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1985

Annual Report of the National Institute of Genetics

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GENERAL STATEMENT

In introducing the Annual Report of our institute that covers 1 year from January to December 1984, I am pleased to begin by noting that, our long-cherished desire to reorganize the institute into a national center for joint use by universities, has been realized by amendment of the related law which took place on April 12 as a result of energetic efforts of the Ministry of Education, Science and Culture.

Looking back upon the history of the institute, its foundation in 1949 was primarily based on the zealous wishes of the Genetics Society of Japan to create a central institute for studies in various branches of genetics, putting emphasis on free scientific and personnel exchanges among universities and institutions without bias towards a particular university or faculty. As such, the institute was placed under the direct control of the Ministry of Education, Science and Culture. (At that time, a national institute for joint use by universities did not exist within the scheme of jurisdiction. The first example of this category was the Institute for High Energy Physics that was established in Tsukuba in 1971.) For 35 years thereafter the institute has been making hard and steady efforts to produce many significant achievements, through which it has become widely known among the scientific community of the world. In the meantime the progress of genetics as the core of life science has been accelerated by various epoch-making developments in biotechnology such as recombinant DNA technique. In accordance with this tendency, it is becoming increasingly necessary to promote collaborative studies among scientists with different disciplines in different institutions. To meet this need, however, there were a number of restrictions imposed by the administrative category to which our institute belonged, so that we had been preparing in the past several years, under the leadership of my predecessor Dr. Y. Tazima, for shifting it to that for joint use by universities. In the course of this preparation many people including related academic societies rendered help and support to us, for which I wish to express my heartfelt thanks.

The main points of reorganization were as follows. Firstly, the pre-existing 10 departments were rearranged into 5 according to the levels of research subjects, that is, Department of Molecular Genetics, Cell Genetics,

Ontogenetics, Population Genetics, and Department of Integrated Genetics. Each department consists of three laboratories, two for regular members and one for visiting professors. This year 3 new laboratories for visiting professors were approved. Secondly, as facilities for joint use, DNA Research Center (DNA Structure Section and Recombinant DNA Section) was newly established, and the Genetic Stock Research Center was expanded from 3 sections (plant, animal and bacteria) to 5, that is, Genetic Resources Section was added and the animal section was divided into Mammalian and Invertebrate Sections. Thirdly, the occupational category of research staff was changed, that is, after due review by an external committee, the department heads, laboratory heads and researchers were appointed Kyōju (professor), Jo-kyōju (associate professor) and Jo-shu (research member, a position to which there is no equivalent in USA), respectively. Fourthly, technicians who had been belonging to each laboratory were put together into Technical Section for the efficiency of labor.

The total expenditure in the 1984 FY was ¥917 million, about 55% of which were for personnel expenses; with respect to the amount allocated for research and related activities, there was a net increase by about ¥83 million as compared with the 1983 FY, which accrued concomitantly with the reorganization. Besides, grants-in-aid in the amount of ¥106 million were rendered to selected staff members by the Ministry of Education, Science and Culture. However, despite increased demand for new activity, there was no increase at all in the full number of regular staff (currently 92), reflecting the present severe state of administrative and financial constraint.

The missions of the new institute, which should be open to scientists in various universities and institutions, are to promote comprehensive and collaborative studies in genetics, taking advantage of the facilities for joint use including various genetic stocks, to cooperate in teaching students in doctor course, and to promote international cooperation and exchange of information. Through these activities we wish to contribute to the development of genetics and related sciences not only in Japan but also in the world.

In the past year two of our colleagues were loaded with honors. Professor Tomoko Ohta, Laboratory of Population Genetics, was elected to a Foreign Honorary Member of the American Academy of Arts and Sciences for her outstanding achievement in theoretical studies on population genetics at the molecular level. Dr. Tadashi Inoue, Laboratory of Mutagenesis, was given Encouragement Award of the Agricultural Chemical Society of

Japan for his biochemical studies on DNA repair and mutagenesis.

Speaking of personnel change, Dr. Tosihide H. Yosida, Head of the Department of Cytogenetics, retired on April 1. Dr. Yosida has been devoting himself enthusiastically to cytogenetic studies of Rodents for about 30 years, during which he produced energetically more than 500 papers. In particular, his extensive studies on karyotype evolution of black rats, developed on the basis of multiple field investigations in South Asia and Oceania, are highly evaluated. Dr. Yosida also served as head of the Genetic Stock Research Center and contributed to its development. The institute conferred on him the title of Honorary Member. He was succeeded by Professor Kazuo Moriwaki. Drs. Akira Ishihama and Ryuji Fukuda were appointed professor and associate professor, respectively, of the Laboratory of Molecular Genetics; they both have been transferred from Institute for Virus Research, Kyoto University. Dr. Yasuo Nakagome was promoted to professor of Laboratory of Human Genetics. Incidentally, under the new system decisions about important items are made by the Advisory Committee which consists of 10 members outside the institute and 10 within the institute.

On April 21, the institute was opened to the public as usual. Some of the research activities of each laboratory were exhibited, and movie films were shown. Double cherry blossoms in the campus were at their best and some 3,000 visitors enjoyed them. On October 27, public lectures were given at the National Science Museum in Tokyo; the titles were "The ecology and evolution of Drosophila" by Associate professor Takao K. Watanabe, and "Damage and repair of DNA" by Dr. Tadashi Inoue. In spite of Saturday afternoon nearly 100 eager people listened to the lectures that were followed by lively discussions.

Two occasional meetings took place in the institute. One, entitled "DNA sequencing and its application" with lectures and technical training course, was organized and held by Dr. E. Soeda of the DNA Research Center on February 14 to 17, as part of 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; there were 121 participants from various parts of Japan, of whom 20 took the training course. The other was an "Oji International Seminar" entitled "Population Genetics and Molecular Evolution"; this meeting was organized and held by staff of the Laboratory of Population Genetics on November 13 to 16, and 33

participants including 9 distinguished foreign scientists discussed the subject most actively. Then the 56th Annual Meeting of the Genetics Society of Japan took place on November 23 to 25 in the campus of Nihon University, Mishima, and most members of our institute served for its organization and operation. It was well attended by about 440 participants from all over Japan. By the way, this was the 4th meeting that was held in Mishima since 1953.

About 80 scientists visited our institute from abroad this year, with whom information and views on recent studies were exchanged actively. Some distinguished guests, including Dr. Linus Pauling, delivered stimulating lectures at Biological Symposium. Those who stayed rather long for technical training and cooperative work were: Dr. P. Thipayathan, Associate professor of Chulalongkorn University, Thailand (accepted by Laboratoty of Microbial Genetics); Dr. N. Houba-Herin, FRFC-Fellow researcher, Liège University, Belgium (Laboratory of Microbial Genetics); Miss P. Barbier, graduate student of Universitè des Sciences et Techniques due Languedoc, Montpellier, France (Laboratory of Agricultural Genetics); Mr. I. Loekman, Researcher and associate investigator, National Atomic Energy Agency, Indonesia (Laboratory of Mutagenesis); Miss L. U. Gadrinab, Scientist II of SEAMEO Regional Center for Tropical Biology, Indonesia (Laboratory of Mutagenesis and Genetic Stock Research Center); Mr. Nai-Kai Zhu, Research associate, Institute of Environmental Chemistry, Chinese Academy of Science, the People's Republic of China (Laboratory of Mutagenesis); and Mr. Bong-Kee Kim, Teaching assistant, Dan Kook University, Korea (Laboratory of Evolutionary Genetics).

The institute has just made the first step toward an interuniversity research institute. Visiting professors and associate professors for the 3 new laboratories have been appointed. We have accepted 17 collaborative programs, 4 workshops, 7 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 DNA Research Center including the establishment of 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. Matsunaga

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.

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.
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, M. Sc.

Laboratory of Cytoplasmic Genetics (Guest member)
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YONEKAWA, Hiromichi, D. Sc., Associate professor

3. ***Department of Ontogenetics***
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Laboratory of Developmental Genetics
SUGIYAMA, Tsutomu, Ph. D., Professor
NAWA, Saburo, D. Sc., Professor
FUJISAWA, Toshitaka, Ph. D.
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.

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.
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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
NAKAGOME, Yasuo, D. Med., Professor
HORAI, Satoshi, D. Med.
NAKAHORI, Yutaka

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.

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

DNA Research Center

MARUYAMA, Takeo, Ph. D., D. Sc., Head of the Department
SOEDA, Eiichi, D. Ag.

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
OIDE, Yukio, Chief of the Finance Section

COUNCIL

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YAMAMURA, Yuichi; President, Osaka University

Vice-chairman

MOROHOSHI, Seijiro; President, Tokyo University of Agriculture and Technology

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EGAMI, Nobuo; Dean, Faculty of Science, University of Tokyo
IINO, Tetsuo; Professor, Faculty of Science, University of Tokyo
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WATANABE, Itaru; Professor, Kitazato University

ADVISORY COMMITTEE

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MATSUNAGA, Ei; Director

Vice-chairman

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FUKUDA, Ichiro; Professor, Faculty of Liberal Arts and Sciences, Tokyo Women's University
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OHBA, Shigeru; Professor, Faculty of Science, Tokyo Metropolitan University
OKADA, Masukichi; Professor, Institute of Biological Sciences, University of Tsukuba
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TSUNEWAKI, Koichiro; Professor, Faculty of Agriculture, Kyoto University
YAMADA, Yukio; Professor, Faculty of Agriculture, Kyoto University

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HIROTA, Yukinori; Professor, Department of Cell Genetics
ISHIHAMA, Akira; Professor, Department of Molecular Genetics
KADA, Tsuneo; Professor, Department of Molecular Genetics
KIMURA, Motoo; Professor, Department of Population Genetics
KURODA, Yukiaki; Professor, Department of Ontogenetics
MARUYAMA, Takeo; Professor, Department of Population Genetics
MORIWAKI, Kazuo; Professor, Department of Cell Genetics
SUGIYAMA, Tsutomu; Professor, Department of Ontogenetics

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 1984

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 and FUKUDA)

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 Chemisry

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)
Genetics and cytogenetics of the silkworm (MURAKAMI)

* Genetics Stock Center

Mutagenesis in germ cells of the silkworm (MURAKAMI)

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

Genetic and cytogenetic studies on retinoblastoma and Wilms' tumor (MATSUNAGA, NAKAGOME and HORAI)
Clinical cytogenetic studies of congenital abnormalities in man (NAKAGOME)
Molecular cytogenetic studies of human chromosomes (NAKAGOME)
Studies on DNA polymorphisms in human populations (HORAI and MATSUNAGA)

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)

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)
Genetic effects of environmental pollution on plant population (IYAMA and MORISHIMA)
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 and HIROTA)
Synthetic ColEl plasmids carrying genes for cell division in *E. coli* (NISHIMURA)

DNA Research Center

Studies on primary structure of DNA (SOEDA)
Rugulatory mechanisms of gene transcription (ISHIHAMA)
DNA data analysis (MARUYAMA)

RESEARCH ACTIVITIES IN 1984

I. MOLECULAR GENETICS

Promoter Selectivity of *E. coli* RNA Polymerase, I. Promoter Strength Recognized by Regular Holoenzyme

Teruaki NOMURA, Nobuyuki FUJITA and Akira ISHIHAMA

Promoter strength is determined by two factors: i) the binding affinity to RNA polymerase and ii) the rate of isomerization from "closed promoter-RNA polymerase complex" to transcriptionally active "open complex". Previously we developed an *in vitro* mixed transcription system for quantitative determination of the promoter strength leading to productive transcription (for details, see Nucleic Acids Res. **11**, 671–686; ibid. **11**, 3873–3889). This year we determined the transcriptional organization and the promoter strength of *dnaQ* and *rnh* genes, which codes for the DNA polymerase III ε subunit and the ribonuclease H, respectively, both involved in DNA replication. Transcription starts from two promoters for *dnaQ* and a single promoter for *rnh*, and proceeds on the same DNA region but into opposite directions (for details, see J. Biol. Chem., **260**, 7122–7125). The three promoters carry different levels of the promoter strength (Fig. 1). The regular holoenzyme appears to recognize the three promoters differentially because the relative level of transcription initiation among the three promoters varied depending on the enzyme concentration. After separation of the three promoters into individual DNA fragments, the activity of *rnh* promoter increased markedly (Nucleic Acids Res., in press). This implies a promoter interference between the convergently transcriped genes.

The promoter strength is being determined for increasing numbers of *E. coli* promoters including those involved in the three mixed operons (*nusA*, *tufB* and *divE*, each composed of tRNA and protein genes), a heat-shock gene (*groE*), two aminoacyl-tRNA synthetase genes (*alaS* and *glnS*), a suppressor tRNA gene (*leuX*) and two catabolite-sensitive genes (*malK* and *malE*).

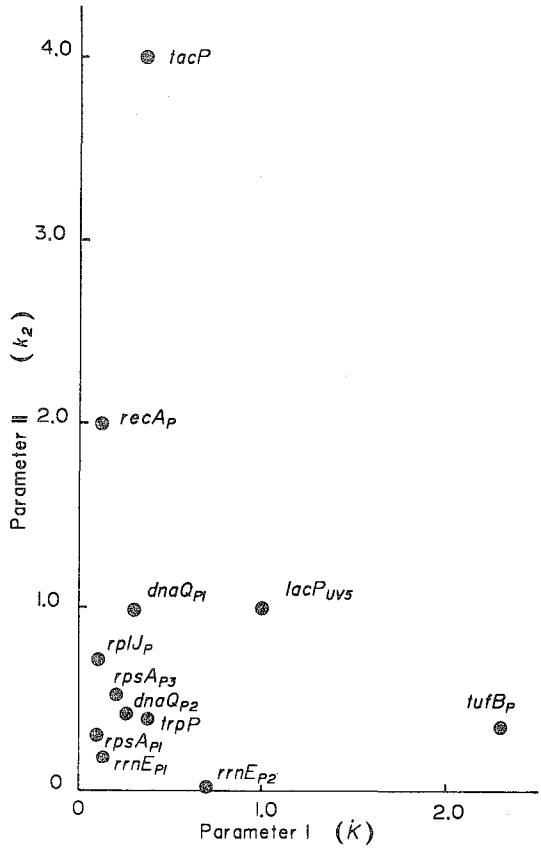

Fig. 1. Map of promoter strength.

Promoter Selectivity of *E. coli* RNA Polymerase, II. Structural and Functional Interconversion of RNA Polymerase by Nucleotide Factors

Akira ISHIHAMA, Teruaki NOMURA and Nobuyuki FUJITA

Increased numbers of evidence indicate that the control of gene transcription in *E. coli* occurs through regulation of the level and specificity of functional RNA polymerase. Some mutations of the genes coding for the four enzyme subunits, β', β, α and σ, affect the promoter selectivity of the RNA

polymerase (for details, see Mol. Gen. Genet. 193, 8–16). RNA polymerase seems to be interconvertible between various forms or states through physical interaction with a number of accessory proteins, termed transcription factors, and nucleotides such as ppGpp and specific tRNA. Using the *in vitro* mixed transcription system, we demonstrated that RNA polymerase lost the recognition property to some specific promoters in the presence of ppGpp, a chemical mediator of stringent control. For example, transcription initiation from the strong promoter P1 of an rRNA gene (*rrnE*) is inhibited by ppGpp but that from P2 is insensitive to it (for details, see J. Biol. Chem. 259, 1951–1957). Likewise, the upstream promoter P1, one of the two major promoters of the ribosomal protein S1 gene (*rpsA*), is sensitive to ppGpp but the downstream P3 is insensitive to it. These observations indicate that not all the promoters belonging to stringently controlled genes are sensitive to ppGpp. A discrimination mechanism seems to operate such that the basal level of gene expression under a poor supply of nutrients is maintained through operation of only the ppGpp-insensitive weak promoters within multiple promoters of stringently controlled genes.

Studies on possible influence of specific tRNA on the promoter selectivity of RNA polymerase is also in progress, in particular with respect to the recognition of promoters within tRNA genes.

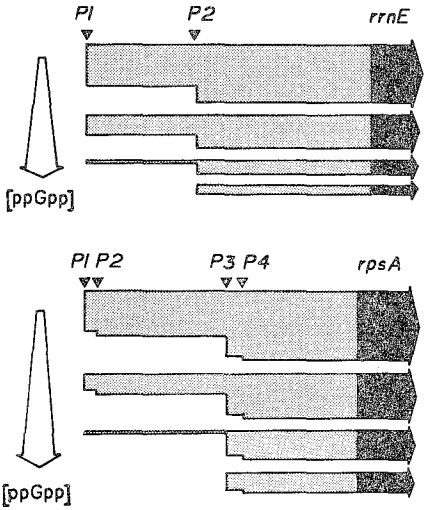

Fig. 1. Differential stringent control.

