for large $4N_e s'$ values. In fact, assuming $2N_e v = 1$ we have $\bar{T} = 54N_e$ for $4N_e s' = 400$, and $\bar{T} = 128N_e$ for $4N_e s' = 1000$. These values are not unrealistically long as compared with $\bar{T} \approx 5N_e$ obtained for $4N_e s' = 0$. The approximate analytical treatment has also been extended to estimate the effect of low rate crossing over in retarding fixation. The bearing of these findings on molecular evolution is discussed with special reference to coupled substitutions at interacting amino acid (or nucleotide) sites within a folded protein (or RNA) molecule. It is concluded that compensatory neutral mutants may play an important role in molecular evolution. For details, see J.

Genet. **64**, 7–19.

Variances and Covariances of Identity Coefficients of a Multigene Family

Tomoko OHTA

By using a model of gene conversion for treating concerted evolution of multigene families, a theory for calculating the variances and covariances of identity coefficients has been developed. Six coefficients on triple identity and 15 coefficients on quadruple identity are needed. The variances and covariances are obtained from the quadruple identity coefficients and divided into within-population and between-population components. The former represent the variation of identity coefficients among individuals within a population, whereas the latter represent the magnitude of fluctuation of population averages. Monte Carlo simulations confirmed the theoretical predictions at equilibrium. For details, see Proc. Natl. Acad. Sci. USA **82**, 829–833.

A Model of Duplicative Transposition and Gene Conversion for Repetitive DNA Families

Tomoko OHTA

A model of duplicative transposition and gene conversion for the evolution of repetitive DNA families was studied. In this model, transposition and conversion (both unbiased) are assumed to occur both within and between the genomes in a diploid cell, and any degree of linkage intensity is incorporated. The transition equations for allelic and nonallelic identity coefficients have been formulated by using the previous results. The results are widely applicable to many repetitive sequences, from dispersed families like transposons to tightly linked multigene families. It has been shown through extensive numerical studies on equilibrium properties that duplicative transposition and gene conversion have very similar effects on nonallelic identity coefficients, but that allelism and allelic identity are greatly influenced by the relative rates of occurrence of the two processes. For details, see Genetics **110**, 513–524.

Reciprocal Altruism and Reciprocal Alliance between Relatives

Kenichi AOKI

The inclusive fitness approach to two-policy games between relatives is shown to be approximately valid, based on a population genetic model which assumes quantitative inheritance of the behavioral trait. The theory is applied to the iterated prisoner's dilemma game, and a condition is derived for TIT FOR TAT reciprocal altruism to evolve in terms of the requisite degree of relatedness between the interacting reciprocal altruists. A simulation of reciprocal altruism between full sibs supports the approximate analytical results. An extension of the model to treat reciprocal alliance is then presented, in which the allies and the common enemy are equally related. It is argued that Packer's description of reciprocal alliance among male anubis baboons is difficult to reconcile with theory. For details see Ohta, T. and Aoki, K. (ed.) *Population Genetics and Molecular Evolution*, pp. 429-441, Jpn. Sci. Press, Tokyo/Springer-Verlag, Berlin.

Gene Diversity in Finite Populations

Naoyuki TAKAHATA

DNA sequence comparison among homologous genes sampled at random from one or two populations allows one to estimate the ultimate amount of genetic variation maintained in a population and to construct the gene genealogy within and between populations. Moreover, if one uses the constant-rate or molecular clock hypothesis, it is possible to infer the divergence pattern and time of populations under study. In this note, various statistics concerning the above are studied when only two genes sampled at random from two related populations are involved. In particular, formulas for estimating the mean and standard error of time, t , of population splitting are derived as

$$\hat{t} = -\frac{3}{8\mu} \log \left(1 - \frac{4}{3}p \right) - 2N$$

$$\sigma(\hat{t}) = \left[\left(1 - \frac{1}{n} \right) (2N)^2 + \frac{1}{n} p (1-p) \right] / \left\{ 2\mu \left(1 - \frac{4}{3}p \right) \right\}^2 \right]^{1/2}$$

where N is the effective size of the ancestral populations, n the number

of nucleotide sites compared, μ the mutation rate per site and p the proportion of nucleotide differences per site. It is seen that the estimated divergence time of homologous sequences always exceeds that of populations by $2N$ and that there is an intrinsic error in $\hat{t}$ even when DNA sequences of infinite length are compared. These results have an important bearing particularly on inference of divergence times of closely related species from DNA sequence comparisons. For details, see *Genet. Res. Camb.* **46**, 107–113.

Gene Genealogy and Variance of Interpopulational Nucleotide Differences

Naoyuki TAKAHATA and Masatoshi NEI

A mathematical theory is developed for computing the probability that m genes sampled from one population (species) and n genes sampled from another are derived from l genes that existed at the time of population splitting. The expected time of divergence between the two most closely related genes sampled from two different populations and the time of divergence (coalescence) of all genes sampled are studied by using this theory. It is shown that the time of divergence between the two most closely related genes can be used as an approximate estimate of the time of population splitting (T) only when $T \equiv t/(2N)$ is small, where t and N are the number of generations and the effective population size, respectively. The variance of Nei and Li's estimate (d) of the number of net nucleotide differences between two populations is also studied. It is shown that the standard error (s_d) of d is larger than the mean when T is small ($T \ll 1$). In this case, s_d is reduced considerably by increasing sample size. When T is large ($T > 1$), however, a large proportion of the variance of d is caused by stochastic factors, and an increase in the sample size does not help to reduce s_d . To reduce the stochastic variance of d , one must use data from many independent unlinked gene loci. For details, see *Genetics* **110**, 325–344.

The Average Frequency of Private Alleles in a Partially Isolated Population

Montgomery SLATKIN and Naoyuki TAKAHATA

An analytic model is developed to explore the relationship between gene

flow, selection, and genetic drift. We assume that a single copy of a mutant allele appears in a finite, partially isolated population and allow for the effects of immigration, genic selection, and mutation on the frequency of the mutant. Our concern is with the distribution of the mutant's frequency before it either is lost from the population or emigrates. Before either of these events, the allele will be a "private allele" and would be found in only one of several populations in a larger collection. Slatkin [(1985) *Evolution* **39**, 53–65] found several simple properties of private alleles in his simulations. We use the method developed by Karlin and Tavaré [(1980) *Genet. Res.* **37**, 33–46; (1981a), *Theor. Pop. Biol.* **19**, 187–214; (1981b) *Theor. Pop. Biol.* **19**, 215–229] for a model similar to ours to obtain a diffusion equation with a "killing term", and obtain the mean and variance of the mutant's frequency and its expected frequency in samples of a specified size. There is only fair agreement between the analytic results from this model and those from Slatkin's (loc. cit.) simulations. The difference indicates that the distribution of private alleles in the simulation results is not due to alleles in the demes in which they first appeared, but due to private alleles after they have spread to other demes. Also, the rescaling method used to obtain the results indicates that if emigration is relatively frequent, the distribution of rare alleles is governed largely by the balance between genetic drift and emigration, with selection, mutation, and immigration playing a lesser role. For details, see *Theor. Pop. Biol.* **28**, 314–331.

Extranuclear Differentiation and Gene Flow in the Finite Island Model

Naoyuki TAKAHATA and Stephen R. PALUMBI

Use of sequence information from extranuclear genomes to examine deme structure in natural populations has been hampered by a lack of clear linkage between sequence relatedness and rates of mutation and migration among demes. Here, we approach this problem in two complementary ways. First, we develop a model of extranuclear genomes in a population divided into a finite number of demes. Sex-dependent migration, neutral mutation, unequal genetic contribution of separate sexes and random genetic drift in each deme are incorporated for generality. From this model, we derive the relationships between gene identity probabilities (between and within demes) and migration rate, mutation rate and effective deme size.

Secondly, we show how within- and between-deme identity probabilities may be calculated from restriction maps of mitochondrial (mt) DNA. These results, when coupled with our results on gene flow and genetic differentiation, allow one to estimate the relative interdeme gene flow when deme sizes are constant and genetic variants are selectively neutral. We illustrate the use of our results by reanalyzing published data on mtDNA in mouse populations from around the world and show that their geographic differentiation is consistent with an island model of deme structure. For details, see *Genetics* **109**, 441–457.

Introgression of Extranuclear Genomes in Finite Populations: Nucleo-Cytoplasmic Incompatibility

Naoyuki TAKAHATA

A 'two locus two allele' model is developed with special reference to the introgression of extranuclear genomes between two species of finite size. The model assumes that one locus, coded by a nuclear genome, causes the reproductive barrier while the other locus, coded by an extranuclear genome, causes nucleo-cytoplasmic incompatibility in particular genotypes. To fully study this model, simulations are conducted, and a diffusion equation is derived when introgression or extranuclear gene flow occurs in one direction. It is shown that although selection against the nuclear genome can reduce the levels of extranuclear gene flow and retard the introgression process, the dynamics are very similar to those without such selection. In contrast, the nucleo-cytoplasmic incompatibility directly affects the dynamics of introgressing extranuclear genomes: in large populations the ability of incompatibility to overcome extranuclear gene flow is conspicuous, but in small populations it is overshadowed by random sampling drift. Paternal leakage of extranuclear genomes, if present, is of evolutionary importance only when the male's migration rate is much larger than the female's. When the sizes of two populations are unequal, the introgression is most likely to occur from the larger population to the smaller one in the absence of mating preferences of backcross progeny. Recent observations on interspecific mitochondrial transfer in various species do not support the ubiquitousness of nucleo-cytoplasmic incompatibility as an efficient reproductive barrier. For details, see *Genet. Res. Camb.* **45**, 179–194.

Genetic Homozygosity in Population which Experience Periodic Bottlenecks

Takeo MARUYAMA and Paul A. FUERST

The amount of variability in a population that experiences repeated restrictions in population size has been calculated. The restrictions in size occur cyclically with a fixed cycle length. Analytical formulas for describing the gene identity at any specific time in the expanded and restricted phases of the cycle, and for the average and second moment of the gene identity, have been derived. It is shown that the level of genetic diversity depends critically on the two parameters that account for the population size, mutation rate and the time of duration for each of the two phases in the cycle. If one or both of these composite parameters are small, the gene diversity will be much reduced, and population gene diversity will then be predictable from knowledge of the harmonic mean population size over the entire cycle. If these parameters take on intermediate values, diversity changes constantly during the cycle, fluctuating steadily from a high to a low value and back again. If these parameters are large, gene diversity will fluctuate rapidly between extreme values and will stay at the extremes for long periods of time. For details, see *Genetics* 111: 691-703 (1985).

Number of Alleles in a Small Population that was Formed by a Recent Bottleneck

Takeo MARUYAMA and Paul A. FUERST

A model is presented in which a large population in mutation/drift equilibrium undergoes a severe restriction in size and subsequently remains at the small size. The rate of loss of genetic variability has been studied. Allelic loss occurs more rapidly than loss of genic heterozygosity. Rare alleles are lost especially rapidly. The result is a transient deficiency in the total number of alleles observed in samples taken from the reduced population when compared with the number expected in a sample from a steady-state population having the same observed heterozygosity. Alternatively, the population can be considered to possess excess gene diversity if the number of alleles is used as the statistical estimator of mutation rate. The deficit in allele number arises principally from a lack of those alleles that are

expected to appear only once or twice in the sample. The magnitude of the allelic deficiency is less, however, than the excess that an earlier study predicted to follow a rapid population expansion. This suggests that populations that have undergone a single bottleneck event, followed by rapid population growth should have an apparent excess number of alleles, given the observed level of genetic heterozygosity and provided that the bottleneck has not occurred very recently. Conversely, such populations will be deficient for observed heterozygosity if allele number is used as the sufficient statistic for the estimation of $4Nv$. Populations that have undergone very recent restrictions in size should show the opposite tendencies. For details, see Genetics 111: 675–689 (1985).

Dynamics of Homozygous Genetic Loads in Connection with the Invasion of Transposons

Terumi MUKAI

For the 1970 and 1984 Raleigh, N. C. population of *Drosophila melanogaster*, homozygous lethal loads (L) and detrimental loads (D) in the second chromosomes were estimated. The results were: $D=0.334$ and 0.237 ; $L=0.501$ and 0.275 . The former figures are the estimates of 1970 (from MUKAI and YAMAGUCHI 1974, Genetics 76: 339–366) and the latter are of 1984 (from Baba, H. et al., unpublished). It is obvious that the amounts of homozygous loads became about one half after 14 years. Without knowing the true nature of the P elements, we have reported the frequency of the P element-carrying (mutator-carrying) second chromosomes to be 0.66 ± 0.059 for the 1970 population (cf. YAMAGUCHI, CARDELLINO and MUKAI 1976, Genetics 83: 409–422). Thus, the above decrease in homozygous loads can be explained as due to the increase in the frequency of the P element-carrying individuals to the level of 100%, where increased mutation rate as one of the syndrome of the P-M hybrid dysgenesis returned to the level of the original mutation-selection balance. Under the above condition, the formulae reported last year [Ann. Report 35: 66–67] were employed for the lethal load and the time when the P elements immigrated into the Raleigh population was estimated to be about 40 (assuming $\alpha=0.25$) and about 50 generations (assuming $\alpha=0.20$) before the summer of 1970, where α is the fraction of individuals of cytotype P in the offspring of M (female) $\times$ P (male) crosses. This may suggest that the rapid invasion of the P element-

carrying individuals into the Raleigh, N. C. population had occurred. In this calculation, the frequency of individuals carrying the *P* elements (P_0) was assumed to be 0.0001, since the effective size of the population was estimated to be ca. 20,000. The results of the above calculation accord well with the change in the frequency of lethal-carrying second chromosomes after the invasion of the *P* elements.

The present experimental results together with those of MUKAI et al. (1985, PNAS USA **82**: 7671–7675) suggest the following process after the invasion of the *P-M* type transposons into the population consisting of the *M* type individuals: When the transposons enter the population, they induce viability mutations. As the results, homozygous loads increase. When all the individuals become the *P* type, the mutation rate returned to the original rate. Accordingly, the homozygous loads gradually return to the standard level.

VIII. EVOLUTIONARY GENETICS

Homology between *P*-Transposable Element of *Drosophila melanogaster* and Bacterial Transposase Gene of Tn3

Yoko SATTA, Takashi GOJOBORI, Takeo MARUYAMA
Kaoru SAIGO and Sadao I. CHIGUSA

With the aid of a computer, each of the four open reading frames (ORFs) in the *P*-transposable element of *Drosophila melanogaster* was aligned with the amino acid sequence of the transposase (TnpA) of bacterial transposon Tn3. The four ORFs, named ORF0, -1, -2, and -3 lined in this order from 5' to 3' direction in the *P* element, turned out to have on the average 18 percent homologies with the Tn3 transposase in the same order without much overlap between adjacent ORFs. Based on the comparison with consensus sequences at exon-intron junctions, three possible introns were predicted in the *P* element. Each of these putative introns covers the region between adjacent ORFs and these splicings do not alter the initially predicted reading frames. It appears, therefore, that a mature mRNA processed from the transcript of the whole *P* element codes a single polypeptide having homology to TnpA. This suggests that the *P* element and the TnpA gene may have diverged from a common ancestor. Taking account of our previous finding that the resolvase of Tn3 has a sequence homology with ORF1 of the *P* element, evolutionary relationships among the *P* element, the resolvase gene and the TnpA gene were discussed. For details, see Jpn. J. Genet. 60: 499-503 (1985).

Tn3 Resolvase-like Sequence in *P* Transposable Element of *Drosophila melanogaster*

Yoko SATTA, Takashi GOJOBORI, Takeo MARUYAMA
and Sadao I. CHIGUSA

Using each of the four open reading frames in the *P* transposable element of *Drosophila melanogaster*, computer search of homology was carried out against the protein sequence data base of NBRF version 4. A deduced polypeptide of about 210 residues in ORF1 of the *P* element appears to have

a significant homology with the resolvases of bacterial transposable elements Tn3 and $\gamma\delta$. The polypeptide seems to possess a region consisting of 22 amino acid residues having homology with a DNA-binding fold of the resolvases of the bacterial transposons Tn3 and $\gamma\delta$, which are known to have a repressor function of the transposable elements. There are a nine base pair region in ORF3 and also in the 31 base pair inverted terminal repeat of the *P* element having homology with a site in the promoter region of the Tn3 and also of $\gamma\delta$ at which the resolvases bind and suppress transcription of transposases. Based on these findings, it was suggested that the ORF1 might code a resolvase protein which mediates site-specific recombination and also suppresses the transposition of the *P* by binding at a specific site in the ORF3 or in the terminal repeat. It was also suggested that the whole *P* might code a transposase. For details, see Jpn. J. Genet. **60**: 261-266 (1985).

Evolutionary Features of Oncogenes

Takashi GOJOBORI

Although the rate of nucleotide substitution for the cellular oncogene is comparable to that for many functional genes in DNA genomes, the rate for the viral oncogene in the RNA genome is roughly a million times greater. Nevertheless, the viral oncogene still retains the general feature of functional genes that the rate of synonymous substitution is much higher than that of nonsynonymous substitution. This suggests that nucleotide substitution in the RNA genome is consistent with Kimura's neutral theory of molecular evolution. Moreover, the phylogenetic tree of cellular oncogenes shows that the *src* family of oncogenes including the *c-mos* gene may share a common ancestral sequence with the yeast cell division control gene, suggesting that the function of oncogenes in the *src* family is related to the control of cell division. For details, see Population Genetics and Molecular Evolution (Eds. Ohta, T. and Aoki, K.), pp. 353-368.

Rates of Evolution of the Retroviral Oncogene of Moloney Murine Sarcoma Virus and its Cellular Homologues

Takashi GOJOBORI and Shozo YOKOYAMA

A method is proposed for computing the rates of nucleotide substitution

for an oncogene of a retrovirus (*v-onc*), its cellular homologue (*c-onc*), and the retrovirus genome simultaneously. The method has been applied to DNA sequences of the *v-mos* gene of Moloney murine sarcoma virus (Mo-MuSV) and the *c-mos* and *gag* genes of Mo-MuSV and Moloney murine leukemia virus (Mo-MuLV). The rates of nucleotide substitution for *c-mos*, the *gag* gene, and *v-mos* are estimated to be 1.71×10^{-8} , 6.3×10^{-4} , and 1.31×10^{-3} per site per year, respectively. The rate of evolution of *c-mos* is comparable to that of many functional genes in DNA genomes, suggesting some important biological function played by cellular oncogenes. The rates of nucleotide substitution in the *v-mos* and *gag* genes are very high and similar to those of RNA viral genes such as the hemagglutinin and neuraminidase genes in the influenza A virus. Thus, oncogenes seem to exemplify a general feature of genome evolution: the rate of evolution of RNA genomes can be more than a million times greater than that of DNA genomes because of a high mutation rate in the RNA genome. For details, see Proc. Natl. Acad. Sci. USA **82**: 4198–4201.

**Complete Nucleotide Sequence of an Infectious Clone
of Human T-Cell Leukemia Virus Type II:
An Open Reading Frame for the Protease Gene**

Kunitada SHIMOTOHNO, Yuri TAKAHASHI, Nobuaki SHIMIZU
Takashi GOJOBORI, David W. GOLDE, Irvin S. Y. CHEN
Masanao MIWA and Takashi SUGIMURA

The entire nucleotide sequence of an infectious clone of human T-cell leukemia virus type II provirus was determined. This provirus consists of 8,952 nucleotides. In addition to long terminal repeats and *gag*, *pol*, *env*, and *X*, a protease gene that is responsible for processing the *gag* precursor protein was found. The protease gene is encoded in a different frame from *gag* and *pol* and was located between the *gag* and *pol* open reading frames. Coding regions of the provirus show about 60% homology with those of human T-cell leukemia virus type I at the nucleotide level. This finding suggests that human T-cell leukemia virus types I and II have diverged from a common ancestral genome. For details, see Proc. Natl. Acad. Sci. USA **82**: 3101–3105.

DNA Polymorphism of AIDS Virus

Takashi GOJOBORI, Shozo YOKOYAMA and Takeo MARUYAMA

To quantify the degree of DNA polymorphism of human retrovirus isolated from patients with the acquired immune deficiency syndrome (AIDS), population genetics theory was applied to restriction endonuclease maps of four closely related clones of HTLV-III. In about 9 kb of the region where the four clones of λ HXB2, λ HXB3, λ BH5/8, and λ BH10 share (Shaw et al., Science **226**: 1165–1171, 1984), we computed the mean number of restriction-site differences ($\nu=2.66$), the number of segregating restriction sites ($k=5$), and the nucleotide diversity ($\pi=0.012$). In particular, our estimate of π was confirmed by the nucleotide sequence comparisons among the AIDS virus genomes (Ratner et al., Nature **313**: 636–637, 1985). These findings provide two different views concerning evolution of the AIDS virus. First, if the virus population is at a steady state, the estimated three quantities (ν , k , and π) consistently imply that $2Nu$ is about 0.01, where N is the effective population size and u is the mutation rate per nucleotide. Hence, if mutation rate is of the order of 10^{-8} , as reported, the effective population size must be extremely small, possibly of the order of 100 or less. Secondly, if the population is in a rapidly expanding state, these values of ν , k , and π reflect the history of the virus, and imply a very recent origin. The probable divergence time appears to be only several years. A quick report on these results has been published in Genetics **100**: s91.

Homology of Kringle Structures in Urokinase and Tissue-Type Plasminogen Activator: The Phylogeny with the Related Serine Proteases

Kei TAKAHASHI, Takashi GOJOBORI and Hiroyuki NAORA

Twelve amino acid sequences of kringle-forming polypeptides were compiled from the known sequences of urokinase A-chain (human), a tissue-type plasminogen activator (human), prothrombin (human and bovine), and plasminogen (human). Their sequence homologies were examined using a computer program. Analyses of the homology alignment and dot matrix showed that they had a great degree of homology. In particular, all the cystein residues responsible for the kringle structures of urokinase

and the tissue-type plasminogen activator were completely preserved. A phylogenetic tree constructed suggests that the kringe-related serine proteases have diverged from a common ancestral element around at 500 million years ago. Thus, the kringe-families seem to have a long evolutionary history. For details, see *Cell Struc. Func.* **10**: 209–218.

**Chromosome Regions Affecting Hybrid Sterilities of
Drosophila simulans/*D. mauritiana* and
D. simulans/*D. sechellia* Males**

Kiyoshi KIMURA, Won Ho LEE, Takao K. WATANABE
and Machiko HATSUMI

Takamura and Watanabe (1979, *Ann. Rep.* **30**, 91) reported preliminary results on the hybrid male sterility of *D. simulans*/*D. mauritiana* in which it was noted that the sterility is markedly affected by genes on the X and third chromosomes. Recently, Coyne (1984, *PNAS* **81**, 4444) pointed out that such sterility may be attributable to interactions between X and Y chromosomes. Both experiments, however, failed to deduce more information about the number and chromosomal location of loci which are responsible for hybrid male sterility, because only one marker gene was used for the X chromosome.

In this study we focused only on the X chromosome which is thought to have a chief effect on hybrid male sterility, and used two marker strains of *D. simulans*, yv^2f and $m^{65}f^{66}$ to study this phenomenon in more detail. The precise map positions of these loci in *D. simulans* are not yet known so that we assumed in the following data analysis that they were the same as those of *D. melanogaster*, namely, $y(0)$, $v^2(33)$, $m^{65}(36.1)$, f and $f^{66}(56.7)$ and centromere (70). The above two strains were crossed with *D. mauritiana* males. The F1 females were backcrossed to *D. simulans* males of the same strain. Eight (four) types of recombinant males for the $yv^2f(m^{65}f^{66})$ strain were examined to determine whether or not seminal vesicles contained motile sperms. If a recombinant male contained motile sperms, it was judged as fertile.

The frequency distribution of the fertile recombinants thus obtained was examined by a chi-square test. The null hypothesis in this test was constructed on the assumptions that (1) there is a single locus on the X chromosome, (2) crossing over occurs twice at most, and (3) the centromere may or may not affect a hybrid fertility change of the map position of such a locus

and inclusion or exclusion of the centromere alters the chi-square value. Through the simulation study which can identify the minimum chi-square value, we found that the most probable location of such a locus is around 40 of the X chromosome and that the proximity of the centromere also plays an important role in hybrid fertility. Thus, it appears that hybrid males become fertile as long as the two regions on the X chromosome, 40 and 70, are the same as those of the paternal species.

The same experiment was carried out using *D. simulans* and *D. sechellia*. The strain we used first was *yf; bw; st* of *D. simulans*. The effect of the X chromosome was larger than that in *D. simulans/D. mauritiana*, and the autosomal effect was almost negligible. Next we used *yv^{2f}* and *m^{88f88}* of *D. simulans* for the precise regions of hybrid sterility on the X chromosome. Again, the regions, 40 and 70, were detected as those involved in hybrid male sterility of *D. simulans/D. sechellia*.

These two experiments indicate that hybrid males carrying the *simulans* Y become fertile if they have the authentic *simulans* 40 and 70 regions even when the rest of the X chromosome is descended from either *D. mauritiana* or *D. sechellia*. However, when we studied the hybrid sterility of *D. simulans/D. sechellia* males carrying the Y chromosome of *D. sechellia*, we identified different regions. These regions are located around 50 and 70. In other words, a different inner region of the X chromosome of *D. sechellia* can recover fertility of hybrid males in this cross. Thus, interactions between X and Y chromosomes, or, more precisely the regions of the X chromosome, which result in hybrid male sterility may be specifically determined by the Y chromosome.

**Notes on Two Interspecific Hybrids of the *Drosophila*
melanogaster Subgroup: *simulans/teissieri*
and *teissieri/mauritiana***

Won Ho LEE and Takao K. WATANABE

A total of eight species are included in the *D. melanogaster* subgroup which can be divided into two complexes. With *D. teissieri* (*yakuba* complex) it is usually difficult to obtain hybrids with *D. simulans* or *D. mauritiana* (both *melanogaster* complex). Matings between *D. simulans* females and *D. teissieri* males were successful only by 7% of the cross while reciprocal matings were unsuccessful. On the other hand, matings between

D. teissieri females and *D. mauritiana* males were 22.1% successful but no successful mating occurred in the reciprocal cross. Among the successful crosses, we observed some sex-specific hybrid lethality. Hybrids, *simulans/teissieri*, were all females with reduced ovaries though the hybrid females mated easily with *D. teissieri* males. Hybrids, *teissieri/mauritiana*, showed continuous variation in sex-ratio, from equal numbers of males and females to all females lacking males. The variations came from using *D. mauritiana* as the paternal strain. The average proportion of F1 males of 14 strains was 12.2%. Two typical *D. mauritiana* strains, one producing 50% males and the other producing no males, were selected and crossed reciprocally to obtain heterozygotes. Then the heterozygous males were crossed with *D. teissieri* females. The percentages of hybrid males were 25% and 22% which was not significantly different and they were close to the mid-parents value (25%). This result suggests that the viability of the hybrid males between *D. teissieri* and *D. mauritiana* is controlled by some autosomal genes of *D. mauritiana*.

Cytogenetic and Biochemical Characteristics of Wild Mice Collected from the Kunming Area, China

KAZUO MORIWAKI, HIROTAMI IMAI, YASUYUKI KURIHARA
HIROMICHI YONEKAWA, CHANG-GENG QIN*
and LI-MING SHI*

In August 1985, we (K. M. and H. I.) visited Kunming, China and collected a number of mice at two localities in that area with the help of the Kunming Institute of Zoology. Taxonomic identification of these mice was kindly achieved by Dr. Wang Ying-Xiang of the Institute, where the air-dried chromosome preparations and proteinase-K digested particulate fractions of liver cells were made from the collected specimens. Later, the chromosome preparations and proteinase digested fractions were brought to our laboratory for analysis of chromosome C-band patterns and RFLP both in the mitochondrial DNA and the ribosomal DNA. Table 1 summarizes the results of these analyses.

Chromosome C band patterns of all mice indicated that they belong to neither *Mus musculus musculus* nor *M. m. molossinus*. This was also demonstrated by the RFLP both in 28S rDNA-NTR and mtDNA. The

* Kunming Institute of Zoology, Academia Sinica, China.

Table 1. Taxonomic, cytogenetic and biochemical characteristics of wild mice collected from the Kunming area

Sample No.	Place of collection	Name of species*	Sex	Body weight	Body length	Tail length	Chromosome C-band
85016	Wang Jiaqiao	Mus domesticus	F	14.5 g	70 mm	70 mm	(n.t.)
85017		homourus	M	12.0	67	75	1-19+/+, X+, Y-
85018			M	5.0	50	58	1-2+/+, 3±/+, 4-19+/+, X+, Y-
85019	Xiao Ximen	Mus castaneus	F	11.8	70	72	1-19+/+, X+/+
85020		tyleri	F	7.6	60	70	1±/±, 2-16+/+, 17±/±, 18-19+/+, X+/+

Sample No.	mtDNA restriction patterns								28S rDNA NTR RFLP	
	Bm	Ec	H2	H3	Hp1	He2	Ps	Bg	EcoRI	BamH1
85016	C	B	C	B	C	C	B	A	7.0, 9.0 kb	6.4 kb
85017	C	B	C	B	C	C	B	A	7.0, 9.0	6.4
85018	C	B	C	B	C	C	B	A	7.0, 9.0	6.4
85019	C	C	B	B	B	B	B	B	7.0, 9.0	6.4
85020	C	C	B	B	B	B	B	B	7.0, 9.0	6.4

* Identified by Dr. Wang.

former could not discriminate between *castaneus* and *bactrianus*, but the latter demonstrated that those from Wang Jiaqiao village were of the *castaneus* type and those from Xiao Ximen village the *bactrianus* type, though both villages are located in the Kunming area. As yet, we do not have any diagnostic markers which can clearly discriminate the nuclear genomes of *castaneus* and *bactrianus*. It is, however, of great interest that two groups of wild mice having mtDNAs being genetically remote from each other inhabit a limited area such as Kunming city.