Promoter Selectivity of *E. coli* RNA Polymerase, III. Structural and Functional Interconversion of RNA Polymerase by Protein Factors

Teruaki NOMURA, Akira ISHIHAMA and Nobuyuki FUJITA

Our systematic search for protein factors was based on the idea that such proteins with regulatory functions might form complexes with RNA polymerase prepared under mild conditions. Attempts were made to isolate and characterize proteins copurified along with RNA polymerase under mild conditions. More than 10 protein species have been identified as possible accessory proteins of RNA polymerase, including NusA and ρ factors, both involved in transcription termination, GroE ATPase and some other heat-shock proteins, and SSP (stringent starvation protein). Among the high molecular-weight protein fraction, we identified a translational initiation factor IF2. Alteration of the promoter selectivity of RNA polymerase, when interacted with these proteins, is being examined using the *in vitro* mixed transcription system.

Phosphorylation of RNA polymerase might also be involved in the control of RNA polymerase activity (for details, see J. Biol. Chem. 259, 526–533). To test this possibility, attempts are being made to develop an *in vitro* system for quantitative phosphorylation of RNA polymerase and to isolate *E. coli* mutants defective in protein phosphorylation.

Promoter Selectivity of *E. coli* RNA Polymerase, IV. Transcription of a Heat Shock Gene

Nobuyuki FUJITA and Akira ISHIHAMA

When *E. coli* cells are exposed to high temperature, the synthesis of a group of proteins, called heat-shock proteins, increases transiently. To approach the molecular mechanism by which the heat-shock response is achieved, transcription of the *groE* gene, whose products were the most prominent among heat-shock proteins in *E. coli*, was analyzed *in vivo* and *in vitro*. Transcriptional start site *in vivo* was determined for the *groE* gene by S1 nuclease mapping and reverse transcriptase mapping. In addition, these experiments clearly showed that the level of *groE* mRNA probe was markedly increased after heat shock and well paralleled the increased rate of protein synthesis, indicating that the regulation of the heat-shock response is

mainly achieved at the level of transcription. An *in vitro* system, in which transcription is initiated from the *groE* promoter, was constructed using core RNA polymerase and purified *htpR* (*rpoH*) gene product, the positive regulator of the heat-shock response in *E. coli*. Using this *in vitro* system, further studies on the mechanism of the action of *htpR* protein, along with the functional relationship between *htpR* protein and sigma factor, are in progress.

Cloning of the Genes for the Possible Transcription Factors of *E. coli*——Analysis of the Gene for Stringent Starvation Protein (SSP)

Ryuji FUKUDA, Hiroaki SERIZAWA and Ryoji YANO*

Until now, up to ten polypeptides have been reported as the possible transcription factors of *E. coli*. In addition, we have found more than ten polypeptides in the enzyme-bound forms in RNA polymerase preparations at the various purification steps. In order to address the physiological functions of these proteins, we have started to clone the genes for these proteins. Cloning allows mapping of the gene on the *E. coli* chromosome, and permits the introduction of mutations useful for understanding the physiological role of these proteins.

This year we tried to isolate the genes for two polypeptides, SSP (stringent starvation protein, 22.5 K) and ω (10 K), which were stably associated with RNA polymerase. Until now, we succeeded to clone the gene for SSP. Usually *E. coli* cells contain about several thousand molecules of SSP. However, when cells are exposed to extreme nutrient starvation, the protein is synthesized predominantly, sometimes occupying more than 50% of total protein synthesis. Reconstitution experiments revealed that SSP binds to RNA polymerase holoenzyme, forming an equimolar complex at the saturation. In contrast, no complex was formed between SSP and core enzyme. Purified SSP considerably influences (mostly reduces) the activity of holoenzyme but not of core enzyme in an *in vitro* transcription system using phage DNAs or synthetic polymers as template.

For isolation of clones containing the SSP gene, we employed a method developed by Suggs *et al.* (1981). We first determined the sequence of the N-terminus of SSP as well as the sequence of two cyanogen bromide-ge-

* Institute for Virus Research, Kyoto University.

nerated peptide fragments. We then chemically synthesized four sets of mixture of oligodeoxyribonucleotides, representing all possible codon combinations for parts of these amino acid sequences. Restriction endonuclease fragments of *E. coli* DNA that hybridized with these probes were cloned into pBR322. After analyses of partial base sequences for the cloned DNA fragments and of proteins encoded by the DNA fragments, we identified a plasmid carrying a complete structural gene of SSP. The complete sequence was determined using various restriction fragments subcloned into the pUC-9 vector.

For mapping of the SSP gene locus on the *E. coli* chromosome, we have integrated the recombinant plasmid containing Amp gene into the chromosome of *E. coli* Hfr stain carrying *polA* mutation. The plasmid was integrated into the chromosomal SSP gene by homologous recombination. Location of the integrated Amp gene and the SSP gene is being determined by Hfr mating and P1 transduction technique (in colaboration with Dr. A. Nishimura, Genetic Stocks Center of this institute).

To elucidate the function of SSP, we are now studying the regulation of SSP synthesis *in vivo* and *in vitro*, and trying to determine whether the protein is essential for cell growth or not, employing the SSP plasmid integration technique. Studies are also in progress to isolate mutants of the SSP gene, using the SSP plasmid.

Structure and Function of RNA-dependent RNA Polymerase Associated with Influenza Virus, I. Isolation and Characterization

Atsushi KATO, Susumu UEDA* and Akira ISHIHAMA

The RNA-dependent RNA polymerase associated with influenza virus plays an essential role in transcription and replication of viral genome in infected cells. To determine the structure and function of the virion-associated RNA polymerase, we made a systematic attempt to isolate the enzyme in an active form from influenza virus A/PR8. The procedure includes: i) disruption of virus particles with a non-ionic detergent; ii) removal of envelope proteins, HA and NA, by cesium trifluoroacetate centrifugation; and iii) removal of M and NP proteins by repeated chromatography on phosphocellulose columns. The resulting RNA polymerase complex is able

* Nippon Institute for Biological Science.

to catalyze not only dinucleotide-primed RNA synthesis but also endonucleolytic cleavage of capped RNA and initiation and elongation of capped oligonucleotide-primed RNA synthesis. This enzyme complex contained only three P proteins, PB1, PB2 and PA, but lacked NP protein. Taken together we concluded that the RNA polymerase is composed of the three P proteins and that NP is no required at least for primary transcription. Purification of the RNA polymerase devoid of viral RNA is in progress. For details, see Virus Research **3**, 115–127.

Structure and Function of RNA-dependent RNA Polymerase Associated with Influenza Virus, II. Possible Proof-Reading Function

Akira ISHIHAMA, Kiyohisa MIZUMOTO* and Atsushi KATO

The RNA-dependent RNA polymerase of influenza virus cleaves capped RNA of host cells at the 5′ side of either A or U residues located about 11 to 13 bases from cap termini, and utilizes the resulting capped RNA fragments as primers for the synthesis of viral mRNA (for details, see Nucleic Acids Res. **11**, 3637–3649). Transcription commences with the addition of GMP residue, which is complementary to the second nucleotide at the 3′-termini of all eight viral RNA segments, to the capped primers. When high concentrations of GTP are added as a sole substrate, however, multiple GMP residues are polymerized to the primers. As illustrated in Fig. 1, the erroneously polymerized GMP residues, other than the first one, were found to be removed prior to incorporation of CMP, the nucleotide complementary

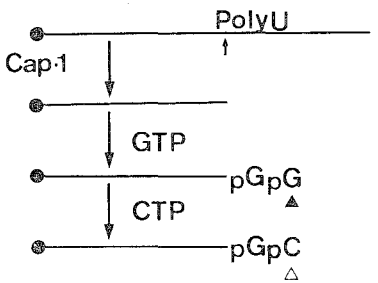

Fig. 1. Proof-reading reaction.

* Institute of Medical Sceince, University of Tokyo.

to the third nucleotide at the 3′-termini of viral RNA. The results suggest that the RNA-dependent RNA polymerase carries a proof-reading function similar to that of DNA polymerases. In contrast to the proof-reading reaction by DNA polymerases, the reaction by the influenza viral RNA polymerase takes place only in the presence of substrates, implying a coupled reaction between the removal of misincorporated nucleotides and the polymerization of correct nucleotides.

Analysis of Temperature-sensitive Mutants of Influenza Virus: The Mutants of RNA Segment 8

Masakazu HASEGAWA, Ryuji FUKUDA and Kazufumi SHIMIZU*

Temperature-sensitive (*ts*) mutants of influenza A virus are useful in understanding the genetic organization of the virus genome and the mechanism of virus replication. Previously Shimizu *et al.* (1982) isolated 83 mutants of influenza A/Udorn/72 virus that were *ts* on primary rhesus monkey kidney (RMK) monolayer cultures. These mutants had been classified into 13 complementation groups and 8 recombination groups corresponding to each of the eight genomic RNA segments. As the first step of this research, we have analyzed these *ts* mutants defective in the segment 8.

Among these groups, the recombination group H had *ts* lesion(s) on the RNA segment 8, as indicated by segregation analysis. The segment 8 encodes two nonstructural polypeptides, NS1 and NS2, the functions of which are entirely unknown. The mRNA encoding NS2 is thought to be produced by splicing of NS1 mRNA. However, the mutants of group H were classified into 4 complementation groups on RMK cells, and 2 or 3 complementation groups on Madin-Darby canine kidney (MDCK) cells. To address the uncertainties in the complementation analysis and assign the mutants to each of the two polypeptides encoded by the segment 8, we performed nucleotide sequence analysis of three mutants. Double-stranded cDNAs prepared from genomic RNA of these mutants were cloned into pBR322. Sequencing of the cloned DNA fragments revealed base substitutions in the segment 8 RNA. This was confirmed by directly sequencing that region of viral RNA, employing the chain terminator method, in which the RNA was transcribed with AMV reverse transcriptase and 5′

* Nihon University, School of Medicine.

^{32}P-labeled restriction fragments as the primer. Two of these mutants, ICR 1629 and SPC 45, have defects in the NS1 coding region which is spliced out for NS2 mRNA. The third mutant, ICR 516 has a defect in the NS2 coding region but downstream of the NS1 coding region. The synthesis of M1 protein, which normally accumulates late in infection, was greatly reduced in MDCK cells infected with those NS1 *ts* mutants at 30°C compared to at 34°C. No significant difference was seen with NS2 *ts* mutant ICR 516 at 40°C on the synthesis of virus-specific protein. The results may indicate that NS1 participates in the switch from early to late protein synthesis. Using an improved hybridization method, we are measuring the synthesis of mRNA, cRNA and vRNA of the eight RNA segments, separately, during the infection of these mutants.

Structure and Function of Reverse Transcriptase Associated with Avian Myeloblastosis Virus

Atsushi KATO, Akihiro NODA*, Susumu UEDA**
and Akira ISHIHAMA

The RNA genome of retroviruses is reverse-transcribed into double-stranded DNA, which is integrated into the host chromosome as the provirus, and both viral mRNA and progeny RNA are produced through transcription of the provirus DNA by DNA-dependent RNA polymerase II of host cells. The reverse transcriptase associated with retroviruses are believed to catalyze not only the multiple step reactions leading to the synthesis of double-stranded DNA from viral RNA but also the integration of viral DNA into host chromosome. Using a new strategy for enzyme purification, we established that most, if not all, avian retroviruses contain three forms, α, $\alpha\beta$ and β_2, of reverse transcriptase. To identify the role of each enzyme form, we improved the purification procedure, which allowed us to purify large amounts of the three enzyme forms of AMV reverse transcriptase to the same extent of homogeneity (for details, see J. Virol Meth. **8**, 325–339). Comparison of the catalytic properties of the three enzyme forms indicated that the β_2-form enzyme catalyzed the RNA-directed synthesis of DNA efficiently whereas the α-form enzyme was the most active in the ssDNA-directed synthesis of dsDNA. Using a purified preparation of p15 endo-

* Central Research Institute, Takara Shuzo Co.
** Nippon Institute for Biological Science.

peptidase, we succeeded in processing *in vitro* of β_2-form enzyme to $\alpha\beta$- and α-form enzymes. Preliminary analysis of alteration of enzyme function indicated that both DNA-dependent DNA polymerase and DNA endonuclease activities increase concomitantly with the processing of β subunit.

Analysis of Mitochondrial DNA in Cytoplasmic Male Sterile Rice

Saburo NAWA, Yoshio SANO and Taro FUJII

Recently, Yamaguchi and Kakiuchi (1983, Japan. J. Genet. 58: 607) discovered mtDNAs, B-1 and B-2, in a cytoplasmic male sterile (cms) strain of rice. To obtain further information on the behavior of mtDNA, induction of a fertile revertant from a cms-strain of rice was investigated using mutagen treatments. Seeds of a cms-strain, (cms-boro)rf_1rf_1, were treated by gamma-rays, ethyl methanesulphonate (EMS) and acridine orange with various intensities or concentrations. Two fertile plants were obtained with EMS treatments among 6500 treated seeds in total. These reverted plants, designated 80–1 and 81–3, indicated reversion of the cytoplasmic factor from cms to normal state, because the revertants showed complete sterility when crossed as the pollen parent to the original cms-strain.

The following 5 rice strains, viz., (cms-boro)rf_1rf_1, (cms-boro)Rf_1Rf_1, (n-boro)rf_1rf_1, 80–1 and 81–3, were used for examination of mtDNA. Because few seeds are available in the cms-strain, a tissue culture technique was utilized with B-5 medium, and callus cells propagated from seeds were analyzed. Calluses were homogenized in mannitol solution and mitochondria were fractionated by repeated centrifugations. DNAs were extracted from the mitochondria in sodium sarkosyl-pronase, followed by purification with phenol, chloroform and RNAse treatments. Mitochondrial DNAs were analyzed in agarose gels. The gel electrophoresis revealed a main high-molecular DNA band in preparations from all of 5 strains. Preparations from male sterile cytoplasms, (cms-boro)rf_1rf_1 and (cms-boro)Rf_1Rf_1, were found to contain two additional fast-migrating bands (B-1 and B-2) whereas no such DNAs were detected in preparations from normal cytoplasm [(n-boro)rf_1rf_1] and 2 revertant cytoplasms, indicating that reversion of cms to fertile cytoplasm was associated with the disappearance of mitrochondrial plasmid DNAs. This suggests a specific involvement of these plasmids in

the male sterility of rice.

The plasmids from cms cytoplasm were further purified by equilibrium centrifugation in CsCl-ethidium bromide gradients. Both of B-1 and B-2 were recovered from the lower bands while the main mitochondrial DNAs were recovered from the upper band. B-1 DNA treated with a restriction enzyme moved slower than untreated DNA in agarose gel electrophoresis. The same was in the case of B-2. From these results, B-1 and B-2 were estimated to be supercoiled circular DNAs (cccDNA) having molecular weight of 2.3 and 1.6 kb, respectively.

Concerted Evolution of the Mouse Immunoglobulin Gamma Chain Genes

H. HAYASHIDA, T. MIYATA, Y. YAMAWAKI-KATAOKA,
T. HONJO, J. WELS and F. BLATTNER

The nucleotide sequences of the immunoglobulin heavy-chain constant region genes, $C\gamma3$, $C\gamma1$, $C\gamma2b$ and $C\gamma2a$, of the mouse, together with that of a human equivalent $C\gamma4$ were compared. It was shown that all the six pairs of genes within the mouse $C\gamma$ gene family contain DNA segments that exhibit marked homology, whereas no such segmental homology was found in the case of inter-species comparison. This result indicates that the four $C\gamma$ gene of the mouse evolved concertedly by exchanging parts of their genetic information with each other through mechanism of either gene conversion or double unequal crossing-over. Another example for such concerted evolution was found in gene regions encoding membrane domains of the mouse $C\gamma$ chains. We further searched such segmental homologies in other mammalian $C\gamma$ gene families and found at least two more examples in man and guinea-pig. In the mouse $C\gamma$ gene family, the silent positions of an exon encoding the third domain of $C\gamma$ chains show divergence in sequence much more strongly than other regions, indicating that the genetic information encoded by this gene region was least scrambled throughout recent evolution. A phylogenetic tree constructed from the nucleotide differences of this exon demonstrates that at least two $C\gamma$ genes had already existed before mammalian radiation. Based on these results, evolution of mammalian $C\gamma$ gene families was discussed. For details, see EMBO J. 3, 2047–2053.