Chromosome C-Banding Patterns of Korean Wild Mice

Kazuo MORIWAKI, Kyoko SUZUKI, Won Ho LEE*
and Wang Su CHO**

In the fall of 1984, a number of wild mice were trapped in the Suweon area of S. Korea. The place of collection and body size of specimen collected are shown in Table 1. Chromosome C-bands of bone marrow cells were analysed by quinacrine Hoechst staining. C-banding patterns of all individuals are summarized in Table 1. All mice examined exhibited several chromosome pairs with quite faint C-bands as well as some pairs with densely stained C-bands. This feature indicates that those mice belong to *Mus musculus molossinus* or *Mus musculus musculus*. Biochemical analysis of RFLP in the mitochondrial DNA demonstrated the same line of evidence as well (Yonekawa *et al.*, Ann. Rep. Nat. Inst. Genet. No. 35, 1985). Even within the *musculus-molossinus* group, minor RFLP of mtDNA has been observed, among which the restriction patterns of Japanese and Korean wild mice are the most similar. The C-banding patterns of the two types of mice are similar as well. For instance, a dense C-band on the subcentromeric region of No. 18 chromosome can be frequently observed in both Japanese and Korean populations. It is probable that *musculus*-like wild mice immigrated Japan through the Korean peninsula, and not directly from northern China.

* Pusan National University, Pusan, Korea.

** Institute of Agricultural Science, Suweon, Korea.

Table 1. Taxonomical and cytogenetical characters of Korean wild mice trapped in Suweon area

Sample No.	Place of collection	Sex	Body weight	Body length	Tail length	C-band pattern							
						1	2	3	4	5	6	7	8
1	Kojuri	F	11.4 g	79.0 mm	68.0 mm	+/+	+/+	-/-	-/-	-/-	+/+	-/-	-/-
2	AC-I	F	14.3	86.0	66.0	+/+	+/+	-/-	-/-	-/-	+/+	-/-	+/+
3	AC-II	F	15.6	82.0	69.0	+/+	+/+	-/-	-/-	-/-	+/+	-/-	+/+
4	IAS-III	M	12.1	84.0	56.0	+/+	-/-	-/-	-/-	-/-	+/+	-/-	+/+

Sample No.	C-band pattern												
	9	10	11	12	13	14	15	16	17	18	19	X	Y
1	±/±	+/+	-/-	-/-	-/-	-/-	-/-	-/-	±/±	+/+	+/+	-/-	
2	+/±	+/+	-/+	-/-	-/-	-/-	-/-	-/-	±/+	+/+	+/+	-/-	
3	±/±	+/+	-/-	-/-	-/-	-/-	-/-	-/-	±/±	+/+	±/±	-/-	
4	+/+	+/+	-/-	-/-	-/-	-/-	-/-	-/-	±/±	+/+	+/+	-	-

AC: Agricultural college, Suweon. IAS: Institute of Agricultural Science.

The Relationship between an Isozyme Locus *Pox-1* and Life-History Traits in Wild Rice

Hiroko MORISHIMA and Reiko SANO

The evolution of life-history in plants has provoked the renewed interest of population biologists during the last decade. However, the genetic control of life-history traits and the coherence mechanism of the relevant genes has remained almost unexplored. The present study was designed to investigate this problem.

In the Asian common wild rice, a perennial-annual continuum is found within the species. The perennial type is characterized by low seed productivity, a high outcrossing rate, late flowering, tall stature, and other characteristics as compared with the annual type which propagates mainly by seeds. In the past studies, we found a strong association between these correlated traits and allelic variation at a polymorphic isozyme locus *Pox-1*, in which three alleles OC, 2A and 4A have been identified. Results of past studies have shown that; 1) Populations of perennials are polymorphic, while those of annuals are exclusively fixed for 2A (rarely OC). 2) In the experimental populations segregating at *Pox-1* locus, the 2A allele tended to increase when propagated by seeds, while the 4A increased when propagated vegetatively. 3) The frequency of 2A increased in a subpopulation selected for early flowering.

In the present study, we studied hybrid progeny obtained from a cross between an annual and a perennial strain of Asian wild rice. In F_3 lines (F_2 derived), the relationships among various life-history traits and allelic variation at the 5 isozyme loci were examined. Most of the character correlations which are consistently found among natural populations bringing about perennial-annual differentiation disappeared among F_3 lines, indicating that they are the product of natural selection. But, high efficiency in seed production, short anther (favoring selfing) and early flowering tended to be associated among F_3 lines. This suggests that linkage or developmental correlation could have partly played a role in perennial-annual differentiation. Further, 2A/2A plants showed a higher efficiency in seed production, shorter anther and earlier flowering than 4A/4A plants. This suggests the existence of major gene or genes for those traits.

In order to search for the neighboring region of *Pox-1* locus, 7 pairs of isogenic lines each of which carried 2A and 4A on the same genetic back-

ground were developed from the same cross by repeating selfing of 2A/4A heterozygotes. Comparison between isogenic lines in F_6 indicated that genes underlying flowering time, anther length, panicle number and plant height seemed to be located near this locus, although these linkages were broken in more than half of the F_6 lines. This chromosomal segment marked by *Pox-1* may be said to be "an adaptive gene block." Why this association persisted so widely and strongly in nature remains unsolved.

Computer Studies on the Molecular Evolution

Takashi MIYATA

The transfer of genetic information from RNA into DNA was thought to be a unique feature of retroviruses, but there is growing evidence that it may be a more general strategy. Both DNA viruses, hepatitis B virus (HBV) and cauliflower mosaic virus (CaMV) as well as eukaryotic transposons, *Drosophila copia* and yeast Tyl elements were found to have RNA intermediates that are reverse transcribed into DNA (Summers, J. and Mason, W. S. 1982, Cell, **29**: 403; Pfeiffer, P. and Hohn, T. 1983, Cell, **33**: 781; Shiba, T. and Saigo, K. 1983, Nature, **302**: 119; Boeke, J. D. *et al.*, 1985, Cell, **40**: 491). Furthermore, these DNA viruses and a *Drosophila copia*-like element 17.6 have polymerases which share striking sequence homologies with retroviral *pol* gene products for a region (RT domain) that is thought to carry reverse transcriptase activity (Toh, H., Hayashida, H. and Miyata, T. 1983, Nature, **305**: 827; Saigo, K. 1984, Nature, **312**: 659).

Another line of evidence for the possibility of nonretroviral reverse transcriptase came from a recent report by Michel and Lang (Michel, F. and Lang, F. Nature, **316**: 641). Following the suggestion for the possibility of a *Neurospora* mitochondrial plasmid being related to transposon, they compared amino acid sequences predicted from the ORFs (open reading frames) of fungal mitochondrial class II introns together with a mitochondrial plasmid with those of polymerases of retroviruses, CaMV, HBV and a *Drosophila* transposon 17.6 and found remarkable homologies within the RT domain. This finding added further evidence for the view that the RT-like sequence can now be recognized as a ubiquitous one distributed over many genetic elements of such a wide evolutionary distance. In addition, such a widespread occurrence of similar sequences suggests that these homologies were derived by divergence, but not by convergence.

Apart from the RT-like sequence, the ORF product of *copia*-like element 17.6 contains another sequence related to the DNA endonuclease (EN) of retroviral *pol* gene products (Toh, H. *et al.*, 1985, EMBO J., 4: 1267), thought to be important in integration of viral DNA into host DNA. This strongly suggests that the transposition mechanism of the *copia*-like element is retrovirus-like. Interestingly CaMV polymerase exhibits a striking homology with ORF2 product of the 17.6 over its entire region, but is completely lacking the EN-like sequence (Toh, H. *et al.*, 1985, EMBO J., 4: 1267). Also no EN-like sequence presents in HBV (Toh, H. *et al.*, 1985,

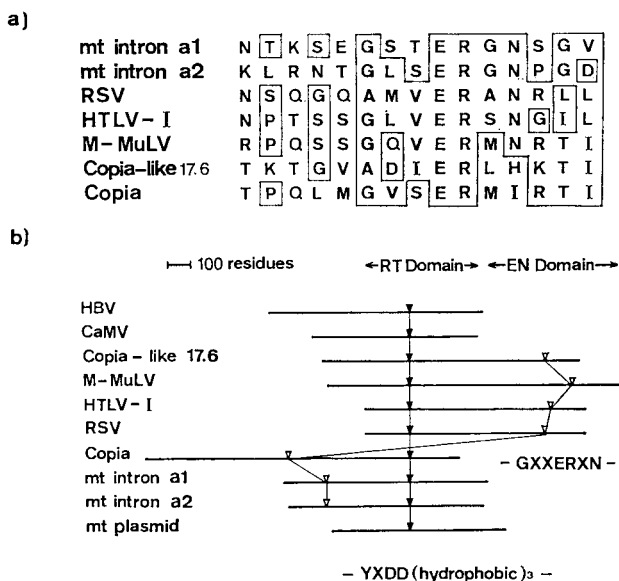


Fig. 1. Alignment of amino acid sequences of viral and transposon polymerases. a), Alignment for a highly conserved segment of the DNA endonuclease (EN) domain. Mt introns a1 and a2, amino acid sequences predicted from ORFs in introns a1 and a2 of yeast mtDNA-coded cytochrome oxidase subunit I gene. Mt plasmid, the sequence predicted from ORF in *Mauriceville* mitochondrial plasmid. Most common amino acids (identical or chemically similar amino acids) were boxed. b), Homology map. Positions of highly conserved stretches of amino acids in the RT (reverse transcriptase) and EN domains were indicated by ▼ and ▽, respectively. Their consensus sequences were also shown (Data from Miyata, T., Toh, H. and Saigo, K. 1985, Proc. Japan Acad., 61B: 464).

EMBO J., 4: 1267). The *Drosophila copia* is distantly related to retroviruses and its putative polymerase shares less extensive homology with retroviral *pol* gene products (Emori, Y. *et al.*, 1985, Nature, 315: 773), but has both highly conserved stretches of amino acids -YXDD- (X, any one of amino acids) flanked by three hydrophobic residues and -GXXERXN- which are diagnostic of the RT and EN domains, respectively (Miyata, T., Toh, H. and Saigo, K. 1985, Proc. Japan Acad., 61B: 464). Similar searches revealed the presence of both sequences in ORFs of mitochondrial introns but the mitochondrial plasmid lacks the latter sequence (Miyata, T., Toh, H. and Saigo, K. 1985, Proc. Japan Acad., 61B: 464). Interestingly the RT and EN domains present in reverse order in the *copia* and mitochondrial introns (Fig. 1).

In conclusion, of all the reverse transcriptase-containing viruses and transposons examined so far, DNA viruses and plasmids lack an endonuclease-related domain, which is in sharp contrast to retroviruses and transposons involving the endonuclease and intrachromosomal phase, thought to be important in their life cycle. Although HBV is known to have an intrachromosomal phase, the mechanism of integration into host DNA possibly differs from that of retroviruses. Mitochondrial plasmids could be transposons that lack an intrachromosomal phase. It may be of interest to know whether or not the relationship between the presence of DNA endonuclease and the chromosomal form (RNA or DNA) could be extended to most reverse transcriptase-containing viruses. Further data will clarify this point.

Application of Mitochondrial DNA Restriction Length Polymorphism to Genetic Monitoring of Laboratory Mice

Hiromichi YONEKAWA¹⁾, Hideki KATOH²⁾, Tomoko SAGAI³⁾
and Kazuo MORIWAKI³⁾

Our previous study showed that most strains of laboratory mice share a single type of mtDNA, which was designated as mtDNA restriction phenotype *d*. However, there were some exceptions: most of the NZB strain showed the another phenotype *b* and strain RR showed *m* (Yonekawa *et al.*,

¹⁾ Laboratory of Cytoplasmic Genetics.

²⁾ The Central Institute for Experimental Animals.

³⁾ Laboratory of Cytogenetics.

1982, Differentiation **22**, 222).

When we examined mtDNA phenotypes among several substrains of NZB, two clearly different phenotypes were found among the substrains. Out of 6 substrains examined, three had *b*-type mtDNA while the rest had *d*-type (Table 1). This suggests that some NZB substrains had undergone genetic contamination by other strains. This suggestion is supported by the fact that only one type of mtDNA should have been detected in the NZB substrains, since all of the substrains used have been maintained by full sister-brother mating. Since the mice of this strain are used widely as models in studies on murine lupus, which is thought to be controlled by a few genes, it is then important to disclose the genetic characteristics of these NZB substrains.

To do this, the authentic NZB strain maintained in the Walter and Elisa Research Institute was used for the restriction analysis. Since the authentic one shows *b*-type mtDNA, the NZB of *d*-type must be a genetic contaminant.

We compared nuclear biochemical markers of the authentic NZB and the 3 contaminated substrains had different chromosome distribution patterns of biochemical markers (Table 2). However, as all loci were fixed as homozygous, the contamination probably occurred a long time ago. Furthermore, *Akp-I^a* and *Es-I^b* were the alleles which have never been found in any of the NZB substrains with *b*-type.

Next the question arises whether the contamination in the NZB substrains was caused by an intercross between NZB and other NZ-strains, since the intercross between NZB and NZW were often carried out to enhance *in vivo* immunoreactions for murine lupus (Shirai, T. 1982, Immunology Today **3**, 182). This possibility, however, was eliminated by the analysis of biochemical markers among four strains of NZ-stocks and NZW. Namely, *Akp-1I* and *Es-1t* could not be found in any of the NZ-strains and NZW.

We then compared biochemical markers between the contaminated NZB substrains and four strains of black-coated mice, since genetic contamination often occurs between strains with the same coat-color and NZB has a black coat-color. The experiments showed that the biochemical markers of C57BL/Ks were very similar to those of contaminated NZB. From these results, we finally concluded that the contamination was probably caused by an intercross between NZB and some black-coated strains.

Here we show that the analysis of mtDNA RFLP is a useful tool for

Table 1. Genetic profiles of NZB sublines, other NZ strains and the NZW strain

Locus (Chr. No.)		Strains*									
		NZB/ KJms	NZB/ Nga	NZB/ Nrs	NZB/ Bl	NZB/ MRC	NZB/ San	NZO/ Bl	NZC/ Bl	NZX/ Bl	NZW/ Wehi
(Contaminated)				(Standard strains)							
<u>Idh-1</u>	(1)	a	a	a	a***	a	a	b	a	a	b
<u>Pep-3</u>	(1)	c	b***	c	c	c	c	c	c	c	b
<u>ASp-1</u>	(1)	b	a	a	b	b	b	b	b	b	b
<u>Hc</u>	(2)	1	0	0	0	0	0	1	0	1	1
<u>Mup-1</u>	(4)	b	a	b	a	a	a	a	b	b	a
<u>Gpd-1</u>	(4)	b	b	b	b	b	b	a	a	b	b
<u>Pgm-1</u>	(5)	b	b	b	b	b	b	a	a	b	b
<u>Hbb</u>	(7)	s	d	d	d	d	d	s	d	s	d
<u>Es-1</u>	(8)	a	a	a	b	b	b	b	b	b	b
<u>Mod-1</u>	(9)	b	b	b	b	b	b	a	b	b	a
<u>Es-10</u>	(14)	b	b	b	b	b	b	a	b	a	a
<u>H-2K</u>	(17)	d	d	d	d	d	d	u	d	d	z
<u>H-2D</u>	(17)	d	d	d	d	d	d	ND**	d	d	z
mtDNA (—)		d	d	d	b	b	b	b	d	d	d

* The details of the strains are given in Materials and Methods.

** ND: not determined.

*** Gothic and italic letters represent the alleles confirmed in ref. 12 and observed to be aberrant in contaminated NZB, respectively.

Other markers examined are as follows: Car-2^a, Ldr-2^a, Lyt-2^b, Gpi-1^a, Es-2^b, Thy-1^b, Trf^b, Es-3^c and Lyt-1^b.

Table 2. Comparison of allele distributions in NZB and other black-coated strains

Locus	Strain					
	AU/SsJ	C58/J	C57BL/6J and C57BL/10Sn	C57BL/Ks	NZB (Contam- inated)	NZB (Standard)
Pep-3	b (+)*	a (—)	a (—)	a (—)	b/c**	c
Akp-1	a (+)	a (+)	a (+)	a (+)	a/b	b
Hc	0 (—)	1 (+)	1 (+)	1 (+)	1/0	0
Mup-1	?	b (+)	b (+)	b (+)	b/a	a
Hbb	p (—)	s (+)	s (+)	s (+)	s/d	d
Es-1	b (—)	b (—)	a (+)	a (+)	a	b
H-2	q (—)	k (—)	b (—)	d (+)	d	d
mtDNA	d (+)	d (+)	d (+)	d (+)	d	b
reference	12, 13	12, 13	12, 13	12, 13	8	8

* (+) and (—) indicate respectively that this allele is “the same as” and “different from” the aberrant allele of contaminated NZB.

** Aberrant allele (Gothic letter)/standard allele.

genetic monitoring of laboratory animals. However, this technique has a weak point in that the animals must be killed for analysis. If live animals are necessary for use, we have to carry out a partial hepatectomic operation, but this is quite time-consuming and not safe for the animals. To overcome this problem, we have developed a very sensitive method of analysis, which requires only 50 μ l of blood to examine one restriction pattern. Fifty μ l of blood can be obtained from the retro-orbital venous plexus and the animals, even mice, will be still alive after this treatment. Thus, this technique enables us to use easily any animal samples for genetic monitoring.

We describe the details of this method below: 50 to 200 μ l of blood was removed from retro-orbital venous plexus with a heparinized glass capillary tubing. Then the blood was spun down and the fraction of cellular components were collected. Five hundred μ l of 0.5 M EDTA-1% sodium lauroyl sarcosinate was added to the pellets. Then the sample was treated with 100 μ g/ml (final concentration) proteinase K at 37°C overnight. Then the samples were treated with 3% NaCl-saturated phenol twice (In these solution conditions, phenol phase was up on the aqueous phase.), 3% NaCl-saturated phenol-chloroform mixture once and then chloroform once. Whole nucleic acids were precipitated with 2 volumes of ethanol and then collected with centrifugation at $10,000 \times g$ for 20 min. The same procedure

was repeated three times in the presence of 2 M ammonium acetate. The pellet of nucleic acids was washed with 90% ethanol, dried under vacuum and finally dissolved into 0.1 mM EDTA (pH 8.0) of an equal volume to original blood. Forty-five μ l of the nucleic acids solution was used for restriction analysis. Digestion of samples with restriction enzymes and agarose gel electrophoresis were carried out as usual (Yonekawa *et al.*, 1982, Differentiation **22**, 222). Southern blotting was performed on Pall Biodyne membrane (BNNG). The conditions for blotting, prehybridization, hybridization and membrane washing were those recommended by the supplier. MtDNA used for probe was purified by two or three cycles of CsCl-ethidium bromide density gradient centrifugation. After linearization with a suitable enzyme, the mtDNA was labeled with 32 P- α -dCTP by oligonucleotide random primer extension method (Feinberg and Vogelstein, 1984 Anal. Biochem. **137**, 266).

IX. HUMAN GENETICS

Mitochondrial DNA Polymorphism in Japanese

Satoshi HORAI and Ei MATSUNAGA

The mitochondrial DNA (mtDNA) of humans is a closed circular molecule which has been completely sequenced for one individual (Anderson *et al.*, 1981). The mtDNA of this individual comprises 16569 base pairs. In a previous study on mtDNA restriction analysis with fifteen enzymes of six base pair recognition, we suggested the existence of a considerably high degree of mtDNA diversity within the Japanese population. In this study an attempt was made to obtain sufficient purified mtDNA from human placenta in order to examine mtDNA polymorphism with a large number of restriction enzymes without using Southern blotting analysis or the end labeling method. In the present study, we have extended this study to include nine enzymes of four or five base pair recognition. The size of the mtDNA fragments produced by digestion by each enzyme were compared after gel electrophoresis. A total of 95 different morphs were observed with nine enzymes, 60 of which have not been so far reported in the previous study by Cann (1982), which is the only comparable study to ours with respect to the population size examined and the number of enzymes used. The high frequency of new polymorphisms is, however, due to the fact that our survey is the first extensive analysis of mtDNA polymorphism in the Japanese population. Although Cann (1982) also analyzed mtDNA from many Orientals, only two Japanese were included. Therefore, this finding may reflect a unique profile of the Japanese population inferred from mtDNA polymorphisms. This is supported by our previous analysis with enzymes of hexanucleotide recognition, where several new polymorphisms with various enzymes were detected. Based on the combination of restriction enzyme morphs observed for each of 116 individuals, we found 62 distinct mtDNA restriction types. Thirteen individuals showed the restriction type derived from the dominant pattern for each digestion of the 9 enzymes (type 21). The next most frequent type was found in 12 individuals (type 1). Although the remaining 60 types occurred at low frequencies, it is informative to measure the index of nucleotide diversity. The average

amount of nucleotide change in human mtDNA can be estimated by the method of cleavage site comparison (Nei and Li 1979). In the present study, an average of 233 sites (equivalent to 932 base pairs, or 5.6% of the whole genome) could be compared. Using pairwise comparison of each restriction type, the average number of nucleotide substitutions per nucleotide site (δ) was estimated to be 0.0026. Phylogenetic analysis of the present data indicates that at least two distinct lineages exist in the Japanese population. The first cluster consisting of 13 types (21 individuals) first diverged from the second cluster. The branching point is estimated as early as about 125,000 years ago. For details, see Human Genetics 72: 105-117.

Analysis of Mitochondrial DNA Polymorphisms in Japanese Monkeys

Satoshi HORAI, Ei MATSUNAGA, Kenji HAYASAKA¹⁾
Takayoshi SHOTAKE¹⁾ and Ken NOZAWA¹⁾

To investigate the genetic relationship between the species of genus *Macaca*, mtDNA was purified from the liver of a Japanese monkey. It was digested with 12 restriction enzymes (Eco RI, Bam HI, Pst I, Hind III, Hinc II, Ava I, Bst EII, Bgl II, Acc II, Sac I, Xba I, Kpn I) and the restriction patterns were analysed by agarose gel electrophoresis. By double digestion analysis, a restriction map with 32 sites was obtained. M. Goerge Jr. (1982) reported mtDNA restriction maps in three species of *Macaca* other than Japanese monkeys and four species of other genera of the Old World monkeys. On the basis of these seven maps and that of the Japanese monkey, we calculated, according to the method by Nei & Li (1979), the index of nucleotide diversity by cleavage site comparison and the number of nucleotide substitution per site (δ) between each pair of the 8 species. A phylogenetic tree was constructed from the values of δ 's by the unweighted pair group method. As expected, the tree showed that the four species of *Macaca* belonged to the same cluster but it also showed some differences as compared with that constructed from the electrophoretic analysis of blood proteins. Analysis with the restriction enzymes which recognize four base pair sequences or sequence analysis is needed in order to clarify their phylogenetic relationships more precisely.

¹⁾ Dept. Variation Res., Primate Res. Inst., Kyoto Univ.

Characterization of 2,7-anhydro-*N*-acetylneuraminic Acid in Human Wet Cerumen

Minoru SUZUKI*, Akemi SUZUKI*, Tamio YAMAKAWA*
and Ei MATSUNAGA

The dimorphism of human normal cerumen, wet (sticky) and dry (flaky), is determined by a pair of autosomal alleles, the wet type being inherited as simple dominant over the dry type. Although there are several reports on the chemical nature of human cerumen, the biochemical basis to distinguish the two types of cerumen is entirely obscure. We have isolated a molecular species of sialic acid in a free form from cerumen of the wet type, but not of the dry type, by an ion-exchange column chromatography and preparative high-performance liquid chromatography. Structural analysis of this sialic acid was performed by gas-liquid chromatography/mass spectrometry with chemical ionization (CI) and electron ionization (EI). In the CI mass spectra, protonated molecular ion of the trimethylsilyl derivative was observed at m/z 580 and that of the methyl ester-trimethylsilyl derivative was at m/z 522. In the EI mass spectrum, the methyl ester-trimethylsilyl derivative gave the characteristic ions at m/z 506, 462, 418, 416, 328, 316, 238, 228, 205, 186 and 173. This mass spectrum was identical to that of 2,7-anhydro-*N*-acetylneuraminic acid, which was already reported by Lifely and Cottee (Carbohydr. Res., **107**, 187–197, 1982) as the mass spectrum of a by-product prepared from *N*-acetylneuraminic acid by methanolysis. These results indicate that the compound in the wet cerumen is 2,7-anhydro-*N*-acetylneuraminic acid. Since this sialic acid species could not be detected in cerumens of the dry type, its formation in the wet cerumen may be controlled by an autosomal dominant gene. For detail, see J. Biochem. **97**: 509–515, 1985.

* Tokyo Metropolitan Institute of Medical Science, Bunkyo-ku, Tokyo 113.

X. APPLIED GENETICS

The Indica-Japonica Differentiation of Rice Cultivars in Thailand and its Neighbouring Countries

Yo-Ichiro SATO, Songkran CHITRAKON¹⁾ and Hiroko MORISHIMA

Two hundred fifty eight native cultivars of the common rice, 97 from Thailand and the rest from Nepal, Bhutan, Northeast India, Bangladesh, Burma and Laos were examined to learn the distribution pattern of the Indica and Japonica types under the different habitat conditions, especially in relation to their photoperiodic sensitivity. The materials tested were divisible into the Indica and Japonica types by a discriminant function which combined measurements of phenol reaction, KClO₃ resistance and apiculus hair length. Japonica types were distributed in upland as well as lowland fields with high elevations in a wide area. They seemed to be adapted in these habitat, where early heading is required. They had weak photoperiodic sensitivities. The glabrous or *nuda* cultivars were frequently found in upland fields. They are also reported in Yunnan China, and Philippines. For detail, refer Proc. Vth Congr. SABRAO (1986).

Variation in Grain Shape of the Indica and Japonica Rice Cultivars

Yo-Ichiro SATO

Grain shape has been used traditionally as a key character for distinguishing between the Indica and Japonica types of rice cultivars. The grain shapes of the Temperate Japonica, Tropical Japonica and Indica types have been recognized to be "round", "large" and "slender", as Matsuo (1952) classified them into a, b and c types, respectively. To evaluate the usefulness of grain shape in the study of varietal differentiation, native cultivars from various Asian countries, 277 in total, were classified into the above-mentioned three types by Oka's (1958) criteria (discriminant scores), and also by grain shape in accordance with Matsuo's scheme, and the con-

¹⁾ Pathum Thani Rice Research Center, Thailand.

cordance of the results obtained by two criteria were examined. Of 111 cultivars classified as Indicas, 83 were of slender or c type, and of 63 classified as Temperate Japonicas, 49 were of round or a type, suggesting that these two varietal groups could be recognized by their grain shape more or less correctly. However, among 103 cultivars of the Tropical Japonica types, 50 were slender, 41 were large, and 12 were round types. On the whole, the coefficients of concordance was 0.625. The concordance differed according to regions; it was very high (0.705–0.813) in Japan, Korea, China, India (other than Assam) and Sri Lanka, but much lower (0.444–0.507) in Assam, Himalayan hill regions, Philippines, Indonesia and Taiwan. For rice cultivars from these regions in which varietal diversity is known to be high, grain shape may not be a reliable criterion for Indica-Japonica classification.

Distribution of Genes for F_1 Weakness in Asian Native Rice Cultivars

Yo-Ichiro SATO and Hiroko MORISHIMA

The F_1 weakness occurring in crosses of a Peruvian cultivar "Jamaica" with some Japanese rice cultivars, first reported by Amemiya and Akemine in 1963, is controlled by a set of complementary genes, *Hwc-1* and *Hwc-2* (formerly designated as *L-2-a* and *L-2-b*). To learn about the distribution of the genes, 348 Asian native cultivars were test-crossed with Jamaica, *Hwc-1* carrier. Among the cultivars tested, 154 carried *Hwc-2*, while the remaining 194 had its recessive allele. One out of 115 Indica types (0.8%) and 153 out of 233 Japonica types (65.8%) were *Hwc-2* carriers, indicating that this allele was Japonica-specific. The *Hwc-2* frequency in the Japonica types differed according to their origins, being high in Japan (94%) and China (92%), and lower in Philippines (17%) and Indonesia (13%). None of 30 strains of the Asian common wild rice tested which were collected from various localities had *Hwc-2* gene. Further, 116 cultivars were also test-crossed with T65 or other *Hwc-2* carriers. None of them had *Hwc-1*, except for Jamaica. Probably, the *Hwc-2* gene was born in the Japonica types after they had evolved from their wild progenitor. This gene could be closely linked with certain adaptive genes differentiating between the Indica and Japonica types, or with some of the genes for intervarietal hybrid sterility.