II. MICROBIAL GENETICS

Purification and Sequencing of the Active Site Tryptic Peptide from Penicillin-binding-Protein 1b of *Escherichia coli*

Robert A. NICHOLAS, Hideho SUZUKI, Yukinori HIROTA and Jack L. STROMINGER

This paper reports the sequence of the active site peptide of PBP 1b. Purified PBP 1b was labeled with [^{14}C] penicillin G, digested with trypsin, and partially purified by gel filtration. Upon further purification by HPLC, two radioactive peaks were observed, and the major peak, representing over 75% of the applied radioactivity, was submitted to amino acid analysis and sequencing. The sequence Ser-Ile-Gly-Ser-Leu-Ala-Lys was obtained. The active site nucleophile was identified by digesting the purified peptide with aminopeptidase M and separating the radioactive products on HPLC. Amino acid analysis confirmed that the serine residue in the middle of the sequence was covalently bonded to the [^{14}C]penicilloyl moiety. A comparison of this sequence to active site sequences of other PBPs and β-lactamases is presented. (For detail, see Biochemistry 1985, **24**, 3448–3453)

Identification of the Active Site in Penicillin-binding Protein 3 of *Escherichia coli*

Robert A. NICHOLAS, Jack L. STROMINGER, Hideho SUZUKI and Yukinori HIROTA

We report the sequence of the active site tryptic peptide of penicillin-binding protein 3 from *Escherichia coli*. Purified penicillin-binding protein 3 was laveled with [^{14}C]penicillin G, digested with trypsin, and isolated by a combination of gel filtration and high-pressure liquid chromatography. The major radioactive peak from high-pressure liquid chromatography was sequenced, and the sequence Thr-Ile-Thr-Asp-Val-Phe-Glu-Pro-Gly-Ser-Thr-Val-Lys, which comprises residues 298–310 in the gene sequence, was obtained. This sequence is compared to the active site sequences from other penicillin-binding proteins and β-lactamases. (For detail, see J.

Bacteriol. 1985)

A Comparison of Amino Acid Sequences of the Active Centers of Penicillin Binding Protein (PBP -1b, -3, Carboxy Peptidase (CPase) and β-lactamase

Hideho SUZUKI, Yukinori HIROTA, Robert A. NICHOLAS and Jack L. STROMINGER

Enzyme Bacteria Amino acid sequence of the active center

Enzyme	Bacteria	Amino acid sequence of the active center				*			*
CPase	*B. subtilis*	Leu	Pro	Ile	Ala	Ser	Met	Thr	Lys
CPase	*B. stearothermophilus*	Leu	Gly	Ile	Ala	Ser	Met	Thr	Lys
PBP-1b	*E. coli*		Ser	Ile	Gly	Ser	Leu	Ala	Lys
PBP-3	*E. coli*	Phe	Glu	Pro	Gly	Ser	Thr	Val	Lys
β-lactamase (ClassA)	*S. aureus*	Phe	Ala	Tyr	Ala	Ser	Thr	Ser	Lys
	B. sereus	Phe	Ala	Phe	Ala	Ser	Thr	Tyr	Lys
	B. licheniformis	Phe	Ala	Phe	Ala	Ser	Thr	Ile	Lys
	E. coli	Phe	Pro	Met	Met	Ser	Thr	Phe	Lys

* Ser and Lys residues of the penicillin reacting peptides are common in all the proteins used. Boxed amino acids, show amino acid residues in common at the relative positions. (1) Waxman, Strominger: J. Biol. Chem., 1980. (2) Yocum, Rasmussen, Strominger: J. Biol. Chem., 1980. (3) Nicolas, Suzuki, Hirota, Strominger: Biochemistry, 1985. (4) Keck, Glauner, Schwarz, Broome-Smith, Spratt: Proc. N. A. S. 1985. (5) Nicolas, Strominger, Suzuki, Hirota: J. Bacteriol, 1985. (6) Ambler: Phil. Trans. R. Soc. Lond., 1980.

Sites of *dnaA* Protein-binding in the Replication Origin of the *Escherichia coli* K-12 Chromosome

Minami MATSUI, Atsuhiro OKA, Mitsuru TAKANAMI, Seiichi YASUDA and Yukinori HIROTA

On the basis of the observation that *dnaA* protein binds preferentially to DNA fragments carrying the *Escherichia coli* chromosomal replication origin (*oriC*), the binding sites were investigated by DNase I footprinting. As a result, three strong binding sites were identified in the minimal *oriC* sequence. The respective binding sites were 16 to 17 base-pairs long and contained a common sequence (5′) T-G-T-G-(G/T)-A-T-A-A-C (3′) in the middle, although their polarities were not the same. Since mutants defective in func-

tion for autonomous replication have been isolated in the corresponding positions of the common sequence at each binding site, *dnaA* protein-binding at these sites seems to be significant for replication initiation. (For detail, see J. Mol. Biol. **184**: 529–533, 1985)

A Mutation Uncouples DNA Replication And Cell Division in *Escherichia coli*

Akiko NISHIMURA

I have isolated a mutant of *E. coli* whose DNA replication and cell division are uncoupled.

Bacterial cell division is tightly coordinated with DNA replication. PAT42, a thermosensitive mutant of DNA replication (*dna*B42) forms filamentous cells when DNA synthesis is blocked at 41° (Hirota *et al.* 1968, Cold Spring Harbor Symp. Quant. Biol. 33: 677).

A culture of PAT42 was treated with 3 cycles of heat pluse (41° for 2 hours) and survival colonies were isolated at 30°. One of these survivors, strain JE6009, had the following properties; (1) JE6009 cells continued to divide at 41° for several hours. Whereas the parental strain PAT42 grew as long filamentous forms (longer than 10 μm) when incubated at 41° for 2 hours, the JE6009 culture contained 30% normal-sized cells (1.5–2 μm) and 70% short filamentous cells (shorter than 6 μm). (2) During this time, the number of survivals were counted by colony forming ability at 30°. Survivals of PAT42 decreased logarithmically after 30 minutes lag, but those of JE6009 were constant over 2 hours at 41°. (3) DNA synthesis of both strains stopped immediately after being shifted to 41°. (4) *dna*B^{ts} mutation was co-transduced with *mal*B^{+} by Pl-phage from JE6009 to W3876 (*mal*B^{-}) strain. These transductants formed long filamentous cells at 41°.

These results indicate that JE6009 carries a mutation, which uncouples DNA replication and cell division. Furthermore, cell division of JE6009 also occurred when hydroxyurea (1.5 mg/ml) or nalidixic acid (10 μg/ml) was used to stop DNA synthesis, whereas division of PAT42 was inhibited completely under these conditions. Colony size of PAT42 at 30° was uniform but size-distribution, containing mini-colonies smaller than that of PAT42, was found in JE6009. The number of colonies at 30° per a given cell, from 30° cultures of JE6009 was less than 1/12 that of PAT42. The mutation in JE6009 might result in the initiation of cell division before

completion of DNA replication and in reduction of survival cells even under permissive conditions for DNA replication.

This mutant gene in JE6009 was not involved in SOS-pathway because the phenotypes mentioned above did not change when *rec*A, *lex*A, *sfi*A, or *sfi*B genes of strain JE6009 were substituted for the genes known to be wild by P1-transduction. The *fts*A protein has been suggested as being involved in a mechanism that coordinates DNA replication and cell division through a pathway independent of the SOS-induced response (Tormo *et al.* 1985 J. Gen. Microbiol. **131**: 239). The mutation in JE6009 was mapped close to the *thy*A gene, and far from the *fts*A gene by mating experiments with Hfr-T42 (*dna*B^{ts}).

The relationship between the expression of a mutatnt gene in JE6009 and *fts*A gene, and/or the SOS-mediated inhibition of division remains to be elucidated.

Effect of the *sacU*^{h}32 Mutation on the Growth and Spore Outgrowth of the *div*-341 Strain of *Bacillus subtilis*

Yoshito SADAIE and Tsuneo KADA

To elucidate the genetic control of asymmetric forespore septum formation in *B. subtilis*, we examined the effect of temperature sensitive septum initiation mutations on sporulation and sporulation associated events in *B. subtilis*. One of such mutations, *div-341*, showed early *spo0* phenotypes at an intermediate permissive temperature (37°C) and its genes product was assumed to be involved in a step required for the excretion of some exoenzymes and cell surface proteins. The *sacU*^{h}*32* mutation on the other hand showed a hyperproduction of exoenzymes and derepressed sporulation in the presence of excess nutrients. Both mutations are closely linked by transformation. A double mutant with *div-341* and *sacU*^{h}*32* was constructed to examine the interaction of both genes. Although the filamentous growth of the *div-341* strain at 42°C was not recovered in the mutant, the slower growth and defective spore outgrowth (twisted outgrowing spore) of the *div-341* strain at 37°C were recovered in the double mutant. These suggest that the *sacU*^{h}*32* gene interacts with the *div-341* gene directly or indirectly in some step of cell growth or spore outgrowth (J. Bacteriol. **163** (2), 1985, 648).

III. IMMUNOGENETICS

A Lethal Gene Mapped within the Mouse Major Histocompatibility Complex.

Toshihiko SHIROISHI, Tomoko SAGAI and Kazuo MORIWAKI

We have produced a series of new congenic strains to introduce the H-2 complex of Japanese wild mouse on the genetic background of C57BL/10J strain. Some of them show enhanced intra-H-2 recombination (Shiroishi *et al.* Nature **300**: 370–372, 1982). More than twenty recombinant H-2 haplotypes were generated from one of such congenic strains, B10. MOL-SGR. A recombinant haplotype aw18 is derived from B10. MOL-SGR/B10. A heterozygote. For the purpose of obtaining aw18 homozygote and fixing this haplotype, we made intercross of aw18/k and aw18/b heterozygotes. Two hundred fourty progeny were subjected to H-2 typing by cytotoxicity test, but none of them was homozygous for the aw18 haplotype. The segregation ratio of each genotypes was; aw18/aw18: aw18/k or aw18/b: k/k or b/b=0: 168: 72. These results strongly suggest that the aw18 haplotype carries recessive lethal gene. Since the lethality is tightly associated with the homozygous state for the aw18 haplotype, it is likely that the lethal gene is linked to the H-2 complex. However, the possibility that this gene is located outside of the H-2 complex can not be ruled out. To make it clear if the gene is mapped within the H-2 complex and to determine the fine location of the gene, we produced further recombinant haplotypes from the heterozygotes of aw18/k and aw18/b. Finally, eight independent recombinants were generated. Their recombination break points were determined based on the identification of the origin of genes within the new recombinant H-2 haplotypes. Subsequently, the lethality of the mouse homozygous for the recombinant haplotype was examined. The lethality was judged from absence of the homozygote in the progeny generated from the intercross of the heterozygotes of the recombinant and k or b haplotype at the age of four weeks after birth. The results are summerized in Table 1. Since a recombinant haplotype aw26 whose proximal region to E_α locus is derived from k haplotype retains lethality, the lethal gene is mapped to distal part to E_α. A haplotype aw20, in which recombination occurred between the

Table 1. The lethality of the homozygote for the aw18-derived recombinant haplotype

Haplotype	Genotype								Lethality[2]
	K	A_β	A_α	E_β	E_α	Slp	Ss	D	
wm7[1]	w	w	w	w	w	w	w	w	Viable
aw18	w	w	w	w	w	d	d	d	**Lethal**
aw20	w	w	w	w	w	d	d | [3]	k	**Lethal**
aw21	w |	k	k	k	k	k	k	k	Viable
aw22	k |	w	w	w	w	d	d	d	**Lethal**
aw23	w |	k	k	k	k	k	k	k	Viable
aw24	w |	b	b	b	b	b	b	b	Viable
aw25	w |	b	b	b	b	b	b	b	Viable
aw26	b	b	b	b	b |	d	d	d	**Lethal**
aw27	w	w	w	w	w	d |	? |	k	**Lethal**

1) Original H-2 haplotype from which aw18 was derived.

2) Lethality was judged from the absence of recombinant homozygote in the offsprings from the cross of R/b×R/b or R/k×R/k at 4 weeks after birth.

3) Vertical bar indicates the position of recombination.

locus of slp and D, and distal region to the D locus came from k haplotype still keeps lethality. Therefore, the lethal gene is located in the proximal region to the D locus. Taken otgether, this gene appears to be mapped to the E_α–D interval of the H-2 complex. All data obtained from the other recombinant haplotypes were completely consistent with the above conclusion. At present, there is no evidence that initial genetic recombination in aw18 directly caused the lethal mutation, but it seems highly likely that the recombination event is related to the emergence of the lethality, because the recombination break point is also located in the E_α–D interval.

In order to determine the stage that the lethality appears, we made the intercross of aw18/b heterozygote. The progeny was scored for the H-2 genotype at different three postnatal stages. As shown in Table 2, thirty six percent of progeny were scored as homozygote at one day after birth. After this stage, the frequency of the homozygote decreased, and none of the homozygotes failed to survive beyond 15 days after birth. The result led to the conclusion that the lethal gene begins to act just after birth. Although we have made preliminary characterization of this lethal mutation, pathological investigation has not been done yet to elucidate the mechanism to cause the lethality. Identification of the product of this lethal gene may

Table 2. The segregation of the H-2 genotypes in the viable progeny generated from the intercross of aw18/b at postnatal stages

Postnatal stage	Litter No.	Genotype/Segregation	% Homozygote
1 day	4 (x=8.3±1.5)	aw18/aw18: aw18/b or b/b	
		12 21	36.4
5 days	4 (x=7.7±1.7)	aw18/aw18: aw18/b or b/b	
		3 23	11.5
15 days	6 (x=7.0±2.0)	aw18/aw18: aw18/b: b/b	
		0 30 13	0.0

x: Mean number of the viable progeny per litter

shed light on understanding the biological function of this gene and the cause of the lethality.

Cytoplasmic Gene Flow in Japanese Mice *Mus musculus molossinus*

Hiromichi YONEKAWA, Osamu GOTOH, Yusaku TAGASHIRA, Shunsuke MIGITA, Ze-Chang Yu, De-Yyan LU, Wang Su CHO, Nobumoto MIYASHITA and Kazuo MORIWAKI

Taxonomists propose that there are two subspecies of house mouse *Mus musculus* in Eastern Asia; i.e. *M. m. castaneus* and *M. m. molossinus*. The former occupies Southeastern Asia and South China, whereas the latter is found in the Far East, especially Japan. The mice belonging to these two subspecies can be easily distinguished from each other by restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA) (Yonekawa *et al.* Jpn. J. Genetics **55**, 289–296: 1980; Genetics **98**: 801–816, 1981) as well as by biochemical markers encoded by the nuclear genome (Bonhomme *et al.* Biochem. Genet. **22**: 275–303, 1984).

As for Japanese mice, we previously showed that there is no heterogeneity in RFLP of mtDNA of mice collected at 15 localities distant from each other (Yonekawa *et al.* Differentiation **22**: 222–226, 1982), although these mice have extensive polymorphism of nuclear genes. On the other hand, considerable polymorphism in both cytoplasmic and nuclear genes are found in other Asiatic subspecies such as *M. m. castaneus* and *M. m. bactrianus*. These results suggest that some special events took place in Japanese mice.

In order to clarify this matter, we collected mice in 17 additional localities

Table 1. The subspecies of *Mus musculus* used, their sources and the set of restriction enzyme cleavage patterns of mtDNAs obtained from each sample

Subspecies Collection locality	Nos. used	Restriction patterns*												
		Bm	Ec	H2	H3	Hp 1	He 2	Ps	Bg	Hp 2	He 3	Tq	Hf	Mb
M. m. bactrianus														
Afganistan Kabul		C	C	B	B	B	B	B	B	B	B	—	—	—**
Pakistan Lahol		C	C	B	B	B	B	B	B	C	C	—	—	—
M. m. castaneus														
Indonesea Bogor		C	B	C	B	C	C	B	A	—	—	—	K	L
Malaysea Kota Kinabal		C	B	C	B	C	C	B	A	—	—	—	L	M
Philippines*** Quezon city		C	B	C	B	C	C	B	A	D	—	—	L	J
Taiwan Taichun		C	B	C	B	C	C	B	A	E	—	—	L	I
M. m. molossinus														
Japan***														
castaneus-type mtDNA		C	B	C	B	C	C	B	A	D	F	G	J	I
molossinus-type mtDNA		D	B	A	B	A	C	B	A	—	—	—	—	—
Korea														
Suweon		D	A	B	A	C	B	A	A	—	—	—	C	B
Kojori		D	B	A	B	A	E	B	A	—	—	—	B	B
China														
Changchun		D	B	A	B	A	C	B	A	A	—	B	D	B
Peking		D	B	A	B	A	C	B	A	—	—	—	—	—
Nanking		D	D	A	B	—	—	C	—	—	—	—	—	—
Shanghai		D	B	A	B	A	C	B	A	A	—	C	G	C
Cengtu		D	B	A	B	A	C	B	A	G	—	C	E	C
Lanzhou		D	B	A	B	A	C	B	A	A	—	C	E	C
Jiayu-guang		D	B	A	B	A	C	B	A	H	—	C	H	C
Turfan		D	B	A	B	A	C	B	A	I	—	E	E	E
Urumuchi		D	B	A	B	A	C	B	A	J	—	F	I	F

* Abbreviation used are Bm: BamHI, Ec: EcoRI, H 2: HindII, H 3: HindIII, He 2: HaeII, Hp 1: HpaI, Ps: PstI, Bg: Bg 1 I, Hp 2: HpaII, He 3: HaeIII, Tq: TaqI, Hf: HinfI and Mb: MboI.