Effect of a Semi-Dwarfness Gene on Morphological Characters in Rice with Different Genetic Backgrounds and Environmental Conditions

Yo-Ichiro SATO and Hiroko MORISHIMA

Semi-dwarfness genes have much contributed to the improvement of the high yielding rice varieties in the world. Surprisingly, those genes proved to be located on the same locus though their origins were different. In the present study, expression of such semi-dwarfness genes on morphological characters, such as culm length, internode length and leaf length were investigated under the different genetic backgrounds and environmental conditions. Materials used were 4 semi-isogenic pairs with and without the semi-dwarfness gene under the genetic background of Taichung 65(T65), Shiokari (SK), Fujiminori (FJ), and an American variety, Calrose (CR), respectively. In the former two pairs, a spontaneous mutant gene carried by Taichung native 1 (*d-47*) was introduced into T65 and SK. The latter two were induced mutants (the allele are *d-49* of FJ and *sd-1* of CR, respectively), and their respective original varieties. The effects of these genes on the organ length depended on genetic backgrounds as well as on environmental conditions. The *d-47* in T65 and SK seemed to give different expression from *d-49* in FJ or *sd-1* in CR in various aspects. The effect of *d-47* was expressed during whole growth duration, whereas *d-49* and *sd-1* were not expressed earlier than flowerbud initiation. This could imply the effect of different alleles at the same locus or the effect of different genetic backgrounds.

Genic Analysis of Isozyme Genes in Rice

Reiko SANO, Ryuji ISHIKAWA, Pascale BARBIER
and Hiroko MORISHIMA

More than 40 isozyme loci are presumed in rice, but formal genic analyses have been conducted only on a limited number of loci. In the present study, Mendelian segregations were confirmed for *Amp-2*, *Amp-3*, *Est-ca*, *Pgd-1* and *Sdh-1*. Linkage relationships were examined for 10 isozyme loci and some marker genes. We found a tight linkage between *Amp-3* and *Est-2* (0.7%). They are located on chromosome 6 in the following sequence, $wx \xrightarrow{(31\%)} Amp-3 \text{---} Est-2 \xrightarrow{(13\%)} Pgi-2$.

To determine chromosomal location of other loci, a series of trisomics of a Japanese variety were crossed as females with a Vietnamese cultivar and also with a strain of Asian common wild rice. So far, segregations of 9 isozyme genes were studied in F_2 s with 8 trisomics. The results obtained indicated that *Amp-2* and *Pgd-1* are located on chromosome 12 and 9, respectively. In other combinations of loci and trisomics, normal segregation or a deficiency of the alleles derived from the trisomic parent (Japonica) which is commonly observed in Indica-Japonica crosses were found.

Electrophoretic Characterization of Rice Endosperms of Indica and Japonica with Four Kinds of Protein Fractions

TORU ENDO

Rice cultivars (*Oryza sativa*) comprise two types, Indica and Japonica, where the latter is believed to be derived from the former. The differences between the two types were investigated with respect to several aspects, including analyses of endosperm protein components, i.e., albumin, globulin, prolamin and glutelin. It is not so difficult to separate the first three protein fractions from rice endosperm as samples applicable to IEF-PAGE (isoelectrofocusing-polyacrylamide gel electrophoresis), but extraction of glutelin as a fraction which can be dissolved in 8M urea solution has so far been difficult.

Separation of the glutelin fraction was attempted as follows: after sequential extraction of the first three protein components, glutelin was extracted with 0.1 M NaOH and centrifuged, and the supernatant was neu-

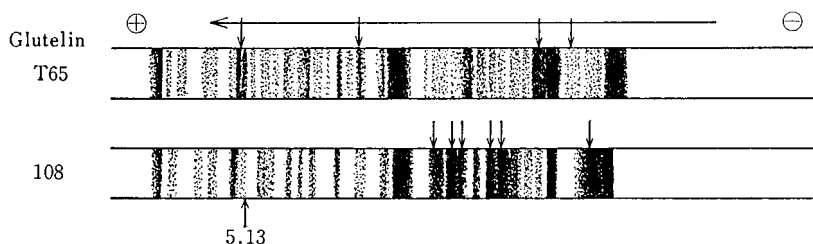


Fig. 1. Comparison of glutelin patterns from rice endosperms of T65 and 108 using the equilibrium system of IEF-PAGE. Arrows at top show major differences between both cultivars.

tralized with 1 M ascorbic acid and centrifuged to get the precipitate of the glutelin fraction. The fraction was found to be soluble in 8 M urea solution, but was insoluble when acids other than ascorbic acid were used.

The four kinds of protein fractions were examined in each of the varieties of Indica (108) and Japonica (T65) types. From the analysis of the experiments a total of at least 126 and 112 bands of the four fractions were counted in T65 and 108, respectively. Characteristic differences were observed between the two types in the protein fractions. Major and medium band differences are the fewest in albumin, are appreciable in globulin and glutelin (Fig. 1), and are considerable in prolamin fractions between both types.

Conservation Methods of Crop Populations with Mixed Selfing and Outcrossing

Katsuei YONEZAWA and Hiko-Ichi OKA

Five different methods of seed propagation as defined in Table 1, i.e., SSD (single seed descent) method, two different types of pedigree and bulk methods each, for rejuvenation of plant populations with mixed selfing and outcrossing were compared with respect to the relative magnitude of random genetic drift represented by the sampling variance of the frequency of genes and genotypes. The result shows that, in predominantly selfing populations, the SSD method causes a much smaller sampling variance than the pedigree and bulk methods. The SSD method requires only about 1/4 as many plants as the bulk method, and about 1/2 as many plants as the pedigree method, to give the same level of random drift in relation to gene frequency. Partial sampling of the plants as harvesting only a small fraction of the plants grown may cause an irrecoverable genetic loss in predominantly selfing populations. The difference due to the propagation methods is largely reduced in predominantly outcrossing populations, while the order of the methods in relative efficiency remains the same as in selfing populations. Controlled selfing by bagging would in many cases be the only practicable way to avoid the contamination by alien pollens. In this situation, the SSD method is recommended to be applied combined with bagging. Population size for a practical conservation project was briefly discussed. (Proceedings of the 5th SABRAO Congress, 1986).

Table 1. Methods of seed propagation investigated

Method	Plants harvested	Harvest manipulation		Seeds for rejuvenation	Sowing
		Seeds/plant	Handling		
I. SSD method					
	All plants	One or, several to many	Bulked A seed lot from each plant	All seeds One seed/plant seed lot	Pedigree families identified or not identified
II. Pedigree method					
P-1 method	All plants	Many	A bulked seed lot for each pedig.	A certain number of seeds/pedig. seed lot	Pedigree families identified
P-2 method	One plant/pedig.	Many	A seed lot from each plant (pedig.)	A certain number of seeds/plant seed lot	Pedigree families identified
III. Bulk method					
B-1 method	All plants	Many	Bulked	Randomly sampled	Pedigree families not identified
B-2 method	Some randomly chosen plants	Many	Bulked	Randomly sampled	Pedigree families not identified

SSD method: Single seed descent method.

Evolutionary Significance of Regulatory Changes in the *Wx* Gene Expression of Rice

Yoshio SANO

Recent interest in the theory of evolution has been focused on the importance of gene regulation. However, there is little evidence relating regulatory changes at the protein level to gross phenotypic changes to be selected for. To examine the importance of *cis*-acting regulatory elements on racial differentiation in rice, the distribution of Wx^a and Wx^b was surveyed in terms of the intensity of *Wx* protein. From the intensity of *Wx* protein in mature endosperms, 193 from 207 strains examined were readily classified as Wx^a or Wx^b and only 14 showed an intermediate intensity, with their allelic states remaining uncertain. Wx^a showed a *Wx* protein level ten fold higher than that of Wx^b as well as a higher level of amylose.

The four species, *O. rufipogon* (= *O. perennis*, a wild progenitor of *O. sativa*), *O. glaberrima*, *O. barthii* (= *O. breviligulata*, a wild progenitor of *O. glaberrima*) and *O. longistaminata*, all exhibited only the Wx^a allele. On the other hand, 103 strains of *O. sativa* had Wx^a and 30 had Wx^b . The frequency distribution of the two alleles was markedly different among three ecogeographical races within *O. sativa*, Wx^b was predominant in the Japonica type while most strains of the Indica type had Wx^a .

Racial differentiation within *O. sativa* has been extensively studied by a number of researchers concerning geographical distribution and morphological, physiological and biochemical characteristics. In addition, the Japonica type is characterized by a lower content of amylose than the Indica type (Lu and Chang 1980). The starch of nonglutinous (nonwaxy) endosperms contains both amylose and amylopectin and amylose content affects the degree of viscosity of cooked rice, so glutinous (*waxy*) cultivars lacking amylose are often called 'sticky rice'. Since amylose content is the major determinant of the eating qualities in rice, factors controlling the amount of amylose must have been selected for or against by farmers. In several cereals including rice, maize, barley and so on, the glutinous (*waxy*) cultivars lacking amylose are found mostly in Southeast Asia, especially in the mountainous regions. The geographical distribution of the *wx* allele in rice appears to be restricted to the regions where the Japonica type with low amylose content prevails. Recently, rice cultivars with an amylose content lower than the Japonica type were widely found in the mountainous regions

of Southeast Asia (Nakagahra *et al.*, 1985). This leads us to consider that the current cultivation of glutinous or low amylose rice cultivars is primarily due to acquired preference or traditional use in the local diet. Hence, an artificial selection seems to strongly operate on differences in amylose content.

Since the wild progenitor of *O. sativa* has Wx^a only, a *cis*-acting regulatory mutation must have produced Wx^a from Wx^b . The frequency of Wx^a which drastically reduces the Wx protein level as well as amylose content in rice endosperm probably increased during domestication leading to the establishment of the Japonica type. The results obtained in this study confirm that the regulatory change controlling the Wx gene expression is closely related to an important agronomical trait which could be selected for (Euphytica, **34**: 587).

**Genetic Control of Alcohol Dehydrogenase and
Estimation of Some Population Parameters in
Hopea odorata Roxb. (Dipterocarpaceae)**

Masaaki IHARA*, Lilian U. GADRINAB**, Ulfah J. SIREGAR**
and Shin-ya IYAMA

The ADH isoenzymes were investigated in seeds, seedlings and leaves of mature trees in a tropical tree species, *Hopea odorata*. The materials were collected from several places in Java, Indonesia as well as Thailand. The isozymes were found to be dimeric and controlled by two loci; *Adh-1* consisted of 3 alleles designated as *Adh-1S*, *Adh-1N* and *Adh-1F*, and *Adh-2* was isoallelic. Because of the differential gene expression of these loci in cotyledons of mature seeds and in leaf blades of seedlings and trees, it was concluded that *Adh-1* was expressed throughout the whole life span of a tree while *Adh-2* only during a limited period in mature seeds.

Using *Adh-1* as a genetic marker, genotype frequencies in a plantation near Bogor, Indonesia were investigated. The plantation consisted of two separate stands, Site I where S/S, S/F and F/F genotypes were found and Site II where S/S, S/F and F/F were found and they were 700 m apart from each other. Genotypes of fallen seeds in Site I included N/F and S/F

* Graduate Division of Biochemical Regulation, Faculty of Agriculture, Nagoya University, Nagoya, 464 Japan.

** Tropical Forest Biology Program, BIOTROP, P.O. Box 17, Bogor, Indonesia.

genotypes besides S/S, S/N and N/N, indicating the gene flow from Site II. Based on the genotype frequencies of breeding trees in Sites I and II, and those of seeds in Site I, interpopulational pollen flow rate ($u=0.315$) was estimated together with outbreeding rate ($t=0.634$) and selection coefficient for N/N genotype ($s=0.486$).

Publications from Genetic Resources Section, Genetic Stocks Research Center

Shin-ya IYAMA

The following publications were released from Genetic Resources Section this year.

1. "Stocks of experimental organisms maintained in the universities in Japan". 42 pages. (in Japanese).

Names of experimental organisms, location (laboratory) where the stocks are maintained, person in charge, and number of stocks were listed according to the universities. In total, 1141 locations in 172 universities and research institutes were included: 336 locations for animal stocks, 125 for plant stocks, 318 for microorganism stocks and 362 for cultured cell stocks.

2. "Drosophila Stock List in Japan 1985." 61 pages.

This list contains 1360 drosophila stocks kept in various places in Japan. Each stock is indicated with the place of maintenance. Wild and mutant stocks of *D. melanogaster*, *D. simulans*, *D. ananassae*, *D. hydei*, *D. virilis* and other species are included.

3. "Rice Genetics Newsletter Vol. 2". 122 pages. (in English).

This volume contains lists of recommended gene symbols and gene marker stocks of rice, a list of about 240 recent publications on rice genetics and 46 research notes on rice genetics. Rice Genetics Newsletter will be published annually.

Data Base Design of DNA Base Sequences

Sanzo MIYAZAWA

Experimental analyses of DNA base sequences have been developed and known DNA base sequences are rapidly growing. To make DNA sequence data, published in scientific journals, readable by computer and accessible to public domain, DNA sequence data banks have been established in Europe

and USA. This recent trend prompts biologists to use computers to analyze DNA base sequences. Typical examples of common analyses are those such as homology search, restriction enzyme site map, and open reading frame search. Search and retrieval of specific base sequences from DNA sequence data are prerequisite to such analyses. Thus, a DNA data base is demanded to efficiently manipulate sequence data. UNIX operating system has been chosen as a target system to develop a data base, because of a portable operating system. By taking advantage of superior tools for interprocess communication in UNIX such as shared memory and pipeline, basic tools for data manipulation will be implemented as commands at the shell level. In addition, a wide variety of application programs for sequence analyses will be incorporated into the data base system.