** "—" represent "not done".

*** The molossinus mice with castaneous—type mtDNA were collected at 9 different localities, i.e. Nemuro, Nakashibetsu, Sapporo, Teine, Oma, Kurihara, Koriyama and Kagoshima. The mice with molossinus-type mtDNA were collected at 28 localities, i.e. Ohzuchi, Niigata, Shinanomachi, Wajima, Kuki, Inamachi, Omiya, Ichikawa, Yotsukaido, Koajiro, Mishima, Numazu, Nishio, Anjo, Mizuho, Gobo, Momoyama, Osaka, Kochi, Mine, Hakozaki, Sasaguri, Izumi, Kagoshima, Miyazaki, Tsushima, Ogasawara and Yonaguni.

in Japan (32 in total), 9 localities in China and 2 in Korea. Then we examined the RFLP of their mtDNA. We found two types of mtDNA in Japanese mice, one of which is closely related to that Chinese mice (*M. m. molossinus* mtDNA), while the other is related to that of the Southeastern Asiatic subspecies *M. m. castaneus*. This shows that some Japanese *molossinus* mice have been invaded by cytoplasmic genes from the subspecies *M. m. castaneus*.

These two types of mtDNA are geographically well separated in Japan: the mice with *molossinus* type mtDNA occupy Central Japan, whereas the mice with *castaneus* type mtDNA are distributed in the two distal ends of Japan. Furthermore, neither of the two mouse populations has any heterogeneity in RFLP of their mtDNA, suggesting that these two populations might have gone through a severe founder effect.

From these results, we propose that Japan suffered two invasions of mice from neighbouring contries, the first from Southeast Asia or South-East China and the second from East-Central China. Since human population movements in Japan are thought to have followed the same pattern, these two mouse populations were probably brought from their native habitat by humans.

IV. DEVELOPMENTAL AND SOMATIC CELL GENETICS

In Vitro Formation of Adult Structures from Lethal Embryonic Cells of *Drosophila melanogaster*

Yukiaki KURODA

Embryos, homozygous for the sex-linked recessive lethal gene, *dor* (*deep orange*; 1–0.3) die during embryogenesis. When undifferentiated cells from embryos at the stage of the post-gastrulation of this mutant were cultured in ecdysterone-free medium, some defects were observed in cell membrane-associated functions such as the syncytium formation of muscle cells, the formation of cellular spheres and the neural secretion of nerve cells. On the other hand, other cells from these lethal embryos developed normally and showed no detectable defects in their morphology and functions.

The author has previously established a procedure for obtaining the *in vitro* formation of adult structures from undifferentiated embryonic cells of the wild type by their cultivation in the presence of ecdysterone. In the present experiment, the ability of *dor* embryonic cells cultured in ecdysterone-containing medium to differentiate into adult structures was examined.

When cells from post-gastrula embryos of the *dor* strain were cultured in the medium K-17 supplemented with 15% fetal bovine serum, 100 μg/ml fetuin and 10 μg/ml ecdysterone, muscle cells differentiated, connected with each other, and formed net-work structures. Some epithelial cells also formed a structure of the adult leg bud.

When embryonic cells of *dor* embryos were cultured in medium containing the wild-type egg extract and ecdysterone, the defects in the adult structures formed were partially repaired. This indicates that undifferentiated cells from *dor* embryos may have an ability to differentiate into adult structures in the presence of the wild-type egg extract and ecdysterone.

Mutagenic Activity of Cytidine Analogs in Cultured Chinese Hamster Cells

Yukiaki KURODA, Kazuo NEGISHI[1] and Hikoya HAYATSU[1]

Among various analogs of nucleic acid bases and nucleosides, 5-bromo-

[1] Faculty of Pharmaceutical Sciences, Okayama University, Okayama.

2′-deoxyuridine, 2-aminopurine, 5-azacytidine and 6-mercaptopurine were mutagenic in cultured mammalian cells. These chemicals induced chromosome aberrations, sister-chromatid exchanges and gene mutations in mammalian cells. Recently, it has been found by the authors that a reaction product of cytidine with hydrazine, N^4-aminocytidine was strongly mutagenic to bacteria and phages. In the present experiment, the mutagenic activity of N^4-aminocytidine and N^4-amino-2′-deoxycytidine in cultured Chinese hamster V79 cells was examined.

The cytotoxic effects of both nucleoside analogs on V79 cells were determined by scoring the colony-forming activity of cells treated with the analogs for 3 hours. The LD_{50} value of N^4-aminocytidine was 22 μg/ml and that of N^4-amino-2′-deoxycytidine was 50 μg/ml.

Cells were treated with each of the analogs for 3 hours, cultured in normal medium for 6 days, replated in fresh dishes, and cultured in 5 μg/ml 6-thioguanine(6TG)-containing medium for 10 days. The number of 6TG-resistant cell colonies formed was scored. N^4-Aminocytidine had a moderate mutagenic activity, giving the induced mutation frequency of 0.12×10^{-6}/μg-hr/ml. N^4-Amino-2′-deoxycytidine was more strongly mutagenic and showed the induced mutation frequency of 1.1×10^{-6}/μg-hr/ml. In microorganisms, N^4-aminocytidine was twenty times more strongly mutagenic than N^4-amino-2′-deoxycytidine. It was suggested that the difference in the mutagenic response of microorganisms and mammalian cells to these analogs is due to the difference in metabolic activity in these organisms.

Studies on Freezing of *Drosophila* Embryos

Yukiaki KURODA and Yuko TAKADA

Drosophila is one of the materials which have been the most extensively used in various fields of genetics. All stocks of wild-type and mutant strains of *Drosophila* are maintained at 18°C to 25°C in most laboratories. The maintenance of many strains in a laboratory consumes a lot of money, time and labor. In the present experiment, an attempt was made to freeze *Drosophila* eggs at −80°C in an electric freezer.

Male and female flies of the Oregon-R strain of *D. melanogaster* were kept in glass tubes at 25°C for 4 hours to collect eggs. Eggs were dechorionated by treatment with 3% sodium hypochloride solution for 30 minutes. Eggs at the appropriate stage of development were selected under a binocular

microscope. They were transferred to salt solution containing 10% glycerol and frozen in small glass vials at −80°C. After several days, eggs were rapidly defrosted, washed by salt solution, and incubated in salt solution at 25°C for 24 hours. Eggs hatched at a very low rate of 0.6%. Hatched larvae developed normally to fertile adult flies.

The low survival of frozen eggs might be due to a low permeability of the vitelline membrane to glycerol and a limited developmental stage appropriate for freezing. Dechorionated eggs were divided into several groups at different developmental stages and frozen with the addition of glycerol. Eggs frozen within 1 hour after fertilization, showed the highest survival after defrosting from −80°C. Studies to enhance the permeability of the vitelline membrane by treatment with chemicals are now being carried out.

Genetical Studies on the Hereditary Mosaic (mo) Strain of *Bombyx mori*: Mosaicism and Fashion of Meiotic Divisions in Oogenic Cells

Akio MURAKAMI

In the domesticated silkworm (*Bombyx mori*), Goldschmidt and Katsuki (1927) found a heritable mosaic strain in which a number of mosaics for both somatic and sexual traits appeared as they were mixed with normal individuals in one batch. The mosaicism is caused by a single recessive gene (*mo*) possessed by the mother silkworm (Goldschmidt and Katsuki, 1928). In cytological observations it was further found that two egg nuclei (or polar-bodies) appeared and each of them was fertilized with a sperm nucleus (Goldschmidt and Katsuki, 1928, 1931). Each fertilized nucleus might develop into each side of the insect body. The presence of two genetically different, fertilizable maternal nuclei within one egg seems to be a sophisticated tool to analyze a fashion of maturation divisions. In *Bombyx mori*, the type of maturation divisions in oogenic cells has not yet been established: some geneticists support a pre-reductional division type and cytogeneticists a post-reductional one.

If the female is heterozygous for the gene involved in the appearance of the serosa-membrane cells, *pe* (yellowish-white) and pe^+ (black), the two egg nuclei and/or polar-bodies may be either alike, pe^+ and pe^+, or *pe* and *pe*, or different, pe^+ and *pe*. Because femininity in *Bombyx* is based on a ZW (or XY) sex chromosomal constitution, two nuclei or polar-bodies may

also either be alike, Z and Z, or W and W, or unlike, Z and W. Consequently, nine different combinations of the autosomal and sexual genotypes of the two nuclei are possible in the case of a post-reductional division type, while there are seven combinations in the case of a pre-reductional type. There is a striking difference in the sex of egg-colour mosaics between the two cases: three different mosaics, are possible, females, males, and gynanders for the post-reductional division type, while there is only one possible in gynanders for the pre-reductional type. From the cross between the hereditary mosaic (*mo*/*mo*) strain females heterozygous for pe^{+} and *pe* genes and males homozygous for the egg-colour gene *pe*, a hundred and twelve mosaic eggs were obtained. According to their anatomical observations, the three different types of mosaics in females, males, and gynanders were detected with more male types than the other two types. The number of gynandromorphic eggs was the same as that of female eggs. The present preliminary data clearly indicate that in *Bombyx* the fashion of meiotic divisions of the oogenic cells is the post-reductional division type (or pre-equational separation in the first meiotic division and post-reductional separation in the second division) rather than the pre-reductional one. However, Kobayashi and Ebinuma (1984) have analyzed this subject by using a hereditary strain, and they tentatively concluded that the type of meiotic divisions in the silkworm females is of the pre-reductional. The discrepancy may be due to a difference in strains used for the experiments.

Genetical Studies on the Hereditary Mosaic (*mo*) Strain of *Bombyx mori*: The Appearance of Polyploids

Akio MURAKAMI

There are some reports in which the hereditary mosaic gene (*mo*) may give rise to the polyploid, besides the mosaics either for sexual or somatic traits. Tanaka (1928) first observed exceptional females which were regarded as due to polyploidy in a sex-mosaic (probably corresponding to the hereditary mosaic) strain. Goldschmidt and Katsuki (1928, 1931) observed cytologically the case in which a female triploid nucleus was present in an egg cytoplasm of a hereditary mosaic strain. Hashimoto (1934) actually detected several tetraploid females and analyzed their origin. However, detailed understanding of the function of the gene (*mo*) responsible for those two striking biological phenomena is still lacking.

In the F_1 offspring of the hereditary mosaic strain females heterozygous for the egg-colour gene (*pe*$^+$/*pe*) crossed with the males homozygous for *pe*, some triploid and tetraploid insects were detected besides a number of somatic or sexual mosaic and normal diploid insects. These tetraploid females appeared as an exceptional egg either black or yellowish-white (hereafter yellow). The progeny from crossing the black tetraploid females to the diploid marker males homozygous for the *pe* was black and yellow eggs at a ratio close to the expected 1: 1. This suggests that the tetraploid is produced by the fusion of two diploid egg nuclei (+/*pe* and *pe*/*pe*) fertilized with the sperm rather than the fertilization of a triploid egg nucleus with a sperm. Thus, it can be said that the tetraploid insect has a +/*pe*/*pe*/*pe* constitution for the chromosome-5 and that two of the three chromosome-5 marked with the *pe* gene might be derived from the sperm. A segregation with a 5: 1 ratio of black and yellow eggs was also rarely detected, suggesting that the origin of this type of tetraploid might be also produced by the fusion of double nucleus eggs (+/+/*pe*/*pe*) already fertilized with sperm. Tetraploid females with all yellow genes (*pe*/*pe*/*pe*/*pe*) were also rarely detected.

Even if triploids were detected, the analysis of their segregation ratio is difficult because of their high sterility. Practically, there were two types of triploids with black or yellow eggs. On crossing the black triploid female to the marker *pe* male a segregation at a ratio of *ca.* 1: 1 for black and yellow eggs, occurred, while a cross of the yellow triploid female to the *pe* male produced only offspring of yellow eggs. From these observations, at least, one chromosome-5 with either + or *pe* of the triploid females may be contributed from the female parent and also one chromosome-5 with the *pe* gene from the male. The presence of +/+/*pe* triploids suggests that the remaining one would be derived from the female parent rather than the male. It should be noted, however, that the tetraploids might primarily occur in the F_1 offspring of the cross between the hereditary mosaic strain female and marker male, but the triploid has a tendency to secondarily occur as the product of a cross between tetraploid females and normal diploid males.

Considering the appearance of both mosaics and polyploids in the hereditary mosaic strain females, the gene responsible for those two biological phenomena may be ascribed to the high affinity among gametic nuclei, ova, polar-bodies, sperm and/or synkaryons, so that the egg nucleus and its sister nucleus can be fused to develop into diploidy in an egg. In *Bombyx*, polyspermy is the rule and the two sperm are readily available for the two

egg nuclei. Consequently, it is also likely to expect that the diploid nucleus can be fertilized with a haploid sperm and leads to a triploidal nucleus. Briefly, in the hereditary mosaic strain, several combinations among the egg nucleus, cellular polar-bodies and sperm in an egg can occur and in consequence form polyploids as well as mosaics.

A Strain with Highly Parthenogenic Tendency in *Bombyx mori*

Akio MURAKAMI

Natural parthenogenesis is one of potential tools for better understanding of the mechanism of sexual reproduction and ontogenesis in bisexual organisms. Spontaneous parthenogenic development of unfertilized eggs takes place rarely in the silkworm (*B. mori*) as reported by several investigators. Accordingly, it is necessary to establish a highly spontaneous parthenogenic line in the insect. In *B. mori*, the developing embryos or eggs are easily discernible from the non-developing egg at the stage of serosa membrane formation. The appearance of coloration or the formation of pigment granules in the serosa membrane cells is generally connected with the development of embryos. An unfertilized egg is light yellowish-white. A fertilized egg on the 3rd day after oviposition starts growing darker and becomes a dark grey peculiar to the silkworm egg around the 5th day. We have made a selection with the intention of obtaining the highly parthenogenic line since 1980, but had not obtained such a line by 1983. In the past year, eight different silkworm strains, C108, Aojuku, J106, Daizo, Uda *pe*, *od*, *pe*: *re* and an F_1 hybrid between C108 and Aojuku were examined. Consequently, the percentage of pigmented eggs or parthenotes per female varied from individual to individual with a few fractions to a few percent of the number of unfertilized eggs per female. It also greatly varied in the strain or stock line. This suggests that the spontaneous parthenogenic phenomena are under the control of genetic factor(s). Among them, a Chinese bivoltine race Daizo showed the highest potency of spontaneous parthenogenesis and the F_1 hybrid had a tendency to some extent. These findings are in good accord with the view that naturally occurring parthenogenesis is generally higher for bivoltine races and hybrid stocks than for univoltine races. It is of interest to note that the egg-laying rate of Daizo was the highest among the strains tested: Daizo completed the egg-laying within one to two days when being place at a room temperature (25°C) after em-

ergence, while the other strains needed around 5–7 days (5 days on the average). This means that unfertilized eggs may have a more vital potency for the natural parthenogenesis in Daizo than those in the other strains used in the experiment. These observations and other indicate that unfertilized eggs may have fair chance for the completion of maturation divisions to remain for several days after oviposition without the penetration of spermatozoa.

In the experiment with the F_1 hybrid between C108 and Aojuku, only one hatched embryo (*ca.* 0.002%) from 150 naturally developed eggs was detected and it grew into a 3rd instar larva. The larva was male, indicating that the parthenote might have resulted from an egg-pronucleus passing through both the reductional and equational maturation divisions. The pronucleus might be divided into two haploid nuclei whose fusion into one cleavage nucleus leads to diploidy in the egg.

The Second Case of a Chromosome Specific Instability in the silkworm, *Bombyx mori*

Akio MURAKAMI

An egg-colour mosaic (*pe*$^+$/*pe*) female mutant was detected in chemically induced mutagenesis experiments by the *pe*: *re* specific-locus method in which wild-type F_1 hybrid (C108×Aojuku) mid-stage female pupae were treated with ethyl methanesulfonate (EMS) and mated to the *pe*: *re* marker strain. The moth that developed from the mosaic mutant was back-crossed to the *pe*: *re* marker male in order to analyze its genetic nature. When the black egg (++/*pe re*) females appearing in the above cross were mated to males homozygous for both *pe* and *re* loci, they laid a very small number of black (*ca.* 4.0%) eggs and an overwhelming number of yellowish-white eggs (*ca.* 90% or more), differing from the expectation of a one to one ratio for black and yellowish-white eggs. In addition, they also produced the same number of red eggs (*ca.* 4.0%) as black eggs. Furthermore, when the black female eggs appearing in the preceeding generation were crossed with the *pe*: *re* marker males, they again laid a very small number of black eggs and the unexpected red eggs. Repetition of this mating procedure revealed a similar tendency in the offspring of following generations. These findings suggest that the unstable and/or mutable line, tentatively MV$^{\text{INSTA}}$-2, may be classified as a line having a chromosome specific genetic instability. It can be also

said that the instability of this line behaves as a semidominant one. Although the genetic instability in the present experiment on the silkworm seems to be derived from EMS-treated oocytes, a possibility that the MV^{INSTA}-2 line might be of a spontaneous origin cannot be excluded. The evidence that the instability affected both sexes is apparently based on the fact that both matings, black females and marker males and marker females and black males, were carried out. No genetic effect of the MV^{INSTA}-2 line on chromosome other than the chromosome-5 was detected, indicating that this line showed a chromosome-5 specific type instability similar to MV^{INSTA} (Murakami, 1975). However, the line did not induce a mosaic type mutation as to egg-colour (serosa membrane) cells dissimilar to the MV^{INSTA} line. The egg-colour mutants detected in the line were of whole-body types for both *pe* and *re* loci. Theoretically, a germ cell mutation occurring or fixed at the stage of germ cells before (and during) DNA replication should be expressed by a whole area of the egg (or a large mutant area), while that occurring at a stage of germ cells after DNA replication should be expressed by a small mutant area. This indicated that the genetic instability of the MV^{INSTA}-2 line chromosome may be, at least, expressed at the germ cell stage in prior to the completion of DNA replication or synthesis.

Lethal Phases of X-linked Lethal Mutations in *Drosophila melanogaster*

Kiyoshi MINATO and Masa-Aki YAMADA

To investigate the mechanism of the expression of embryonic lethal genes, the lethal phase of development was examined on 28 strains of X-linked recessive lethals induced by ethyl methanesulfonate (2.5×10^{-3} M) in *Drosophila melanogaster*.

Twenty-two strains among 28 strains died during larval stage, 6 strains died during pupal stage. No strains, however, died during embrionic stage of development. As compared to the previous reports (Hadorn and Chen, 1952, Oster, 1952, and D. T. Suzuki, 1970), the ratio of embryonic lethals was extremely low. This may be partly due to a mild procedure for inducing mutation used in our experiment, since it has been found that the milder the procedure for inducing mutation was, the less the embryonic lethals were obtained (Seto, 1954). Because the defects in gene activity produced by a mild procedure may reflect the defects in the stage-specific gene ac-

tivity, the above low ratio of embryonic lethals is of interest, suggesting the relative independency of embryonic development from the control by the zygotical genes.

Nematocyte Differentiation in Mutant Strains of Hydra with Altered Developmental Gradients

Chiemi NISHIMIYA, Toshitaka FUJISAWA, Nancy WANEK and Tsutomu SUGIYAMA

Nematocyte differentiation from the interstitial stem cells in hydra occurs non-uniformly along the body column. The relative ratios of the 4 nematocyte types produced vary gradually from head to foot along the body axis (Bode and Smith, 1977). To find out whether this regional variation in nematocyte differentiation is related to the gradients of the head-activation and head-inhibition potentials, nematocyte differentiation patterns were examined in strains which have significantly different developmental gradients along their body columns.

It was found that the regional variations in the nematocyte differentiation were similar in all the strains examined, and that no significant differences of the variation existed that could be attributed to the differences of the morphogenetic potentials in these strains. This suggests that nematocyte differentiation is strongly affected by the axial position along the body column, but that the gradients of the morphogenetic potentials involved in head formation are not involved in this effect. Instead, some other parameter(s) of axial position not directly associated with these gradients must be responsible for the positional effect on nematocyte differentiation.

Elimination of Excess Epithelial Cells by Phagocytosis in a Mutant Strain of Hydra (L4)

Eucaly KOBATAKE and Tsutomu SUGIYAMA

The mechanism of removal of "excess" epithelial cells was examined in a mutant hydra strain which has a very reduced budding capacity (L4).

In a well-fed and exponentially growing population of normal hydra, the epithelial cells produced by cell division are largely utilized to increase the tissue mass (David and Campbell, 1972; Otto and Campbell, 1977). In contrast, these cells produced in the mutant strain L4 are not effectively

utilized to increase the tissue mass (Takano *et al.*, 1980). Instead, a large number of them are apparently eliminated from tissue by some mechanism (Takano, 1984).

In order to elucidate the mechanism of elimination of excess epithelial cells in strain L4, its tissue was macerated and the resultant dissociated cells were examined after Feulgen staining. It was found that a large number of L4's epithelial cells had in their cytoplasm large phagocytic vacuoles that contained partially-degraded cells, whose nuclei had highly condensed and intensely Feulgen-positive chromatin granules. This and other features of the entire phagocytic structure were very similar to those of the phagocytic structure recently found in the starved animals of a normal strain by Bosch and David (1984).

The incidence of epithelial cells having phagocytic vacuoles was relatively high when L4 animals were cultured under the conditions which allowed a large number of excess epithelial cell production, but relatively low when cultured under other conditions allowing little or no excess epithelial cell production. These results suggest that epithelial cells produced but not utilized in strain L4 were removed, at least in part, by phagocytosis by neighbouring cells.

V. CYTOGENETICS

Karyological Survey of Indian Ants

H. T. IMAI, C. Baroni URBANI[1], M. KUBOTA[2], G. P. SHARMA[3], M. N. NARASIMHANNA[4], B. C. DAS[4], A. K. SHARMA[5], A. SHARMA[5], G. B. DEODIKAR[6], V. G. VAIDYA[6], and M. R. RAJASEKARASETTY[7]

The karyotypes of 94 species of Indian ants were examined. Their chromosome numbers range almost continuously between $n=5$ and 38, though the frequency distribution is bimodal with a remarkable antimode at $n=11$ and two modal points at $n=10$ and 15. Based on this bimodal distribution, Indian ants were classified into two groups: Lower-numbered species ($n\leq 11$) and higher-numbered species ($n>11$), the former being characterized by metacentric-rich karyotypes, and acrocentrics predominating in the latter. The three major subfamilies (Ponerinae, Myrmicinae, and Formicinae) showed a highly divergent distribution in chromosome number, ranging between $n=7$–38, 6–35, and 8–27, respectively, suggesting a convergence in karyotype evolution of each subfamily. Another three subfamilies, of which only a few species were examined, had moderate or lower numbers, i.e., $n=5$–14 in Dolichoderinae, $n=14$ in Cerapachynae, and $n=12$ in Dorylinae. We found four Robertsonian polymorphisms, two pericentric inversion polymorphisms, and four reciprocal translocations, three of which were fixed. Robertsonian polymorphisms were found only in higher-numbered species, while translocation were restricted to lower-numbered species. A possible biological significance for this nonrandom distribution of rear-

[1] Zoological Institute of the University, Rheinspring 9, CH-4051 Basel, Switzerland, and Naturhistorisches Museum Basel, CH-4001 Basel, Switzerland.

[2] Nakasone 13, Odawara, Kanagawa-ken 250, Japan.

[3] Department of Zoology, Panjab University, Chandigar-160014, India.

[4] Central Tasar Research Station, Ranchi-834005, India.

[5] Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Calcutta-700019, India.

[6] Department of Zoology, Maharashtra Association for the Cultivation of Science, Law College Road, Poona-411004, India.

[7] Department of Zoology, Manasa Gangotori, Mysore-560006, India.

rangements is discussed with reference to karyotype evolution in ants. For details see Jpn. J. Genet. **59**: 1–32, 1984.

Chromosome Observations on Tropical Ants from Indonesia

Hirotami Y. IMAI, Masao KUBOTA[1], William L. BROWN Jr.[2], Masaaki IHARA[3], Machmud TOHARI[4], and Rafael I. PRANATA[4]

The senior author has been promoting a karyological survey of world ants. As a part of this project, IMAI and KUBOTA had a chance to survey chromosomes of Indonesian ants in cooperation with IHARA, TOHARI and PRANATA. Collection of ants and chromosome preparations were made from March 3 to March 15 in 1984. A total of 88 colonies including 4 subfamilies, 32 genera, and 64 species were examined. The colonies collected were labelled as HI84-(1～88) and their localities are as follows: Bogor (HI84-1～15, 44～52, 54～62, 71～88), Chibodas (HI84-16～43, 53), and Darmaga (HI84-63～70). The identification of species was made mainly by BROWN and partly by BOLTON (British Museum) for *Tetramorium*. One set of alcohol specimens was deposited with BIOTROP, which is maintained by PRANATA. The results are summmarized in Table 1.

Table 1. Chromosome numbers of Indonesian ants

Taxa (Colony number)	Chrom. number (n) $2n$
PONERINAE	
Discothyrea sp. near *bryanti* (HI84–56)	30
Leptogenys kraepelini (HI84–17, 24, 30, 31)	26
L. diminuta (HI84–7)	32
L. iridescens (HI84–57)	46
L. myops (HI84–13, 62)	48
L. peuqueti (HI84–6, 75)	54
Diacamma sp. (HI84–4)	66
Odontoponera transversa (HI84–86)	42
Pachycondyla astuta (HI84–25)	18
P. astuta (HI84–54)	22

[1] Nakasone 13, Odawara, Kanagawa-ken, 250, Japan.
[2] Department of Entomology, Cornell University, Ithaca, New York, 14853, U.S.A.
[3] JICA Scientific Expert, Tropical Forest Program BIOTROP, Bogor, Indonesia.
[4] Tropical Agricultural Pest Biology, BIOTROP, Bogor, Indonesia.

Table 1. (Continued).

Taxa (Colony number)	Chrom. number (*n*) 2*n*
P. rubra (HI84–16, 34)	(10) 20
P. sp. near *obscurans* (HI84–64)	44
Ponera sp. (HI84–28)	12
Hypoponera pruinosa (HI84–58)	24
H. confinis (HI84–61)	38
Odontomachus latidens (HI84–23, 33)	32
O. simillimus (HI84–12, 44)	(22) 44
Anochetus modicus (HI84–22)	30
A. graeffei (HI84–5, 76)	38
MYRMICINAE	
Pheidole capellinii (HI84–2)	20
P. hortensis (HI84–37, 53)	20
P. binghami (HI84–40)	20
P. plagiaria (HI84–60)	20
P. sp. 5 (HI84–66)	18
P. sp. 6 (HI84–84)	18
P. sp. 7 (HI84–38, 43)	18
P. sp. 8 (HI84–39)	32
Crematogaster sp. **1** (HI84–74, 77)	24
C. sp. 2 (HI84–88) B-chromosome polymorphism	56/58
Meranoplus bicolor (HI84–11, 15)	16
Monomorium sp. 1 (HI84–47)	22
M. sp. 2 (HI84–51)	22
Oligomyrmex sp. 1 (HI84–69, 80)	34
O. sp. 2 (HI84–49)	42
Vollenhovia sp. (HI84–29)	36
Myrmicaria sp. (HI84–18, 21)	44
Myrmecina sp. 1 (HI84–32)	66
M. sp. 2 (HI84–19)	68
Tetramorium kheperra (HI84–81)*	14
T. brevidentatum (HI84–35, 36)*	(10) 20
T. adelphon (HI84–10)*	22
T. insolens (HI84–9, 14)*	22
T. pacificum (HI84–87)*	22
T. smithi (HI84–8)*	26
Smithistruma sp. (HI84–27, 71)	38
Kydris mutica (HI84–73)	36
Strumigenys doriae (HI84–20)	22
S. godeffroyi (HI84–72)	44

Table 1. (Continued).

Taxa (Colony number)	Chrom. number (*n*) 2*n*
DOLICHODERINAE	
Dolichoderus bituberculatus (HI84–50)	30
Iridomyrmex anceps (HI84–48)	48
Tapinoma melanocephalum (HI84–3, 67)	10
Technomyrmex sp. 1 (HI84–83)	28
T. sp. 2 (HI84–78)	30
FORMICINAE	
Anoplolepis longipes (HI84–82)	34
Acropyga sp. (HI84–63)	32
Plagiolepis sp. (HI84–70)	18
Pseudolasius sp. (HI84–46)	30
Paratrechina longicornis (HI84–1)	(16)
P. sp. 2 (HI84–41)	30
P. sp. 3 (HI84–42, 68, 79, 85)	30
Camponotus sp. 1 (HI84–45, 52) Robertsonian polymorphism	38/39
C. sp. 2 (HI84–65)	38
Polyrhachis gribodoi (HI84–26)	48
P. illaudata (HI84–55, 59)	(14) 28

The *Tetramorium* species with asterisks indicates samples for which the determination has been checked and confirmed by Bolton.

The Reproductive Cycle of the Queenless Ant *Pristomyrmex pungens*

Tomio ITOW[1], Kazuo KOBAYASI[1], Masao KUBOTA[2], Kazuo OGATA,[3] Hirotami T. IMAI, and Ross H. CROZIER[4]

The life cycle of the myricine ant *Pristomyrmex pungens* was investigated. Colonies of this species are usually composed of several thousand small workers, although a few males (2–3%) occasionally appear during June and July in mature colonies, and large workers with ocelli and abortive sper-

[1] Department of Biology, Faculty of Education, Shizuoka University, Shizuoka 422, Japan.

[2] Nakasone 13, Odawara, Kanagawa-ken, 250, Japan.

[3] Faculty of Agriculture, Kyushu University, Fukuoka, 812, Japan.

[4] School of Zoology, University of New South Wales, P. O. Box 1, Kensington, N.S.W. 2033, Australia.

mathecae (here termed *ergatoid queens*) were observed extremely rarely. We found that the virgin small workers can lay eggs and that these develop into further small workers. Cerebral ganglion cells and oogonial cells had the diploid chromosome number ($2n=24$), but the haploid number ($n=12$) was observed in oocytes at pachytene, and also in spermatocytes from the rare males. Males have functionally normal copulatory organs and their spermatogenesis is normal, but they probably do not mate, because small workers have no spermatheca and their copulatory organs are degenerative. These observations indicate that reproduction in *P. pungens* is carried out predominantly or, probably, wholly by the small workers and hence is thelytokous. We discuss the evolution of the *P. pungens* life cycle, pointing out the difficulty of applying the "queen" concept to this species or even in deciding whether or not it is "eusocial". In view of the apparent genetic isolation between colonies, the mode of selection maintaining sociality in this case is probably interdemic group rather than kin selection. For details see Insectes Sociaux **31**: 87–102, 1984).

Molecular Analyses of Transposable Elements in *Drosophila simulans*

Yoshihiro H. INOUE, Takao K. WATANABE, Kazuo MORIWAKI, and Masatoshi YAMAMOTO

Transposable elements have been studied in most detail in *Drosophila melanogaster*. And it is considered that the biological role of transposable elements is to increase genetical variability by spontaneous mutations and chromosomal rearrangements. In order to examine whether this applies to other organism, its sibling spieces *D. simulans* was studied. Although *D. simulans* is morphologicaly similar to *D. melanogaster*, it has some different genetical characters from *D. melanogaster*; The frequency of chromosomal rearrangements found from wild population is lower than that of *D. melanogaster*. And further, the dispersed middle repetitive DNA, to which transposable elements belong, is possesed about one seventh fewer than *D. melanogaster*. Here we report an unstable *white* allele, w^{mky} in *D. simulans*, which was spontaneously isolated from an isofemale line of wild population in Japan.

The w^{mky} of *D. simulans* is an unstable *w* allele and a few new eye colour mutants were derived from the inbred line stock. The mutation frequency

of w^{mky} was measured at 4×10^{-5}. From the w^{mky} subline 1, w^{cho} and w^{apl} were isolated. On the other hand, w^{psm1}, w^{psm3}, w^{psm4} were obtained from the w^{mky} subline 2. The genetical analyses showed that the derivatives from the subline 1 were the mutation at the *w* locus and those derived from the subline 2 were atributed to *su*(w^{mky}) on the third chromosome. The *su*(w^{mky}) were closely linked to *cu*(3–90.0). This is the first evidence of an unstable gene locus in *D. simulans*. A molecular analysis of w^{mky} indicated that an about 15 Kb long DNA fragment of unknown origin was inserted into the *w* locus. These genetical and molecular data suggest that the insertion is a transposable element which causes the instability of the *w* allele.