PUBLICATIONS

- ACKERMANN, J. and SUGIYAMA, T.: Genetic analysis of developmental mechanisms in hydra. X. Morphogenetic potentials of a regeneration-deficient strain (reg-16). *Develop. Biol.* **107**: 13-27, 1985.
- AOKI, K.: Reciprocal altruism and reciprocal alliance between relatives. In "*Population Genetics and Molecular Evolution*" (ed. by T. Ohta and K. Aoki), 429-441, Japan Sci. Soc. Press, Tokyo/Springer, Berlin, 1985.
- ENAMI, M., FUKUDA, R. and ISHIHAMA, A.: Transcription and replication of eight RNA segments of influenza virus. *Virology* **142**: 68-77, 1985.
- ENDO, T. and IHARA, M.: Notes on extractant-dependent leaf peroxidase and malate dehydrogenase zymograms from twenty plant species. *Jpn. J. Breed.* **35**: 340-345, 1985.
- ENOMOTO, T., SUZUKI, M., TAKAHASHI, M., KAWASAKI, K., WATANABE, Y., NAGATA, K., HANAOKA, F. and YAMADA, M.: Purification and characterization of two forms of DNA polymerase α from mouse FM3A cells. *Cell Struc. Func.* **10**: 161-171, 1985.
- FUJII, T. and INOUE, T.: Absence of mutagenic activity of benzo(a)pyrene in the soybean test system. *Env. Exp. Bot.* **25**: 139-143, 1985.
- FUJITA, N. and ISHIHAMA, A.: Regulation of transcription initiation in prokaryotes. (in Japanese). *Jpn. J. Genet.* **60**: 505-525, 1985.
- FUKUDA, M., WAKASUGI, S., TSUZUKI, T., NOMIYAMA, H., SHIMADA, K. and MIYATA, T.: Mitochondrial DNA-like sequences in the human nuclear genome: Characterizations and implications in the evolution of mitochondrial DNA. *J. Mol. Biol.* **186**: 257-266, 1985.
- FUKUDA, R., YANO, R., FUKUI, T., HASE, T., ISHIHAMA, A. and MATSUBARA, H.: Cloning of the *E. coli* gene for the stringent starvation protein. *Mol. Gen. Genet.* **201**: 151-157, 1985.
- FURUSATO, T., TAKANO, J., JIGAMI, Y., TANAKA, H. and YAMANE, K.: Two tandemly located promoters, artificially constructed, are active in a *Bacillus subtilis* α -amylase secretion vector. *J. Biochem.* (in press).
- GOJOBORI, T.: Evolutionary features of oncogenes. In "*Population Genetics and Molecular Evolution*" (ed. by T. Ohta and K. Aoki), 353-368, Japan Sci. Soc. Press, Tokyo/Springer, Berlin, 1985.
- GOJOBORI, T. and YOKOYAMA, S.: Evolutionary rates of the retroviral oncogene of Moloney murine sarcoma virus and the cellular homologues. *Proc. Natl. Acad. Sci.* **82**: 4198-4201, 1985.
- GRONOSTAJSKI, R. M., ADHYA, S., NAGATA, K., GUGGENHEIMER, R. A. and HURWITZ, J.: Site-specific DNA binding of nuclear factor I: analysis of cellular binding sites. *Mol. Cell. Biol.* **5**: 964-971, 1985.
- HAYASHIDA, H. and MIYATA, T.: On the direction of gene conversion. *Proc. Japan Acad.* **61** (B): 204-207, 1985.

- HAYASHIDA, H., TOH, H., KIKUNO, R. and MIYATA, T.: Evolution of influenza virus genes. *Mol. Biol. Evol.* **2**: 289-303, 1985.
- HAYASHIDA, H., KIKUNO, R., YASUNAGA, T. and MIYATA, T.: A classification of gene conversion and its evolutionary implications. *Proc. Japan Acad.* **61** (B): 149-152, 1985.
- HAYASHIDA, H. and MITAYA, T.: Sequence similarity between epidermal growth factor precursor and atrial natriuretic factor precursor. *FEBS Letts.* **185**: 125-128, 1985.
- HIDAKA, S., TSUNASAWA, S., YOON, J., NARITA, K., TAKANAMI, Y., KUBO, S. and MIURA, K.: Messenger RNA structure participating in the initiation of synthesis of cucumber mosaic virus coat protein. *J. Biochem.* **97**: 161-171, 1985.
- HOUBA-HÉRIN, N., HARA, H., INOUE, M. and HIROTA, Y.: Binding of penicillin to thiolpenicillin-binding protein 3 of *Escherichia coli*: identification of its active site. *Mol. Gen. Genet.* **201**: 499-504, 1985.
- HURTWITZ, J., ADHYA, S., FIELD, J., GRONOSTAJSKI, R., GUGGENHEIMER, R. A., KENNY, M., LINDENBAUM, J. and NAGATA, K.: Synthesis of adenoviral DNA with purified proteins. In "*Genetics, Cell differentiation and Cancer*", 15-24, Academic Press, 1985.
- IDA, K., HIROTA, Y. and SCHWARZ, U.: Mutants of *Escherichia coli* defective in penicillin-insensitive murein DD-endopeptidase. *Mol. Gen. Genet.* **189**: 215-221, 1983.
- IKEMURA, T.: Codon usage, tRNA content, and rate of synonymous substitution. In "*Population Genetics and Molecular Evolution*" (ed. by T. Ohta and K. Aoki), 385-406, Japan Sci. Soc. Press, Tokyo/Springer, Berlin, 1985.
- IKEMURA, T.: Codon usage and tRNA content in unicellular and multicellular organisms. *Mol. Biol. Evol.* **2**: 13-34, 1985.
- INOUE, S., NOGUCHI, M., SAKAKI, Y., TAKAGI, Y., MIYATA, T., IWANAGA, S., MIYATA, T. and TSUJI, F. I.: Cloning and sequence analysis of cDNA for the luminescent protein aequorin. *Proc. Natl. Acad. Sci. USA* **82**: 3154-3158, 1985.
- ISHII, R., YOSHIKAWA, K., MINAKATA, H., KOMURA, H. and KADA, T.: Specificities of bio-antimutagens in plant kingdom. *Agric. Biol. Chem.* **48**: 2587-2591, 1985.
- ISHIKAWA, Y., KANO, M., TAMIYA, N. and SHIMADA, Y.: Acetylcholine receptors of human skeletal muscle: a species difference detected by snake neurotoxins. *Brain Res.* **346**: 82-88, 1985.
- IZUI, K., MIWA, T., KAJITANI, M., FUJITA, N., SABE, H., ISHIHAMA, A. and KATSUKI, H.: Promoter analysis of the phosphoenolpyruvate carboxylase gene of *Escherichia coli*. *Nucleic Acids Res.* **13**: 59-72, 1985.
- JASPERS, N. G. J., PAINTER, R. B., PATERSON, M. C., KIDSON, C. and INOUE, T.: Complementation analysis of ataxia-telangiectasia. In "*Ataxia-Telangiectasia: Genetics, Neuropathology, and Immunology of a Degenerative Disease of Childhood*" (ed. by R. A. Gatti and M. Swift), Alan R. Liss Inc., New York, 147-162, 1985.
- KADA, T., KANEKO, K., MATSUZAKI, S., MATSUZAKI, T. and HARA, Y.: Detection and chemical identification of natural bio-antimutagens. A case of the green tea factor. *Mutation Res.* **150**: 127-132, 1985.
- KADA, T., SADAIE, Y. and INOUE, T.: Tritium effects on DNA. Tritium concentration dependency of RBE in aqueous solution. NIRS-M-52 (Proc. 2nd Workshop on

- Tritium Radiobiology and Health Physics): 64-74, 1985.
- KAMIMURA, T., TSUCHIYA, M., URAKAMI, K., KOURA, K., SEKINE, M., SHINOZAKI, K., MIURA, K. and HATA, T.: Synthesis of a dodecaribonucleotide, GUAUCAAUA-AUG, by use of "fully" protected ribonucleotide building blocks. *J. Am. Chem. Soc.* **106**: 4552-4557, 1984.
- KATAGIRI, F., KODAKI, T., FUJITA, N., IZUI, K. and KATSUKI, H.: Nucleotide sequence of the phosphoenolpyruvate carboxylase gene of the cyanobacterium *Anacystis nidulans*. *Gene* **38**: 265-269, 1985.
- KATO, A., MIZUMOTO, K. and ISHIHAMA, A.: Purification and enzymatic properties of an RNA polymerase-RNA complex from influenza virus. *Virus Res.* **3**: 115-127, 1985.
- KATO, J.-I., SUZUKI, H. and HIROTA, Y.: Overlapping of the coding regions for α and γ components of penicillin-binding protein 1b in *Escherichia coli*. *Mol. Gen. Genet.* **196**: 449-457, 1984.
- KATO, J.-I., SUZUKI, H. and HIROTA, Y.: Dispensability of either penicillin-binding protein-1a or -1b involved in the essential process for cell elongation in *Escherichia coli*. *Mol. Gen. Genet.* **200**: 272-277, 1985.
- KATOH, H., ESAKI, K., SHOJI, Y., NOMURA, T., MORIWAKI, K., YONEKAWA, H.: Demonstration of genetic profiles in various lines of AKR and NZB by a genetic monitoring system. In "The Contribution of Laboratory Animal Science to the Welfare of Man and Animals: 8th ICLAS/CALS Symposium, Vancouver, 1983" (ed. by J. Archibald & others), 495-502, Gustav Fisher, Stuttgart, 1985.
- KATOH, Y., HASEGAWA, T., SUZUKI, T. and FUJII, T.: Changes in the amounts of putrescine, spermidine and spermine in Hiproly barley callus after auxin withdrawal. *Agric. Biol. Chem.* **49**: 1027-1032, 1985.
- KAWAKAMI, K., NOGUCHI, S., NODA, M., TAKAHASHI, H., OHTA, T., KAWAMURA, M., NOJIMA, H., NAGANO, K., HIROSE, T., INAYAMA, S., HAYASHIDA, H., MIYATA, T. and NUMA, S.: Primary structure of the α -subunit of *Torpedo californica* ($\text{Na}^+ + \text{K}^+$)-ATPase deduced from cDNA sequence. *Nature* **316**: 733-736, 1985.
- KAWAKAMI, K., MIZUMOTO, K., ISHIHAMA, A., YAMAGUCHI-SHINOZAKI, K. and MIURA, K.: Activation of influenza virus-associated RNA polymerase by cap-1 structure. *J. Biochem.* **97**: 655-661, 1985.
- KIKUNO, R. and MIYATA, T.: Sequence homologies among mitochondrial DNA-coded URF2, URF4 and URF5. *FEBS Letters*, **189**: 85-88, 1985.
- KIKUNO, R., HAYASHIDA, H. and MIYATA, T.: Rapid rate of rodent evolution. *Proc. Japan Acad.* **61** (B): 153-156, 1985.
- KIMURA, M.: Diffusion models in population genetics with special reference to fixation time of molecular mutants under mutational pressure. In "Population Genetics and Molecular Evolution" (ed. by T. Ohta and K. Aoki), 19-39, Japan Sci. Soc. Press, Tokyo/Springer, Berlin, 1985.
- KIMURA, M.: Genes, populations and molecules: a memoir. In "Population Genetics and Molecular Evolution" (ed. by T. Ohta and K. Aoki), 459-481, Japan Sci. Soc. Press, Tokyo/Springer, Berlin, 1985.
- KIMURA, M.: Natural selection and neutral evolution. In "What Darwin Began" (ed.

- by L. R. Godfrey), 73-93, Allyn and Bacon, Boston, 1985.
- KIMURA, M.: The neutral theory of molecular evolution. *New Scientist*, No. 1464 (11 July): 41-45, 1985.
- KIMURA, M.: Neutralisme dans l'évolution. *Sciences & Avenir*, Numero Special: 29-34, 1985.
- KIMURA, M.: The role of compensatory neutral mutations in molecular evolution. *J. Genetics* **64** (1): 7-19, 1985.
- KITAMURA, N., KITAGAWA, H., FUKUSHIMA, D., TAKAGAKI, Y., MIYATA, T. and NAKANISHI, S.: Structure organization of the human kininogen gene and a model for its evolution. *J. Biol. Chem.* **260**: 8610-8617, 1985.
- KOMINAMI, R., MURAMATSU, M., SUDO, K., YOSHIKURA, H., SUZUKI, H., MORIWAKI, K. and HILGERS, J.: The origin and meiotic instability of a polymorphic repetitive sequence RPI family. In "*The BALB/c Mouse: Genetics and Immunology*" (ed. by M. Potter), 58-65, Springer-Verlag, Berlin, 1985.
- KOMINAMI, R., SUDO, K., YOSHIKURA, H., SUZUKI, H., MORIWAKI, K., HILGERS, J. and MURAMATSU, M.: A polymorphic repetitive-sequence PRI family evidence for meiotic instability. *J. Mol. Biol.* **183**: 301-309, 1985.
- KUBO, T., NODA, M., TAKAI, T., TANABE, T., KAYANO, T., SHIMIZU, S., TANAKA, K., TAKAHASHI, H., HIROSE, T., INAYAMA, S., KIKUNO, R., MIYATA, T. and NUMA, S.: Primary structure of δ subunit precursor of calf muscle acetylcholine receptor deduced from cDNA sequence. *Eur. J. Biochem.* **149**: 5-13, 1985.
- KURIHARA, Y., MIYASHITA, N., MORIWAKI, K., PETRAS, M. L., BONHOMME, F., CHO, W. S. and KOHNO, S.: Serological survey of T-lymphocyte differentiation antigens in wild mice. *Immunogenet.* **22**: 211-218, 1985.
- KURODA, Y., YOKOIYAMA, A. and KADA, T.: Assays for the induction of mutations to 6-thioguanine resistance in Chinese hamster V79 cells in culture. *Progress in Mutation Research* (ed. by J. Ashby & others), Vol. 5: 537-542, 1985.
- KURODA, Y., HAYATSU, H. and NEGISHI, K.: Mutagenic activity of cytidine analogs in cultured mammalian cells. *Mutation Res.* **147**: 262-263, 1985.
- KURODA, Y.: Aging of cells. (in Japanese) In "*Development and Aging*" (ed. by G. Eguchi and N. Osawa), II, 89-100, Nakayama-shoten, Tokyo, 1985.
- KURODA, Y.: Clonal culture of cells and growth factors. (in Japanese) In "*Studies on Control of Aging*" (ed. by Institute of Physical and Chemical Research), 218-223, Sozo Life Science Kenkyukai, Tokyo, 1985.
- KUSUDA, J., ONIMARU, K., NOGUCHI, T. and YAMASHITA, O.: Maturation and hatching of eggs from silkworm ovaries preserved in liquid nitrogen. *J. Insect Physiol.* **31**: 963-967, 1985.
- MARUYAMA, T. and FUERST, P. A.: Non-equilibrium models in population genetics: The first arrival times and the number of alleles in evolving populations. In "*Population Genetics and Molecular Evolution*" (ed. by T. Ohta and K. Aoki), Japan Sci. Soc. Press, Tokyo/Springer, Berlin, 1985.
- MARUYAMA, T. and FUERST, P. A.: Population bottleneck and non-equilibrium models in population genetics. II. Number of alleles when a small population is derived from a large steady state population by means of a bottleneck. *Genetics* **111**: 675-

689, 1985.