Thus, a transposable element which was able to induce an unstable mutation was found in *D. simulans*. This shows that the active transposable element which contributes to induction of the spontaneous mutation is carried in not only *D. melanogaster* but *D. simulans*.

VI. MUTAGENESIS AND RADIATION GENETICS

So called "Kada Effect" of the Tritium

Tsuneo KADA and Yoshito SADAIE

We previously reported about the *in vitro* inactivation efficiencies of tritiated water on DNA (Proc. 7th ICRR, B1-18, 1983). Transforming DNA was extracted from *B. subtilis* cells (Arg^+), purified, dissolved in SSC (5 μg/ml) containing tritiated water of different concentrations (100 μCi/ml, 10 μCi/ml, 1 μCi/ml . . . etc.) and kept at 4°C. When the transforming activities ($Arg^- \rightarrow Arg^+$) were determined after different periods of the tritium treatment, we found that the inactivation efficiencies increased markedly by decreasing the concentrations of tritiated water. Such a concentration effect of tritium as to the *in vitro* inactivation of DNA was called the "Kada effect".

Characteristics of the above observation may be the following.

(1) When damages are produced in DNA by β-irradiation from tritum, they should remain unchanged in a buffer solution at low temperatures, there is no chance of being repaired.

(2) To obtain similar levels of the radiation exposure of different doses that are proportional to the values μCi/ml$\times$(days of treatment), it takes, for example, 10 times more days in a treatment sample having 10 times more diluted tritium concentration. Therefore the increase of inactivation efficiencies should be proportional to the period of treatment.

We previously supposed that certain stable trit um-generated radicals might be generated and play a role in the "Kada effect". If this is the case, the inactivation event may be proportional to the period of treatment and not to the concentration of tritium. Recently Dr. Watanabe and his collaborators (Tokyo Isotope Inst.) and Dr. Yamamoto (Hiroshima Univ.) found generation of hydrogenperoxide (H_2O_2) of considerable levels in the course of stocks of tritiated water. We carried out experiments to check if H_2O_2 might inactivate DNA in a similar fashion as tritiated water, and found very similar effects both for H_2O_2 and tritiated water.

In Vitro and *In Vivo* Analysis of Antimutagenesis

Tsuneo KADA

We carried out screenings of bio-antimutagens from tissues of different plants using radiation and chemically treated bacterial strains such as *E. coli* B/r WP2, *Salmonella* TA100, etc. Results of our studies on isolation of bio-antimutagens in plants and animals and their actions indicated the following mechanisms.

1) Activation of error-free DNA repair:

In the case of cobaltous chloride, the spectrum of bio-antimutagenic activity is very very wide. It reduced the induced mutations of UV, γ-ray, MNNG, Trp-P-1, etc. Such a wide spectrum can not be explained by the action of the agent on the path-way specific to each mutagen. We found that the survivals of 4NQO-treated bacteria were higher in the presence of bio-antimutagens than in their absence. Similar observations were made with cinnamaldehyde. The increase in survival was dependent on the *recA*$^+$ function. *In vitro* formation of D-loop from homologous double strand DNA and single strand DNA by means of the *recA* protein was promoted by the presence of cobaltous chloride.

2) Promotion of DNA repair-pathways specific to UV and UV-mimetic chemicals: Collaborative studies with K. Shimoi and I. Tomita (Shizuoka College of Pharmaceutical Sciences) showed that tannic acid reduced the UV-indiced mutation when present for as long as 10 minutes after irradiation. Liquid-holding experiments as well as other experiments indicated that this chemical might promote excision repair of DNA possessing UV-induced lesions.

We are also studying mechanism of other bio-antimutgens such as human placenta factor and green tea factor. It is interest to us whether these anti-mutagens might also work in higher plants and animals (Mutation Res. **150**: 127, 1985; Proc. 4th Inter. Conf. Env. Mutagens).

Gamma-Induced Chromosome Aberrations in *Caenorhabditis elegans*

Yoshito SADAIE and Tamiko SADAIE

A simple method for observing chromosome aberrations in *Caenorhabditis elegans* was established. Gravid worms were irradiated with gamma-rays

and metaphase chromosomes of early embryonic origin were examined. The frequency of chromosome aberrations increased with increasing doses of gamma-rays in *C. elegans* var. Bristol strain N2, and decreased upon incubation of the irradiated gravid worms. About 5 times as many spontaneous chromosome aberrations were observed in *C. elegans* var. Bergerac strain BO which carries ten times more transposons than the Bristol strain.

Age-dependent and Tissue-specific Expression of a DNA Repair Enzyme in the Wasted Mouse, a Model Animal of a Human Genetic Disease Ataxia Telangiectasia (AT)

Hideo TEZUKA, Tadashi INOUE, Takehiko NOGUCHI[1], Tsuneo KADA and Leonard D. SHULTZ[2]

Biological, cytogenetic and biochemical methods were used to study various tissues from a stock of mouse mutant, wasted, after weaning. In affected homozygotes, a marked age-dependent decrease in the ratio of spleen or thymus to body weight was observed. This tendency was, however, observed neither in the liver nor in the kidney. An age-dependent increase was observed in the frequency of both spontaneous and gamma-ray-induced chromosomal aberrations in bone marrow cells of wasted mice. In littermate control mice, neither of these alterations was observed in an age-dependent manner. Activities of primer activating enzyme and apurinic endonuclease were measured in extracts from spleens or livers of affected homozygotes and control mice. The only alteration was an age-dependent decrease in primer activating enzyme activity in the spleens but not in the livers of homozygotes. No change in primer activating enzyme activity was found in the control group while neither group showed alterations in apurinic endonuclease activity.

The present results suggest that this mouse mutant may serve as a useful animal model for studying the relationship between DNA metabolism and lymphoid tissue differentiation.

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[1] Genetic Stocks Research Center; deceased on November 24, 1983.

[2] The Jackson Laboratory, Bar Harbour, Maine 04609, USA.

Effect of DNA Damaging Agents on Isolated Spleen Cells and Lung Fibroblasts from the Mouse Mutant "Wasted", a Putative Animal Model for Ataxia-Telangiectasia

Tadashi INOUE, Hideo TEZUKA, Tsuneo KADA, Katsuhiro AIKAWA[1] and Leonard D. SHULTZ[2]

Ataxia-telangiectasia (A-T) is a human autosomal recessive disease characterized by a progressive neurological disorder, a marked IgA deficiency, an increased predisposition to cancer, and a spontaneous chromosomal instability. The hypersensitivity of A-T to ionizing radiation, expressed as reduced viability and increased cytogenetic damage in cultured fibroblasts, has suggested defective DNA repair in this disease. We have previously demonstrated the reduced level of the primer activating enzyme in cellular extract from A-T cells which enhances the priming activity of gamma-irradiated DNA for purified DNA polymerase, thus indicating that one of the causal factors of A-T is a DNA repair defect.

Recently, Shultz *et al.* have described a mouse mutant, wasted (*wst*), which shows pathological changes in both central nervous and lymphoid systems, and exhibits increases in the frequency of spontaneous, as well as gamma-ray-induced, chromosomal aberrations. These findings prompted us to evaluate the mouse mutant as an animal model for A-T.

Isolated cells of wasted mouse were examined as to their responses to DNA damaging agents including bleomycin and gamma-rays. DNA synthesis was found to be more resistant to bleomycin, gamma-rays and 4-nitroquinoline 1-oxide in wasted mouse spleen cells than in control mouse spleen cells whereas both types of cells exhibited similar responses to UV light. The amount of bleomycin-stimulated repair synthesis in wasted mouse spleen cells was quite similar to that in control cells. Primary lung fibroblasts derived from wasted mouse were not hypersensitive to the killing effect of gamma-rays. These data indicate that the defect in DNA metabolism of spleen cells of wasted mouse, if any, is specific for damages induced by gamma-rays or by an agent which mimics gamma-rays and that the expression of defects is somewhat different between wasted mouse cells and ataxia-telangiectasia fibroblasts.

[1] Institute for Animal Industry, Kukizaki, Ibaraki-ken 305 (Japan).
[2] The Jackson Laboratory, Bar Harbor, Maine 04609 (U.S.A.).

Mutagenic Activity of 3′-deoxyadenosine in the Soybean Test System

Taro FUJII

The study of somatic mosaicism in soybean plants offers a sensitive and reliable method for screening and monitoring environmental mutagens. Mutagenic effects of some chemicals in higher plants were examined with this test system and the mutagenicities of known carcinogens, such as AF-2 and L-ethionine etc., were clearly demonstrated. Mutagenic activity of 3′-deoxyadenosine (3′-dA) and 3′-deoxyguanosine (3′-dG), a known repair inhibitor for radiation induced damage, were investigated with soybean test system, and 2′-dA and 2′-dG were used as control drugs.

2′- and 3′-dA: Concentrations of 0 to 2 mM of 3′-dA were applied to seeds in the pilot experiment. Seedling growth was inhibited with increasing drug concentrations, gradually, and all seedlings at 1.0 and 2.0 mM treatments showed shrunken or deformed leaf development. Based on the above information, much lower concentrations of drug, 0.05 and 0.1 mM, were used in the next experiment. 2′-dA was used at the same concentrations. Spotting frequencies of 1.1 and 1.0 per leaf obtained from 0.05 and 0.1 mM treatments of 2′-dA, respectively, were similar to that of 0 mM treatment. On the other hand, 3′-dA induced numerous spots with 4.7 and 8.5 per leaf with 0.05 and 0.1 mM treatments, respectively, being observed. Moreover, some leaves at 0.1 mM treatment showed 30 or more spots per leaf. Further experiments with more diluted concentrations, viz., 0.025, 0.05 and 0.075 mM, were conducted to check the dose versus frequency relationships. The results clearly demonstrated mutation induction by 3′-dA, and when the data are plotted on the figure, spotting frequency increased more effectively with increasing concentrations.

From the experiments, it is evident that 2′-dA is not mutagenic but 3′-dA has strong mutagenic activity in the present test system. A severe toxic effect of 3′-dA on plant growth was also noticed with concentrations higher than 0.1 mM.

2′- and 3′-dG: To obtaine further information concerning the mutagenic activity of 3′-dA, experiments were extended with another repair inhibitor, 3′-dG. Seeds were treated with 0.1, 0.25 and 0.5 mM solutions of 2′- and 3′-dG. 2′-dG showed no mutagenic activity; spotting frequencies of the 0.1–0.5 mM treatments were not much different from that of the 0 mM

treatment. On the contrary, 3′-dG indicated mutagenic activity with the number of spots per leaf increasing with increasing of drug concentrations. However, the activity of 3′-dG is much less than that of 3′-dA for induction of somatic mutations.

VII. POPULATION GENETICS

Evolution of an Altruistic Trait through Group Selection as Studied by the Diffusion Equation Method

Motoo KIMURA

A diffusion model is formulated which incorporates the process of group selection (i.e. interdeme competition) in addition to mutation, migration, individual selection, and random genetic drift. Let us assume a diploid species consisting of an infinite number of competing groups ("demes") each having N_e breeding members and in which mating is at random. Consider a locus at which a pair of alleles A and A' are segregating, where A' is the "altruistic allele," which has selective disadvantage s' relative to A with respect to individual selection within demes, but which is beneficial for a deme in competition with other demes; namely, we assume that a deme having A' with relative frequency x has the advantage $c(x-\bar{x})$ relative to the average deme, where c is a positive constant and $\bar{x}$ is the average of x over the entire species. Let $\phi=\phi(x;\, t)$ be the distribution function of x at time t such that $\phi(x;\, t)\Delta x$ represents the fraction of demes whose frequency of A' lies in the range $(x,\, x+\Delta x)$. Then, we obtain the following diffusion equation.

$$\frac{\partial\phi}{\partial t}=\frac{1}{2}\frac{\partial^2}{\partial x^2}\{V_{\delta x}\phi\}-\frac{\partial}{\partial x}\{M_{\delta x}\phi\}+c(x-\bar{x})\phi\ ,$$

where

$$V_{\delta x}=x(1-x)/(2N_e)$$

and

$$M_{\delta x}=v'(1-x)-vx+m(\bar{x}-x)-s'x(1-x)\ ,$$

in which v' and v are mutation rates to and from the altruistic allele A', and m is the migration rate per deme per generation (assuming Wright's island model).

By investigating the above diffusion equation, I obtained a condition for group selection to prevail over individual selection leading to the evolution of an altruistic trait: Let

$$D_K=c/(v+v'+m)-4N_es' ,$$

then, group selection overrides individual selection if $D_K>0$, and the altruistic allele predominates in the species. It can also be shown that, if $D_K<0$, individual selection prevails so that the altruistic allele becomes rare and cannot be established in the species. For details, see Kimura, M. (1984), IMA Jour. of Math. Appl. in Med. & Biol. 1: 1–15.

Population Genetics Theory of Concerted Evolution and Its Application to the Immunoglobulin V Gene Tree

Tomoko OHTA

The previous simple model for treating concerted evolution of multigene families has been revised to be compatible with various new observations on the immunoglobulin variable region family and other families. In the previous model, gene conversion and unequal crossing-over were considered, and it was assumed that genes are randomly arranged on the chromosome; neither subdivision nor correlation of gene identity and chromosomal distance were considered. Although this model satisfactorily explains the observed amino acid diversity within and between species, it fails to predict the very ancient branching of the mouse immunoglobulin heavy chain V-gene family. By incorporating subdivided structure and genetic correlation with chromosomal distance into the simple model, the date of divergence may be satisfactorily explained, as well as the rate of nucleotide substitution and the amino acid diversity. The rate at which a V-gene is duplicated or deleted by conversion or by unequal crossing-over is estimated by the new model to be on the order of 10^{-6} per year. The model may be applicable to other multigene families, such as those coding for silkmoth chorion or mammalian kallikrein. For details, see J. Mol. Evol. 20: 274–280.

Population Genetics of Transposable Elements

Tomoko OHTA

Transposable elements are DNA sequences that can move or duplicatively transpose on the chromosome. A simple model of transposable elements was studied, and the frequency spectrum, allelism, and identity coefficients at equilibrium were obtained. In the analyses, the two cases of asexual

haploid with no recombination and diploid with free recombination were treated separately. It was shown that the multiple allele theory of population genetics is applicable to the problems of frequency spectrum and allelism. On the other hand, identity coefficients may be formulated analogously to the treatment of concerted evolution of multigene families. Results indicate that, except for allelic identity, the model of transposable elements and that of multigene families predict similar values of identity coefficients. For details, see IMA Jour. Math. Appl. Med. & Biol. **1**: 17–29.

Some Models of Gene Conversion for Treating the Evolution of Multigene Families

Tomoko OHTA

A simple model of gene conversion presented earlier has been compared with a more general model under the assumption of no bias. The former is based on a simple concept that, by conversion, a gene (or a segment of a gene) is transferred from one locus to another in a multigene family. Intrachromatid symmetric conversion may result in reciprocal exchange of genes within a multigene family, and the previous theory of identity coefficients needs modification when it occurs. The relationship of various parameters of the two models was made clear in formulating the transition equations of identity coefficients. As an example of extending the analyses, a model of preferential conversion that is relevant to the observed uneven polymorphisms of class I genes in the major histocompatibility complex has been investigated. It differs from the biased conversion, in that directional process is through location of genes on the chromosome. It is shown that the model satisfactorily explains the observed data, even though the effect of preferential conversion is difficult to distinguish from that of natural selection. For details, see Genetics **106**: 517–518.

The Cohesive Population Genetics of Molecular Drive

Tomoko OHTA and Gabriel A. DOVER*

The long-term population genetics of multigene families is influenced by several biased and unbiased mechanisms of nonreciprocal exchanges (gene conversion, unequal exchanges, transposition) between member genes, often

* Dept. of Genetics, Univ. of Cambridge.

distributed on several chromosomes. These mechanisms cause fluctuations in the copy number of variant genes in an individual and lead to a gradual replacement of an original family of n genes (A) in N number of individuals by a variant gene (a). The process for spreading a variant gene through a family and through a population is called molecular drive. Consideration of the known slow rates of nonreciprocal exchanges predicts that the population variance in the copy number of gene a per individual is small at any given generation during molecular drive. Genotypes at a given generation are expected only to range over a small section of all possible genotypes from one extreme (n number of A) to the other (n number of a). A theory is developed for estimating the size of the population variance by using the concept of identity coefficients. In particular, the variance in the course of spreading of a single mutant gene of a multigene family was investigated in detail, and the theory of identity coefficients at the state of steady decay of genetic variability proved to be useful. Monte Carlo simulations and numerical analysis based on realistic rates of exchange in families of known size reveal the correctness of the theoretical prediction and also assess the effect of bias in turnover. The population dynamics of molecular drive in gradually increasing the mean copy number of a variant gene without the generation of a large variance (population cohesion) is of significance regarding potential interactions between natural selection and molecular drive. For details, see Genetics **108**: 501–521.

F_{ST} and G_{ST} Statistics in the Finite Island Model

Naoyuki TAKAHATA and Masatoshi NEI

In Wright's island model of population structure it is assumed that a population consists of an infinite number of subpopulations each of the same size N, and in each subpopulation the proportion m of the total gene pool is derived from immigrants that may be considered a random sample of the entire population. The extent of genetic differentiation of subpopulations is measured by the fixation index (F_{ST}). The equilibrium value of F_{ST} is given by

$$F_{ST}=1\Big/\left[1+2N\left\{\frac{1}{(1-m)^2}-1\right\}\right]$$

which has been derived by considering a pair of alleles at a locus without mutation.

With the development of the infinite allele model of neutral mutations, a number of authors have extended this model to the case of a finite number of subpopulations. Nei defined G_{ST} as the ratio of the intersubpopulational gene diversity to the total gene diversity. G_{ST} is an extension of F_{ST} to the case of multiple alleles, but Nei's formula for G_{ST} is quite complicated, and its relationship with F_{ST} is not clear. Recently Takahata derived a simple equation for G_{ST} using diffusion approximations: yet, its relationship with Wright's or Nei's is not immediately clear. We therefore examined Nei's exact formula to clarify its relationship with other related formulas, and obtained that the exact formula for G_{ST} becomes

$$G_{ST}=1\Big/\left[1+2N\left(\frac{s}{s-1}\right)\left\{\frac{1}{(1-m)^2(1-v)^2}-1\right\}\right]$$
$$\approx 1\Big/\left[1+4N\left(\frac{s}{s-1}\right)(m+v)\right]$$

where v is the mutation rate per generation and s is the number of subpopulations. We also studied the non-equilibrium behavior of G_{ST} for its use in data analysis. For details, see Genetics **107**: 501–504.

Mitochondrial Gene Flow

Naoyuki TAKAHATA and Montgomery SLATKIN

To account for the transmission of mitochondrial DNA between conspecific species *Drosophila pseudoobscura* and *D. persimilis* in sympatry reported by J. R. Powell [Powell, J. R. (1983) Proc. Natl. Acad. Sci. USA **80**: 492–495], a simple model of gene flow and selection in infinite populations is analyzed. The model assumes two alleles at each of two loci, one of which is coded by an autosome and the other by mitochondrial DNA. Viability selection is presumed to be underdominant—i.e., heterozygous inferiority to the homozygotes—at an autosomal locus, and neutral or deleterious at a mitochondrial locus, with the combined action being multiplicative. Extremely strong selection against heterozygotes may prevent the transmission of mitochondrial DNA between two species, but otherwise the transmission can easily occur over species boundaries. The rate of approach to equilibrium is determined by the level of gene flow and is not affected much by selection against an autosomal locus. The divergence of the nuclear genomes of the two species is reexamined. Based on published data on enzyme loci,

we conclude that there has been mitochondrial gene flow between these species for a long enough time that several nuclear loci examined could diverge because of accumulation of neutral mutations. For details, see Proc. Natl. Acad. Sci. USA **81**: 1764–1767.

A Model of Extranuclear Genomes and the Substitution Rate under Within-Generation Selection

Naoyuki TAKAHATA

A single locus model of extranuclear genomes is developed under the assumption of the complete action of within-generation drift which is caused by random transmission of multiple copy genomes during cell division in a generation. Within-generation drift segregates different copy genomes in a cell into different cells, resulting in homoplasmic cells. Under some conditions, the present model reduces to that for haploid nuclear genomes. A point overlooked in previous models is that the multiplicity also admits of the possibility of selection occurring within a cell or between cells in an individual (within-generation selection). If there is selection mediated by, for instance, differential proliferation of genomes, then a haploid model no longer explains the dynamics of extranuclear genomes. Rather a model analogous to biased gene conversion at a single locus (Nagylaki, 1983; Walsh, 1983) is more appropriate. An application of this model to either the fixation probability or substitution rate of new mutations shows that strictly maternal inheritance does not allow the fullest use of mutations, as it obscures the effect of within-generation selection. But if there is appreciable paternal contribution, within-generation selection could be a strong evolutionary force to which nuclear genomes are never exposed. For details, see Genet. Res., Camb., **44**: 109–116.

A Quantitative Genetic Model of Two-policy Games between Relatives

Kenichi AOKI

In a behavioral interaction between two diploid individuals of the same generation in which two policies are possible, assume that changes in fitness proportional to the following occur: α for policy 1 against policy 1, β for policy 1 against policy 2, γ for policy 2 against policy 1, δ for policy 2 against

policy 2. Let the probability that an individual adopts policy 1 as opposed to policy 2 in any interaction be a quantitative genetic trait, C, determined by many additively acting genes of small effects and an independent environmental component. If interactions occur at random between all members of the population, the change per generation in $\bar{C}$, the population mean of C, is given by

$$\Delta\bar{C}=V_t[(\beta-\delta)+(\alpha-\beta-\gamma+\delta)\bar{C}]/\overline{W}\,.$$

V_t and $\overline{W}$ stand for the total genotypic variance and the mean fitness of the population, respectively. On the other hand, if interactions occur between relatives of the average degree of relatedness, r, where r is the average correlation between their genotypic values, the corresponding equation is

$$\begin{aligned}\Delta\bar{C}=V_t\{&\beta-\delta+(\alpha-\beta-\gamma+\delta)[\bar{C}+Cov(\bar{C}_s, V_s)/V_t]\\&+r[\gamma-\delta+(\alpha-\beta-\gamma+\delta)(\bar{C}+T_b/V_b)]\}/\overline{W}\,.\end{aligned}$$

It is assumed that each group of relatives and the number of such groups is sufficiently large. Here, V_b is the between group genotypic variance, $Cov(\bar{C}_s, V_s)$ is the covariance of group means and within group genotypic variances, and T_b is the third central moment of group means. For a quantitative genetic trait with the additional assumption of unlinked loci, it is reasonable to assume that $Cov(\bar{C}_s, V_s)/V_t$ and T_b/V_b are both negligible, in which case this becomes

$$\begin{aligned}\Delta\bar{C}=V_t\{&\beta+r\gamma-(1+r)\delta\\&+[(1+r)\alpha-(\beta+r\gamma)-(\gamma+r\beta)+(1+r)\delta]\bar{C}\}/\overline{W}\,.\end{aligned}$$

This equation can be heuristically derived from the first by substituting the corresponding inclusive fitness effects. Note that when per locus selection coefficients are small and linkage equilibrium holds, r can be equated approximately with Wright's coefficient of relationship. Thus, the quantitative genetic model provides a genetic basis for the inclusive fitness approach toward games between relatives. By contrast, in a monogenic system with major gene effects $Cov(\bar{C}_s, V_s)/V_t$ and T_b/V_b cannot be neglected, and we obtain substantially different results which contradict those obtained by the inclusive fitness approach in game theory. Applications are made to the Hawk-Dove game, and the simple and iterated forms of the Prisoner's Dilemma. For details, see J. Theor. Biol. **109**: 111–126, 1984.

A Population Genetic Model of the Evolution of Oblique Cultural Transmission

Kenichi AOKI

Let there be two haploid asexual genotypes A and a, each capable of expressing either of two phenotypes 1 and 2, acquired by cultural transmission from the parental generation. I assume viability selection with selection coefficient $s(>0)$ against phenotype 2. Among adults after viability selection, denote the frequency of individuals of genotype A by p and phenotype 1 by y. The phenotype of a newborn of the next generation is determined as follows: If of genotype A, it expresses phenotype 1 with probability $f(y)$ and phenotype 2 with probability $1-f(y)$; if of genotype a, it expresses phenotype 1 with probability $g(y)$ and phenotype 2 with probability $1-g(y)$. Then the coevolutionary recursion equations in y and p are

$$\bar{W}y'=pf(y)+(1-p)g(y)$$
$$\bar{W}p'=pf(y)+(1-s)p[1-f(y)]$$

where a prime indicates the next generation, and $\bar{W}=1-s[1-pf(y)-(1-p)\times g(y)]$ is the mean fitness. For example, let genotype A be imitative and genotype a be contrary. We make the reasonable assumption that acquisition of phenotype 1 which confers a selective advantage is error prone, with respective error rates u and v ($0\leqq u, v<1$) for the two genotypes. Then the transmission functions are

$$f(y)=(1-u)y\ ,\quad g(y)=(1-v)(1-y)\ .$$

When $(1-s)(1-uv)-(1-u)^2>0$, we obtain the possibly counterintuitive result that contrariness prevails over imitativeness. For details, see Proc. Jap. Acad. **60(B)**: 310–313, 1984.

Evolution of Alliance in Primates: A Population Genetic Model

Kenichi AOKI

Alliance behaviors in anubis baboons, chimpanzees, Japanese monkeys, and rhesus monkeys are briefly described. Alliance differs from simple altruism or cooperation between 2 individuals in that a third conspecific individual, the common enemy, is adversely affected. Two models of alliance formation are considered, one in which support is given unilaterally

and the other in which both parties can profit. It is assumed that the allies are equally related to each other and to the common enemy. Using a quantitative genetic model, conditions are derived for alliance behavior to be selectively advantageous. The models are applied to reciprocal altruism between adult male anubis baboons and manipulation by adult male chimpanzees. It is argued that reciprocally altruistic alliance in baboons as described is difficult to reconcile with theory. For details, see J. Ethol. **2**: 55–61, 1984.

Average Coefficient of Relationship Within Troops of the Japanese Monkey and Other Primate Species with Reference to the Possibility of Group Selection

Kenichi AOKI and Ken NOZAWA

The average coefficient of relationship within troops of the Japanese monkey (*Macaca fuscata*) is .1667±.0433; this corresponds to an F_{ST} between troops of .0935±.0277. The estimate is based on polymorphic variation at 12 blood protein loci in a sample of about 1,500 individuals distributed over 33 troops. The average coefficient of relationship among adult females (the sedentary sex) is .1886±.0670 and that among adult males (immigrants) is .1067±.0382. The difference, though not statistically significant, is in the direction expected from the observed sex asymmetry in dispersal. We give an approximate formula for the coefficient of relationship between specified relatives. The coefficient of relationship in the Japanese monkey is compared with corresponding estimates in five other terrestrial primate species and in tribal man. It is suggested that the best informed guess of the average coefficient of relationship within bands of Pleistocene man may have been about 10 to 15%. This estimate is relevant to the possibility of the evolution of altruism in our ancestors by group selection. For details, see Primates **25**: 171–184, 1984.

Group Selection for a Polygenic Behavioral Trait: Estimating the Degree of Population Subdivision

James F. CROW and Kenichi AOKI

For assessing the degree of population subdivision, and therefore the extent to which group selection might favor an altruistic trait, an appro-

priate measure is Nei's G_{ST}, defined by $(F_0-\bar{F})/(1-\bar{F})$. F_0 is the probability that two alleles drawn from the same group are identical in state and $\bar{F}$ is the probability for two alleles drawn at random from the entire population. These probabilities can be assessed from molecular polymorphisms. G_{ST} has a number of properties that make it useful for empirical studies. When the mutation rate is small relative to the migration rate and the reciprocal of the group size, G_{ST} depends mainly on the absolute number of migrants per generation, moves rapidly to near equilibrium, and is independent of the number of alleles. The relative homogenizing effect of migration in the island and stepping-stone models is not as different as might be expected; one immigrant chosen randomly for the rest of the population is only one to two times as effective as one from a neighboring group, appreciably exceeding 2 only when there are 1000 or more groups. The use of molecular data to estimate the degree of population subdivision may permit testable predictions of the extent of altruistic behavior. For details, see Proc. Natl. Acad. Sci. USA **81**: 6073–6077.

Mathematical Model of Rapid Invasion of the P-M Type Transposon to a Local Population of *Drosophila melanogaster*

Terumi MUKAI and Fumio TAJIMA

The following assumptions were made for cytotype: In the crosses of P(♀)×P(♂), P(♀)×M(♂), M(♀)×M(♂), the cytotypes of the progeny are the same as that of the mother and the mutation rate is not increased. (2) In the cross of M(♀)×P(♂), the cytotype of α fraction of the progeny changes to P, while that of $(1-\alpha)$ remains M, but the mutation rate in all the progeny increases at the increment of v. If the frequency of the P cytotype is P and that of M is $(1-P)$ and generation is expressed by t, the following approximate equations can be obtained:

$$P \cong \frac{P_0 e^{\alpha t}}{1+P_0(e^{\alpha t}-1)}$$

$$t \cong \frac{1}{\alpha} \ln \frac{P(1-P_0)}{(1-P)P_0}$$

where P_0 is the frequency of individuals carrying the P cytotype at the starting generation.

If we assume that the frequency of the transposon-induced detrimental allele is q and that q is much smaller than 1, furthermore, if hs (selection against heterozygote) is much smaller than α, then,

$$q \cong e^{-hst}\left\{\frac{v(1-P_0)P_0(e^{\alpha t}-1)}{\alpha[1+P_0(e^{\alpha t}-1)]}+q_0\right\}$$

where s is the selection coefficient against the mutant homozygote and q_0 is the frequency of transposon-induced detrimental allele at the starting generation.

Using these formulae, the case of the Katsunuma population was analyzed. The detail will be published in Proc. Natl. Acad. Sci. U.S.

Population Bottlenecks and Nonequilibrium Models in Population Genetics. I. Allele Numbers when Populations Evolve from Zero Variability

Takeo MARUYAMA

A simple numerical method was developed for the mean number and average age of alleles in a population that was initiated with no genetic variation following a sudden population expansion. The methods are used to examine the question of whether allele numbers are elevated compared with values seen in equilibrium populations having equivalent gene diversity. Excess allele numbers in expanding populations were found to be the rule. This was true whether the population began with zero variation or with low levels of variation in either of two initial distributions (initially an equilibrium allele frequency distribution or initially with loci occurring in only two classes of variation). Although the increase of alleles may persist for only a short time, when compared with the time which is required for approach to final equilibrium, the increase may be long when measured in absolute generation numbers. The pattern of increase in very rare alleles (those present only once in a sample) and the persistence of the original allele were also investigated. (Genetics **108**: 745–763.)

VIII. EVOLUTIONARY GENETICS

A Mathematical Model of Codon Substitution and the Constancy of Evolutionary Rate

Takashi GOJOBORI

A mathematical model for codon substitution is presented. This model is constructed by a 61×61 transition probability matrix for the 61 non-terminating codons, taking into account unequal mutation rates among different nucleotides and selective constraint against amino acid changes. A computer simulation under this model is conducted to study the numbers of silent (synonymous) and amino acid altering (nonsynonymous) nucleotide substitutions. It is shown that, when the mutation rates are not equal among different nucleotides, the numbers of synonymous substitutions estimated by the methods of Perler *et al.* (CELL, 1980) and Miyata and Yasunaga (J. Molec. Evol., 1980) both show a general trend of nonlinear increase with the time, though the true number of synonymous substitutions increases linearly. It is, therefore, possible that the 'saturation' of synonymous substitutions observed by Perler *et al.* is due to the inefficiency of their method in detecting all synonymous substitutions. However, it is also shown that, when the pattern of mutation is similar to that of nucleotide substitution in pseudogenes, the estimate of synonymous substitutions obtained by Miyata and Yasunaga's method increases linearly. This seems to be expected, for Miyata and Yasunaga incorporated the empirically obtained pattern of amino acid changes into their method. For details, see Proceedings of the XIIth International Biometric Conference (Invited papers), pp. 287–297.

Rates of Nucleotide Substitution for Cellular and Viral Oncogenes

Takashi GOJOBORI and Shozo YOKOYAMA[1)]

With the aim of quantitative analyses of evolutionary dynamics of oncogenes, we devised a method of computing the rates of nucleotide substitution for c-*onc*, v-*onc*, and the retrovirus genome simultaneously. Using

[1)] Washington University Medical School.

the method, we compared DNA sequences of an oncogene (v-*mos*) of Moloney murine sarcoma virus (M-MuSV) and its cellular homologue (c-*mos*), and *gag* genes of M-MuSV and Moloney murine leukemia virus (M-MuLV). The estimated rates (per site per year) of nucleotide substitution for c-*mos*, *gag* gene, and v-*mos* are the order of 10^{-9}, 10^{-4}, and 10^{-3}, respectively. The present results imply that v-*oncs* fit in with a general evolutionary feature that the evolutionary rate of the RNA genome can be more than a million times greater than that of the DNA genome because of a high mutation rate in the RNA genome. A quick report for these results has been published in Genetics 107: s39.