- MARUYAMA, T. and FUERST, P. A.: Population bottlenecks and non-equilibrium models in population genetics. III. Genetic homozygosity in populations which experience periodic bottlenecks. *Genetics* **111**: 691-703, 1985.
- MATSUI, M., OKA, A., TAKANAMI, M., YASUDA, S. and HIROTA, Y.: Sites of *dna A* protein binding in the replication origin of the *Escherichia coli* K-12 chromosome. *J. Mol. Biol.* **184**: 529-533, 1985.
- MATSUNAGA, E.: Monitoring of birth defects: present status and problems. (in Japanese). *J. Jpn. Med. Assoc.* **93**: 1322-1324, 1985.
- MINAKATA, H., KOMURA, H., TAMURA, S. Y., OHFUNE, Y., NAKANISHI, K. and KADA, T.: Antimutagenic unusual amino acids from plants. *Experientia* **41**: 1621-1623, 1985.
- MIYASHITA, N., MORIWAKI, K., MINEZAWA, M., YONEKAWA, H., BONHOMME, F., MIGITA, S., YU, Z. C., LU, D. Y., CHO, W. S. and THOHARI, M.: Allelic constitution of the hemoglobin beta chain in wild population of the house mouse, *Mus musculus*. *Biochem. Genet.* **23**: 975-986, 1985.
- MIYASHITA, N., SUZUKI, K. and MORIWAKI, K.: The H-2 complex affects the frequency of urethan-induced chromosomal aberrations. *Jpn. J. Cancer Res.* **76**: 1141-1145, 1985.
- MIYATA, T., TOH, H., HAYASHIDA, H., KIKUNO, R., INOKUCHI, Y. and SAIGO, K.: Sequence homology among reverse transcriptase-containing viruses and transposable genetic element: functional and evolutionary implications. In "*Population Genetics and Molecular Evolution*" (ed. by T. Ohta and K. Aoki), 313-331, Japan Sci. Soc. Press, Tokyo/Springer, Berlin, 1985.
- MIYATA, T., HAYASHIDA, H., KIKUNO, R., TOH, H. and KAWADA, Y.: Evolution of interferon genes. In "*Interferon 6*" (ed. by Grosser), 1-30, Academic Press, London, 1985.
- MIYATA, T., TOH, H. and SAIGO, K.: Reverse transcriptase-containing DNA virus and plasmids lack integrase. *Proc. Japan Acad.* **61B**: 464-466, 1985.
- MORISHIMA, H.: Habitat, genetic structure and dynamics of perennial and annual populations of the Asian wild rice *Oryza perennis*. In "*Genetic Differentiation and Dispersal in Plants*" (ed. by P. Jacquard, & others), 179-190, Springer-Verlag, Berlin, 1985.
- MORITA, K., NISHIMURA, Y. and KADA, T.: Chemical nature of a desmutagenic factor from burdock (*Arctium lappa* Linne). *Agric. Biol. Chem.* **49**: 925-932, 1985.
- MORIWAKI, K., MIYASHITA, N. and YONEKAWA, H.: Genetic survey of the origin of laboratory mice and its implication in genetic monitoring. In "*Contribution of Laboratory Animal Science to the Welfare of Man and Animals: 8th ICLAS/CALS Symposium, Vancouver, 1983*" (ed. by J. Archibald & others), 237-247, Gustav Fischer, Stuttgart, 1985.
- MUKAI, T.: Experimental verification of the neutral theory. In "*Population Genetics and Molecular Evolution*" (ed. by T. Ohta and K. Aoki), 125-145, Japan Sci. Soc. Press, Tokyo/Springer, Berlin, 1985.
- MUKAI, T., BABA, M., AKIYAMA, M., UOWAKI, N., KUSAKABE, S. and TAJIMA, F.: Rapid

- change in mutation rate in a local population of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **82**: 7671-7675, 1985.
- MURAKAMI, A.: Dose-response relationships for mutations induced by chemicals in the silkworm. In "Problems of Thresholds in Chemical Mutagenesis" (ed. by Y. Tazima & others), Environm. Mutagen Soc. Japan, 5-13, 1984.
- MURAKAMI, A.: A new mutant at the *pe*⁺ locus, black eyed light yellowish-brown egg in the silkworm, *Bombyx mori*. Sericologia **25**: 327-342, 1985.
- NICHOLAS, R. A., STROMINGER, J. L., SUZUKI, H. and HIROTA, Y.: Identification of the active site in penicillin-binding protein 3 of *Escherichia coli*. J. Bacteriol. **164**: 456-460, 1985.
- NICHOLAS, R. A., SUZUKI, H., HIROTA, Y. and STROMINGER, J. L.: Purification and sequencing of the active site tryptic peptide from penicillin-binding protein 1b of *Escherichia coli*. Biochemistry **24**: 3448-3453, 1985.
- NOMURA, T., AIBA, H. and ISHIHAMA, A.: Transcriptional organization of the convergent overlapping *dnaQ-rnh* genes of *Escherichia coli*. J. Biol. Chem. **260**: 7122-7125, 1985.
- NOMURA, T., FUJITA, N. and ISHIHAMA, A.: Promotor selectivity of *E. coli* RNA polymerase: analysis of the promotor system of convergently-transcribed *dnaQ-rnh* genes. Nucleic Acids Res. **13**: 7647-7661, 1985.
- OHNISHI, S. and WATANABE, T. K.: Genetic analysis of color dimorphism in *Drosophila montium* subgroup. Jpn. J. Genet. **60**: 355-358, 1985.
- OHTA, T.: Variances and covariances of identity coefficients of a multigene family. Proc. Natl. Acad. Sci. USA **82**: 829-833, 1985.
- OHTA, T.: A model of duplicative transposition and gene conversion for repetitive DNA families. Genetics **110**: 513-524, 1985.
- OHTA, T. and AOKI, K. (eds.): "Population Genetics and Molecular Evolution", Japan Sci. Soc. Press, Tokyo/Springer, Berlin, 1985.
- OHTA, T.: Genetic variation of multigene families. In "Population Genetics and Molecular Evolution" (ed. by T. Ohta and K. Aoki), 233-242, Japan Sci. Soc. Press, Tokyo/Springer, Berlin, 1985.
- ONO, M., TOH, H., MIYATA, T. and AWAYA, T.: Nucleotide sequence of the Syrian hamster intracisternal A particle gene: Close evolutionary relationship of type A particle gene with type B and D oncovirus genes. J. Virol. **55**: 387-394, 1985.
- SADAIE, Y. and KADA, T.: *Bacillus subtilis* gene involved in cell division, sporulation, and exoenzyme secretion. J. Bacteriology **163**: 648-653.
- SANO, Y., MAEKAWA, M., and KIKUCHI, H.: Temperature effects on the *Wx* protein level and amylose content in the endosperm of rice. J. Heredity **76**: 221-222, 1985.
- SANO, Y., KATSUMATA, M. and AMANO, E.: Correlations between the amounts of amylose and *Wx* protein in rice endosperm. SABRAO J. 1985 (in press).
- SANO, Y.: Interspecific cytoplasm substitutions of an Indica strain of *Oryza sativa* L. and *O. glaberrima* Steud. Euphytica **34**: 587-592, 1985.
- SATO, Y.-I. and HAYASHI, K.: Varietal variations in basic vegetative phase and photo-sensitive phase among Japanese native cultivars of rice. (in Japanese). Jpn. J. Breed. **65**: 72-75, 1985.
- SATO, Y.-I. and HAYASHI, K.: The genetic control of basic vegetative phase in early-

- maturing native rice varieties of Japan. (in Japanese). *Jpn. J. Breed.* **35**: 160-166, 1985.
- SATTA, Y., GOJOBORI, T., MARUYAMA, T. and CHIGUSA, S. I.: Tn3 resolvase-like sequence in P transposable element of *Drosophila melanogaster*. *Jpn. J. Genet.* **60**: 261-266, 1985.
- SATTA, Y., GOJOBORI, T., MARUYAMA, T., SAIGO, K. and CHIGUSA, S. I.: Homology between P-transposable element of *Drosophila melanogaster* and bacterial transposase of Tn3. *Jpn. J. Genet.* **60**: 499-503, 1985.
- SHIMADA, Y. and ISOBE, Y.: Cytoskeletal organization in embryonic chick skeletal muscle cells *in vitro* revealed by the detergent-extraction, freeze-dry method. In "*Molecular Biology of Muscle Development*" (ed. by C. Emerson, & others), (in press) Alan R. Liss, New York, 1985.
- SHIMIZU, N. and SHIMADA, Y.: Immunochemical analysis of tropoin T isoforms in adult, embryonic, regenerating and denervated chicken fast muscles. *Develop. Biol.* **111**: 324-334, 1985.
- SHIMOI, K., NAKAMURA, Y., NORO, T., TOMITA, I., FUKUSHIMA, S., INOUE, T. and KADA, T.: Methyl cinnamate derivatives enhance UV-induced mutagenesis due to the inhibition of DNA excision repairs in *Escherichia coli* B/r. *Mutation Res.* **146**: 15-22, 1985.
- SHIMOI, K., NAKAMURA, Y., TOMITA, I. and KADA, T.: Bio-antimutagenic effects of tannic acid on UV and chemically induced mutagenesis in *Escherichia coli* B/r. *Mutation Res.* **148**: 17-23, 1985.
- SHIMOTOHNO, K., TAKAHASHI, Y., SHIMIZU, N., GOJOBORI, T., CHEN, I. S. Y., MIWA, M. and SUGIMURA, T.: Complete nucleotide sequence of an infectious clone of human T-cell leukemia virus type II: A new open reading frame for the protease gene. *Proc. Natl. Acad. Sci.* **82**: 3101-3105, 1985.
- SHIROISHI, T., EVANS, G. A., APPELLA, E. and OZATO, K.: In vitro mutagenesis of a mouse MHC class I gene for the examination of structure-function relationships. *J. Immunol.* **134**: 623-629, 1985.
- SLATKIN, M. and TAKAHATA, N.: The average frequency of private alleles in a partially isolated population. *Theor. Pop. Biol.* **28**: 314-331, 1985.
- SRIMAL, S., MIYATA, T., KAWABATA, S., MIYATA, T. and IWANAGA, S.: The complete amino acid sequence of coagulogen isolated from southeast Asian horseshoe crab, *Carcinoscorpius rotundicauda*. *J. Biochem.* **98**: 305-318, 1985.
- SUGIYAMA, T. and SUGIMOTO, N.: Genetic analysis of developmental mechanisms in hydra. XI. Mechanisms of sex change by parabiosis. *Develop. Biol.* **110**: 413-421, 1985.
- SUZUKI, M., SUZUKI, A., YAMAKAWA, T. and MATSUNAGA, E.: Characterization of 2,7-anhydro-N-acetylneuraminic acid in human wet cerumen. *J. Biochem.* **97**: 509-515, 1985.
- TACHIBANA, H. and ISHIHAMA, A.: Correlation of the formation rate of open promoter complexes with the melting property of DNA. In "*Physical Foundation of Protein and Nucleic Acid Functions*" (ed. by A. Wada), Jpn. Sci. Soc. Press/Elsevier, 1985.
- TACHIDA, H. and MUKAI, T.: The genetic structure of natural populations of *Drosophila*

- melanogaster*. XIX. Genotype-environment interaction in viability. *Genetics* **111**: 43-55, 1985.
- TAKAHASHI, K., GOJOBORI, T., and NAORA, H.: Homology of kringle structures in urokinase and tissue-type plasminogen activator: The phylogeny to the related serine proteases. *Cell Struc. Func.* **10**: 209-218, 1985.
- TAKAHATA, N.: Introgression of extranuclear genomes in finite populations: nucleo-cytoplasmic incompatibility. *Genet. Res. Camb.* **45**: 179-194, 1985.
- TAKAHATA, N.: Gene diversity in finite populations. *Genet. Res. Camb.* **46**: 107-113, 1985.
- TAKAHATA, N. and PALUMBI, S. R.: Extranuclear differentiation and gene flow in the finite island model. *Genetics* **109**: 441-457, 1985.
- TAKAHATA, N. and NEI, M.: Gene genealogy and variance of interpopulational nucleotide differences. *Genetics* **110**: 325-344, 1985.
- TAKAHATA, N.: Population genetics of extranuclear genomes: A model and review. In "*Population Genetics and Molecular Evolution*" (ed. by T. Ohta and K. Aoki), 195-212, Japan Sci. Soc. Press, Tokyo/Springer, Berlin, 1985.
- TEZUKA, H., SASAKI, Y. F., INOUE, M., UCHIDA, A., MORIYA, M. and SHIRASU, Y.: Heritable translocation study in male mice with trimethyl phosphate. *Mutation Res.* **157**: 205-213, 1985.
- TEZUKA, H., INOUE, T., NOGUTI, T., KADA, T. and SHULTZ, L. D.: Evaluation of the mouse mutant "wasted" as an animal model for ataxia telangiectasia. I. Age-dependent and tissue-specific effects. *Mutation Res.* (in press).
- TOH, H., HAYASHIDA, H., KIKUNO, R., YASUNAGA, T. and MIYATA, T.: Sequence similarity between EGF receptor and α_1 -acid glycoprotein. *Nature* **314**: 199, 1985.
- TOH, H., ONO, M., SAIGO, K. and MIYATA, T.: Retroviral protease-like sequence in the yeast transposon Tyl. *Nature* **315**: 691, 1985.
- TOH, H. and MIYATA, T.: Is the AIDS virus recombinant? *Nature* **316**: 21-22, 1985.
- TOH, H., ONO, M. and MIYATA, T.: Retroviral *gag* and DNA endonuclease coding sequences in IgE-binding factor gene. *Nature* **318**: 388-389, 1985.
- TOH, H., KIKUNO, R., HAYASHIDA, H., MIYATA, T., KUGIMIYA, W., INOUE, S., YUKI, S. and SAIGO, K.: Close structural resemblance between putative polymerase of a *Drosophila* transposable genetic element 17.6 and *pol* gene product of Moloney murine leukaemia virus. *EMBO. J.* **4**: 1267-1272, 1985.
- TSUNEWAKI, K., SPETSOV, P. and YONEZAWA, K.: Increasing genetic variability in common wheat by utilizing alien cytoplasm—Cytoplasmic effects on the performance and interplant variability of the F_1 and F_2 generations of the cross, *Triticum aestivum* cv. Norin 26 $\times$ cv. Norin 61. Japan. J. Breed. **35**: 398-412, 1985.
- WATANABE, K. and MIURA, K.: Specific interaction between tRNA and its cognate amino acid as detected by circular dichroism and fluorescence spectroscopy. *Biochem. Biophys. Res. Comm.* **129**: 679-685, 1985.
- WEIR, B. S., OHTA, T. and TACHIDA, H.: Gene conversion models. *J. Theor. Biol.* **116**: 1-8, 1985.
- YAMADA, M. A., WATANABE, T. K. and KOANA, T.: Absence of resistance genes against male-killing action of the SRO in *Drosophila melanogaster*. *Jpn. J. Genet.* **60**: 93-

102, 1985.

YASUDA, S., NAKAYAMA, N., JIKUYA, H. and SOEDA, E.: An improved method for shotgun DNA sequencing. *Agr. Biol. Chem.* **49**: 1525-1526, 1985.

YONEKAWA, H., KATOH, H., ESAKI, K., SAGAI, T., MORIWAKI, K. and TAGASHIRA, Y.: Maternal contamination of some NZB sublines and genetic profiles of NZ-strains. *Lab. Anim. Sci.* (in press, 1985).

YONEZAWA, K.: A definition of the optimal allocation of effort in conservation of plant genetic resources—with application to sample size determination for field collection. *Euphytica* **34**: 345-354, 1985.

YOSHIMARU H. and MUKAI, T.: Relationships between the polygenes affecting the rate of development and viability in *Drosophila melanogaster*. *Jpn. J. Genet.* **60**: 307-334, 1985.

YOSIDA, T. H., UDAGAWA, T., ISHIBASHI, M., MORIWAKI, K., YABE, T. and HAMADA, T.: Studies on the karyotypes of the black rats distributed in the Pacific and South Pacific Islands, with special regard to the border line of the Asian and Oceanian type black rats on the Pacific Ocean. *Proc. Jpn. Acad.* **61B**: 71-74, 1985.

YUKUHIRO, K., HARADA, K. and MUKAI, T.: Viability mutations induced by the P elements in *Drosophila melanogaster*. *Jpn. J. Genet.* **60**: 261-266, 1985.

ABSTRACTS OF DIARY FOR 1985

Biological Symposium

- 234th meeting Feb. 7 Molecular population genetics of *Drosophila*: transcriptional units and transposable elements (C. H. Langley)
- 235th — Feb. 26 Molecular analysis of a "sex chromosome associated" repeated DNA sequences in *Drosophila melanogaster* (P. R. Simpson)
- 236th — Mar. 18 Genetic dissection of RNA polymerase (R. E. Glass)
- 237th — Mar. 18 Hereditary variation of the normal electroencephalogram (EEG): a model system in human behavior genetics (F. Vogel)
- 238th — Apr. 17 Use of DNA probes to study the population genetics of intracellular bacteria (genus *Rickettsia*) (P. A. Fuerst)
- 239th — May 30 The role of calcium influxes during hydrozoan development (G. Freeman)
- 240th — June 7 Geographic origins, genetic diversity and the molecular clock hypothesis in the *Oryzae* (G. Second)
- 241st — Sept. 19 Human cancer susceptibility genes and oncogenes (W. F. Benedict)
- 242nd — Sept. 20 Analysis of the transcriptional signals of bacterial rRNA genes (P. Venetianer)
- 243rd — Oct. 21 Molecular genetics of the mouse major histocompatibility complex (J. A. Frelinger)
- 244th — Nov. 20 Models of biological pattern formation (H. Meinhardt)
- 245th — Nov. 19 Genes phylogeny and species phylogeny in the genus *Mus* (F. Bonhomme)
- 246th — Dec. 5 Influence of DNA inhibitors on the biological effects of physical and chemical mutagens (A. T. Natarajan)

Mishima Geneticists' Club

- | | | | |
|-------|---------|---------|--|
| 295th | meeting | Feb. 14 | The structure of the gene for the human major histocompatibility antigen, HAL Class II (Kiyotaka Okada) |
| 296th | — | Feb. 19 | Game between males for possession of females —emergence curve of butterflies and sex ratio of frogs (Yoh Iwasa) |
| 297th | — | Mar. 11 | Quantitative analysis of biological morphogenesis (Hiroshi Shimizu) |
| 298th | — | Mar. 13 | Reverse transcriptase involved in the DNA replication of cauliflower mosaic virus—cloning of the gene for the reverse transcriptase and its expression in yeast cells (Johe Ikeda) |
| 299th | — | Apr. 11 | Immunological self-recognition in killer-T cell as a model (Nobutaka Shinohara) |
| 300th | — | Apr. 25 | An adenovirus vector for expression of cloned genes in mammalian cells (Masao Yamada) |
| 301st | — | May 31 | <i>Bacillus subtilis</i> <i>spoO</i> genes and the major sigma factor (Fujio Kawamura) |
| 302nd | — | June 25 | Recent findings on MR system in <i>Drosophila melanogaster</i> (Yuichiro Hiraizumi) |
| 303rd | — | July 22 | Analysis of molecular genetic variation at the loci encoding amylase and ADH (Hidenori Tachida) |
| 304th | — | Sep. 18 | Theoretical analysis of promoter strength in transcription (Hideki Tachibana) |
| 305th | — | Sep. 19 | Search for new oncogenes; use of newly developed cDNA expression cloning vector system (Hiroto Okayama) |
| 306th | — | Oct. 21 | Altered expression of the human non- α -globin gene complex by the mutation (Takashi Imamura) |
| 307th | — | Nov. 28 | Sexual differentiation and hormones of snails (Naokuni Takeda) |
| 308th | — | Nov. 26 | Mouse major histocompatibility class I gene expression in early-stage embryos (Keiko Ozato) |

- 309th — Dec. 24 Expression of recombinant genetic element in
the teratocarcinoma stem cell (Makoto Taketo)

FOREIGN VISITORS IN 1985

March 9, 1984– February 28, 1985	Kim, Bong Kee, Dankook University, Korea
March 16, 1984– April 30, 1985	Houba-Herin, Nicole, Liège University, Belgium
December 22, 1984– February 9, 1985	Lee, Won Ho, Pusan National University, Korea
September 29, 1984– December 19, 1985	Loekman, Irwansyah, National Atomic Energy Agency, Indonesia
September 29, 1984– December 19, 1985	Gadrinab, Lilian U., SEAMEO Regional Center for Tropical Biology, Indonesia
January 19	Tetsuji, Matayoshi, Centro de Genetica Medica, Argentina
January 25–February 15	Choo, Jong Kil, Chung Ang University, Korea
February 6–12	Simpson, Paul, Australian National University, Australia
February 6–7	Paik, Yong Kyun, Hanyang University School of Medicine, Korea
February 8	Langley, C. W., National Institute of Environmental Health Sciences, NIH, U.S.A.
February 26– March 1	Boursot, Pierre, Universite Montpellier, France
March 18–19	Huang, Yi-de, Shanghai Institute of Plant Physiology, Academia Sinica, China
March 18–19	Glass, Robert E., University Medical School, Nottingham, England
March 18–19	Vogel, F., Institut für Anthropologie u. Humangenetik der Universität Heidelberg, West Germany
March 21–28	Boussy, Ian, Australian National University, Australia
March 26–May 4	Fuerst, Paul A., Ohio State University, U.S.A.
April 1–4	Guhardja, Edi, Institut Pertanian Bogor, Indonesia
April 1–November 9	Jain, Ajay Kumar, King George Medical Col-

	lege, India
April 5	Lu, Hong Sheng, Institute of Sericulture, Chinese Academy of Agricultural Sciences, China
April 6	Majewski, Tomasz, Institute of Botany, Polish Academy of Sciences, Poland
April 22	Zhang, Xu Jing, Qinghai Academy of Veterinary Medicine, China
	Fang, Xi Ye, Experimental Animal Center, Chinese Academy of Medical Sciences, China
	Zhang, Cheng Gui, Yunnan Primate Center of Experimental Animals, China
May 22	Ruffié, Jacques, Laboratoire d'Anthropologie Physique, Collège de France, France
May 22-24	Glass, Robert E., University Medical School, Nottingham, England
May 23	Richards, S., British Council, Tokyo
May 30	Rabson, Robert, Office of Basic Energy Sciences, U.S.A.
June 3	Second, Gérard, Centre d'Etudes Phytosociologiques et Ecologiques, France
June 12-30	Crow, James F., University of Wisconsin, U.S.A.
June 12-13	de Azevedo, Joao Lucio, University of São Paulo, Brazil
June 19	Haselkorn, Robert, University of Chicago, U.S.A.
July 5	Warid, Warid A., University of Cairo, Egypt
July 16	Sudarwanti, Sri, Bandung Institute of Technology, Indonesia
August 1	Yung, Sun Kang, Seoul, Korea
September 2	Mukherjee, T. K., University of Malaya, Malaysia
September 7-20	Jacquard, Pierre, Centre d'Etudes Phytosociologiques et Ecologiques, France
September 9	Jia, Shi Rong; Wang, Ji Fang; Chuan, Huan Ying; Vegetable Research Institute, Chinese Academy of Agricultural Sciences, China
	Ping, Ji Ming; Zhang, Ru Yu, Ministry of

	Agriculture, Animal Husbandry and Fishery, China
September 11-	Lee, Won Ho, Pusan National University, Korea
September 17	Park, Hyeng Shik, Korea
September 19	Benedict, William F., Children's Hospital of Los Angeles, U.S.A.
September 20	Fallon, Ann M., UMDNJ-School of Osteopathic Medicine, U.S.A.
September 20-21	Ventetianer, Pal, Hungarian Academy of Sciences, Hungary
October 11-	Barbier, Pascale, Universite des Sciences et Techniques du Languedoc, Montpellier, France
October 15-	Qiu, Yuan Sheng, Guangdong Microbiology Research Institute, China
October 21	Frelinger, Jeffrey A., University of North Carolina, U.S.A.
November 13	Riley, Ralph, Agricultural Research Council, England
November 19	Bonhomme, Francois, Institut des Sciences de l'Evolution, Université Montpellier, France
November 19-21 ¹	Meinhardt, Hans, Max-Planck-Institut für Virusforschung, West Germany
November 26	Czarnomska, Alina, Institute of Oncology, Poland
December 4-6	Natarajan, A. T., University of Leiden, The Netherlands

AUTHOR INDEX

AOKI, K.	72	INOUE, M.	32
AOTA, S.-I.	28	ISHIHAMA, A.	17, 18, 19, 20, 23, 24
BARBIER, P.	102	ISHIKAWA, R.	102
BENZ, R. D.	64	ISHIMI, Y.	26
BROWN, W. L. Jr.	57	IYAMA, S.	107, 108
CARSTEN, A. L.	64	JONES, S. T.	17
CHEN, I. S. Y.	81	KADA, T.	62, 63, 65, 66
CHIGUSA, S. I.	79	KANO, Y.	20
CHITORAKON, S.	100	KATO, A.	24
CHO, W.-S.	87	KATO, J.-I.	33
ENDO, T.	103	KATOH, H.	92
FUERST, P. A.	76	KIMURA, K.	83
FUJII, T.	30, 66	KIMURA, M.	70
FUJITA, N.	17, 18, 19, 20	KITAZAWA, T.	46
FUKASE, Y.	49	KOBAYASHI, M.	50
FUKUDA, R.	21, 22, 25	KOIZUMI, A.	42
GADRINAB, L. U.	107	KOJIMA, H.	41
GLASS, R. E.	17	KONISHI, H.	41
GOJOBORI, T.	79, 80, 81, 82	KUBOTA, M.	57
GOLDE, D. W.	81	KURIHARA, Y.	39, 55, 85
GOTWALD, W. H. Jr.	57	KURODA, Y.	40, 41, 42, 43, 44, 49
HARA, H.	32	KUSUDA, J.	54
HARADA, Y.	38, 55, 68	LEE, W. H.	56, 83, 84, 87
HASEGAWA, M.	25	LEH, C.	57
HATADA, E.	25	MARUYAMA, T.	76, 79, 82
HATSUMI, M.	83	MATSUNAGA, E.	97, 98, 99
HAYASAKA, K.	98	MINATO, K.	45, 46
HIROTA, Y.	32, 33	MIWA, M.	81
HONDA, A.	23	MIYASHITA, N.	36, 37
HORAI, S.	97, 98	MIYATA, T.	90
HOUBA-HÉRIN, N.	32	MIYAZAWA, S.	108
IHARA, M.	107	MIZUMOTO, K.	23
IIDA, K.	32	MORIMOTO, K.	42
IKEMURA, T.	28, 29	MORISHIMA, H.	89, 100, 101, 102
IKUSHIMA, T.	64	MORIWAKI, K.	35, 36, 37, 38, 39, 55, 56, 68, 85, 87, 92
IMAI, H. T.	56, 57, 58, 59, 85	MUKAI, T.	77
IMAMOTO, F.	20	MURAKAMI, A.	46, 48, 49, 50
INOUE, T.	62, 63, 65	MURAKAMI, K.	62, 68
INOUE, Y.	27, 52		

NAGATA, K.	23, 24, 26	SHIROISHI, T.	35
NAORA, H.	82	SHOTAKE, T.	98
NATSUUME-SAKAI, S.	35	SIREGAR, U. J.	107
NAWA, S.	30	SLATKIN, M.	73
NEI, M.	73	SUGIMOTO, N.	52
NENE, V.	17	SUGIMURA, T.	81
NISHIMURA, A.	22	SUGIYAMA, T.	52
NOMURA, T.	17, 18, 19	SUZUKI, A.	99
NOZAWA, K.	98	SUZUKI, H.	33
OHTA, T.	71	SUZUKI, K.	87
OHTSUKI, Y.	46	SUZUKI, M.	99
OKA, H. I.	104	TACHIBANA, H.	20
ONIMARU, K.	54	TAKADA, Y.	43
OOTSUYAMA, A.	63	TAKAHASHI, J.	63
OSATO, K.	20	TAKAHASHI, K.	82
QIN, C.-G.	85	TAKAHASHI, Y.	81
PALUMBI, S. R.	74	TAKAHATA, N.	72, 73, 74, 75
SADAIE, T.	64	TAKEUCHI, K.	23
SADAIE, Y.	64	TAMAI, K.	62
SAGAI, T.	35, 92	TANO, S.	66
SAIGO, K.	79	TAYLOR, R. W.	59
SAKAIZUMI, M.	55	TEZUKA, H.	62, 63, 64
SANO, R.	89, 102	TJAN, K. N.	56, 57
SANO, Y.	30, 106	TOMITA, T.	38
SATO, Y.-I.	100, 101, 102	TSUCHIYA, K.	55
SATTA, Y.	79	TUTIKAWA, K.	68
SCHWARZ, U.	32	UEDA, S.	24
SERIZAWA, H.	21, 22	WATANABE, T. K.	83, 84
SHI, L.-M.	85	YAMAKAWA, T.	99
SHIMADA, Y.	44	YAMANE, K.	33
SHIMIZU, K.	25	YOKOYAMA, S.	80, 82
SHIMIZU, N.	81	YONEKAWA, H.	85, 92
SHIMOTOHNO, K.	81	YONEZAWA, K.	85, 104
SHINKAWA-TACHI, K.	42	YONG, H.-S.	57

国立遺伝学研究所年報 第36号

昭和61年10月25日 印刷

昭和61年10月30日 発行

発行者 松 永 英

国立遺伝学研究所内

編集者 木村資生・池村淑道

国立遺伝学研究所内

印刷者 笠 井 康 弘

東京都新宿区高田馬場 3-8-8

印刷所 株式 国際文献印刷社
会社

東京都新宿区高田馬場 3-8-8

発行所 国立遺伝学研究所

〒411 静岡県三島市谷田 1111

電話 代表 (0559) (75) 0771