Concerted Evolution of the Immunoglobulin V_H Gene Family

Takashi GOJOBORI and Masatoshi NEI[1)]

With the aim of understanding the concerted evolution of the immunoglobulin V_H multigene family, a phylogenetic tree for the DNA sequences of 16 mouse and five human germline genes was constructed. This tree indicates that all genes in this family have undergone substantial evolutionary divergence. The most closely related genes so far identified in the mouse genome seem to have diverged about 6 million years (MY) ago, whereas the most distantly related genes diverged about 300 MY ago. This suggests that gene duplication caused by unequal crossing-over or gene conversion occurs very slowly in this gene family. The rate of occurrence of gene duplication in the V_H gene family has been estimated to be 5×10^{-7} per gene per year, which seems to be at least about 100 times lower than that for the rRNA gene family. This low rate of concerted evolution in the V_H gene family helps retain intergenic variability that in turn contributes to antibody diversity. Because of accumulation of destructive mutations, however, about one-third of the mouse and human V_H genes seem to have become nonfunctional. Many of these pseudogenes have apparently originated recently, but some of them seem to have existed in the genome for more than 10 MY. The rate of nucleotide substitution for the complementarity-determining regions (CDRs) is as high as that of pseudogenes. This suggests that there is virtually no purifying selection operating in the CDRs and that germ line mutations are effectively used for generating antibody diversity. For details, see Mol. Biol. Evol. **1**: 195–212.

[1)] The University of Texas at Houston.

Classification and Measurement of DNA Polymorphism

Masatoshi NEI[1], Fumio TAJIMA[2], and Takashi GOJOBORI

Statistical methods for studying DNA polymorphism by using restriction endonucleases are presented. It is shown that the most fundamental measure of DNA polymorphism is nucleotide diversity (heterozygosity per nucleotide site), and this quantity can be estimated by several different methods. Data on restriction site polymorphisms as well as on nucleotide sequences indicate that nucleotide diversity is generally 0.002 to 0.02 in eukaryotic genes. In the influenza A virus hemagglutinin gene, however, it amounts to 0.51. This high degree of DNA polymorphism is apparently caused by a high rate of mutation in RNA viruses. To evaluate the effects of insertion and deletion, a method of estimating the number of gap nucleotides per nucleotide site is introduced. In some genes such as the alcohol dehydrogenase gene in *Drosophila melanogaster* the effect of insertion and deletion on nucleotide sequence variation is larger than that of nucleotide substitution. Statistical analyses of available data suggest that a large part of DNA polymorphisms in natural populations are caused by mutation and random genetic drift. For details, see Human Population Genetics: The Pittsburgh Symposium (ed. A. Chakravarti), pp. 307–330, Van Nostrand Reinhold Company, New York.

Evolutionary Phylogenies of the *Drosophila montium* Subgroup

Bong K. KIM and Takao K. WATANABE

Ohnishi and Watanabe (1984) constructed a biochemical phylogeny of the *D. montium* subgroup by an electrophoretic technique (2DE). Twenty nine species were classified to three complexes and others. Seventeen species belonging to *D. kikkawai* complex (6 spp), *D. jambulina* complex (4 spp) and *D. auraria* complex (7 spp) were genetically tested by crossing interspecifically. Mating successes during two days mating were examined by dissecting females. Percent of unsuccessful matings between species significantly correlated with the genetic distances estimated by 2DE (r=0.58, d.f.=79).

From the difference of successful matings between pairs of reciprocal

[1] The University of Texas at Houston.
[2] Faculty of Science, Kyushu University.

hybrid matings, we speculated the direction of species evolution in each complex according to Watanabe and Kawanishi (1979); that is females of a derived species do not mate well with the ancestoral males but females of the ancestral species readily mate with derived males. In the *D. kikkawai* complex, species evolved as *kikkawai* → *leontia* → *pennae* → *lini-like* →*lini* → *bocki*. In the *D. auraria* complex, it was *punjabiensis* → *punjabiensis-like* → *jambulina* → *barbarae*. In the *D. auraria* complex, it was *quadraria* → *asahinai* → *rufa* → *subauraria* → *biauraria* → *triauraria* → *auraria*. Ancestral species such as *kikkawai* and *punjabiensis* distributed in the widest area among the *D. kikkawai* complex and the *jambulina* complex. In case of *D. auraria* complex, *rufa* is found in the wide area of Asia, although *quadraria* and *asahinai* are restricted in Taiwan and its near islands. These results support the stasipatric speciation model by Watanabe and Kawanishi (1983) in which old species distribute in wide or restricted area and young species in a small and central area of each sibling group.

Genetic Profile of Wild Mouse Subspecies Collected from South Pacific Islands

Kazuo MORIWAKI, Nobumoto MIYASHITA, Hiromichi YONEKAWA, Tosihide YOSIDA, Tomoko SAGAI, Hitoshi SUZUKI, Yasuyuki KURIHARA and Tomomasa WATANABE*

"The scientific survey on the wild rodents in the Pacific Islands" supported by a Grant-in-Aid for the Overseas Scientific Survey was held from July to August in 1984, in which three members, Dr. Udagawa (Azabu Univ.), T.Y. and K.M., joined. In this survey, we collected a number of mice, *Mus musculus*, in New Caledonia, Fiji and Tonga and analyzed various genetic characters of them. Those are chromosome C-band patterns, antigenic specificities of lymphocytes, RFLPs of mitochondrial DNA and ribosomal DNA, electrophoretic mobilities of hemoglobin-beta, Proteinases and Amylases as summarized in Table 1. In these mice, C-bands of each chromosomes are almost positive as similar as European and Central- and Southeast Asian subspecies, except negative C-bands of No. 16 and X chromosomes. Anti-H-2 monoclonal antibodies, Hd-1, Hd-37 and Hd-38, well reacted with H-2 public specificities in the European subspecies in our previous survey. Hd-39 exhibited much crossreaction with the Asian subspecies.

* Aichi Coloney Research Institute.

Table 1. Genetic characters of wild mice collected from South Pacific Islands

Sample No.	Place of collection	Sex	Chromosome C-bands pattern
1	Port Lagoerre (New Caledonia)	F	1−11+/+, 12+/±, 13−19+/+, X+/±
2	〃	F	1−15+/+, 16+/−, 17−19+/+, X±/±
3	Katiramona (New Caledonia)	M	1−19+/+, X⁻Y
4	〃	M	1−16+/+, 17±/±, 18−19+/+, X⁻Y
5	Lakena (Fiji)	F	1−19+/+, X+/−
6	Longo (Tonga)	F	1−/−, 2+/−, 3+/+, 4+/−, 5−15+/+, 16−/−, 17−19+/+, X+/−

Sample No.	mAb anti-H-2				mtDNA RFLP	rDNA RFLP	Hbb
	Hd-1	Hd-37	Hd-38	Hd-39			
1	weak	3+	+5	—	domesticus-type	domesticus-brevirostris-type	S
2	+3	+4	+5	weak	〃	〃	S
3	—	+4	+5	+3	〃	〃	DS
4	—	+4	+5	—	〃	〃	S
5	+4	+3	+5	—	〃	〃	S
6	+4	weak	+4	—	brevirostris-type	〃	D

Sample No.	Lymphocyte Antigens				Proteinases		Amylase
	Thy-1	Ly-1	Ly-2	Ly-3	Prt-1 & 3	Prt-2	Amy-2
1	2	2	1	2	BB	A	B
2	2	2	1	2	BB	A	B
3	2	2	1	2	BA	A	B
4	2	2	1	2	BB	A	AB
5	2	2	1, 2	2	BA	A	A
6	2	2	1	2	BB	A	A

MtDNA and rDNA mostly demonstrated *M. m. domesticus*-nature of those mice. Hbb^s allele is restricted to the European subspecies. All the lymphocyte antigenic specificities, Proteinases (Prt-1 & 3 and Prt-2) and Amylase (Amy-2) were European type. Thus, wild mouse subspecies in South Pacific Islands here examined is almost similar to the European sub-

species *M. m. domesticus*, though those in Tonga may closely related to *M. m. brevirostris*. We could have hardly found the effect of Southeast Asian subspecies, *M. m. castaneus* in these area.

Allelic Constitution of the Hemoglobin Beta Chain in Wild Populations of the House Mouse, *Mus musculus*

Nobumoto MIYASHITA, Shunsuke MIGITA* and Kazuo MORIWAKI

We surveyed the allelic constitution of the hemoglobin beta chain locus in the wild populations of house mouse in Asia and surrounding areas. A total of 222 mice at 45 localities was examined to the electrophoretic analysis (Table 1).

One hundred seventeen mice were collected in 22 localities in Asia excluding Japan. In these populations, Hbb^p and hbb^d were common although their frequency distribution was rather heterogeneous. In seven localities in northern China and two localities in Korea, solely Hbb^p homozygotes were observed. Both Hbb^p and Hbb^d were common in the other eight localities in Asia. In five localities of Southeast Asia and Sri Lanka, only Hbb^d was observed.

Sixty-three wild mice were caught in 16 localities in Japan. Three phenotypes, Hbb-P, -PD and -D, were observed in the wild populations of

Table 1. The allelic frequency of the Hbb locus in wild subspecies of *Mus musculus*

	No. of mice	Allelic frequency		
		p	d	s
Subspecies				
M. m. molossinus	63	0.86	0.14	0.00
M. m. castaneus	23	0.33	0.67	0.00
M. m. urbanus	10	0.35	0.65	0.00
M. m. bactrianus	7	0.57	0.42	0.00
(unidentified)				
Central and northern China, and Korea	58	1.00	0.00	0.00
China, coastal areas (Beijing and Shanghai)	19	0.66	0.34	0.00
Chichi Island (Ogasawara Is.)	18	0.00	0.28	0.72
Australia (Sydney) and South Pacific Is.	12	0.00	0.08	0.92
Seychelles and Mauritius	12	0.00	0.46	0.54

* Cancer Research Institute, Kanazawa University.

Japanese house mouse, *Mus musculus molossinus*, as well as Asian house mouse subspecies. The overall allelic frequencies of Hbb^p and Hbb^d were 86 and 14%, respectively. Moreover, interregional differences in these frequencies have been observed. Geographically, the Hbb^p allele was prevalent in the central part of Japan, while the Hbb^d was common in the peripheral regions.

Forty-two wild mice were collected from Chichi Island (the Ogasawara Is., Japan), Sydney (Australia), Numea (New Caledonia), Suva (Fiji), Nuku'alofa (Tonga), Mahe Island (Seychelles) and Réduit (Mauritius), in which only three phenotypes, Hbb-S, -SD and -D were identified. The Hbb phenotypes of these wild mice were similar to those of European wild mice, *M. m. domesticus* and *M. m. brevirostris* (Miyashita *et al.*, *Biochem. Genet.* 23: 975–986 (1985)).

Restriction Fragment Length Polymorphism of rDNA in European Wild Mice with Robertsonian Translocations

Yasuyuki KURIHARA, Hitoshi SUZUKI, Kazuo MORIWAKI, Heinz WINKING[1], Ryo KOMINAMI[2] and Masami MURAMATSU[2]

Genetic diversity of ribosomal DNA (rDNA) in wild mice (*Mus musculus*) with Robertsonian translocations (Rb) was estimated by southern blot analysis using 18S- and 28S rDNA probes, and the data were compared with our previous study (Ann. Rep. Natl. Inst. Genet. 33: 26, 1982) about restriction fragment length polymorphism of rDNA in various mouse subspecies. The comparison revealed that restriction patterns could be interpreted as a mixture between two types; one of which is found in Asian subspecies and the other is European subspecies. For example, CB mice from central Italy exhibited a 5.5 kb band, specific to *M. m. brevirostris* from France, and a 9.0 kb band, characteristic of Asian mice, when hybridized with the 28S rDNA probe after EcoR I digestion. Moreover, Zadar mice from Yugoslavia exhibited a 2.3 kb band, characteristic of European mice, and a band over 20 kb, characteristic of Asian mice, when hybridized with 28S rDNA probe after BamH I digestion. Similar results were obtained in the other Rb-mice. Although more detailed works are desired, we may assume that a genetic introgression from Asian subspecies could have

[1] Medizinische Hochschule Lübeck, Lübeck.
[2] Tokyo University.

occurred in European subspecies.

On the other hand, rDNA clusters which are cytologically identified as NORs are generally located at centromeric regions on several chromosomes in *Mus musculus*. But, exceptionally, in some Rb-mice named WMP and Cremona (Rb-mice^t), one of the rDNA clusters is located in the telomeric region of chromosome No. 4. We have also studied these Rb-mice^t in the same way: commonly in Rb-mice^t, our 18S rDNA probe hybridized with an 8.7 kb band after Pvu II digestion, and our 28S rDNA probe hybridized with an 18- and a 14 kb bands after Hind III digestion. These bands were not observed in the other Rb-mice. Conceivably, the structures of the telomeric rDNA units are largely different from those of the centromeric rDNA units.

A 5′-end Sequence of the Fibroin Gene from Wild Mulberry silkworm, *Bombyx mandarina*

Jun KUSUDA, Osamu NINAKI, Yoshiaki SUZUKI
and Yataro TAZIMA

Morphological and cytological studies have suggested that *Bombyx mandarina* is a possible ancestor of domesticated silkworm, *B. mori*. In order to elucidate their evolutionary relationship, the structures of their fibroin genes were compared. The fibroin genes of *B. mori* have already been cloned from some strains and the sequences around 5′-ends are known. We constructed the DNA library of *B. mandarina* DNA and screened its fibroin gene hybidizing with the cloned DNA containing the fibroin gene of *B. mori*. One clone (λMa3) contained 14.5 Kb *B. mandarina* DNA and the insert was cut out for three fragments with BamHI; 5.1 Kb, 4.9 Kb and 3.5 Kb.

Since the probe of *B. mori* hybridized only with the 4.9 Kb fragment, the region inserted to vector seems to be the end of fibroin gene or the junction of exon-intron. To analyse this region precisely, 4.9 Kb BamHI fragment was recloned into pUC12. One clone (pMaf7) was cleaved with several restriction enzymes and the physical map was constructed. The restriction sites resembled those around 5′-end of fibroin gene of *B. mori*. A 2.6 Kb fragment corresponding 5′-end of *B. mori* was sequenced by M13 vector-dideoxy method. The nucleotide sequences of both fibroin genes were almost identical except for some base exchanges and deletions or insertions.

Comparison of the base exchanges of *B. mandarina* with those of *B. mori* strains revealed that *B. mandarina* is closer to Chinese bivoltine strains than European univoltine strains. These results support the idea that *B. mandarina* is a closely related species to *B. mori* and a probable proto-type of domesticated silkworm.

IX. HUMAN GENETICS

Use of Retinoblastoma and Wilms' Tumor as Sentinel Phenotypes for Population Surveillance

Ei MATSUNAGA and Kensei MINODA*

A vast majority of retinoblastoma (RB) and Wilms' tumor (WT) occur sporadically, and they are generally arising from a germinal or somatic mutation. Although the two tumors are not usually detectable at birth, in countries where systematic registration of childhood malignancies has been established, data on the incidence of the two tumors may readily be used for population surveillance of environmental mutagens.

In Japan, the ongoing nation-wide registration of all childhood malignancies is supported by a private foundation and maintained by voluntary cooperation of physicians. The current data, however, are not satisfactory because of underreporting (Hanawa, Y. All Japan Children's Cancer Registration, 1969–73. Children's Cancer Assoc. Japan, Tokyo, 1975), with the exception of those for RB reported in the initial stage of registration (Minoda, K. 1976. Acta Soc. Ophthalmol. Jpn. **80**: 1648). On the other hand, fairly reliable data at the prefectural level are available in Kanagawa, where the ascertainment is made by reviewing the application form submitted by the patients to the local government for defraying the cost of medical treatment (Nishihira, K. 1983. Kanagawa Children's Med. Center Jour. **12**: 62); since 1971 childhood malignancies have been among a number of "designated diseases" for which, upon application, such assistance is rendered by central and local governments. From these data the live-birth frequencies of RB and WT are estimated to be about 1: 15,000 and 1: 20,000 in Japan, respectively.

With approximately 1.5 million births a year, the annual number of new cases of the two tumors combined would be about 175; if this is taken as a standard, then the border-line above which a rise in the incidence is statistically significant at the 1% level would be 210. If a significant rise in the incidence of sporadic cases could be detected, a case-control study has to be undertaken. In bilateral cases either paternal or maternal exposure prior

* Tokyo Metropolitan Yoiku-In Hospital.

to the conception of the index child should be suspected, whereas in unilateral cases the child's exposure before or after birth should be suspected. The Ministry of Health and Welfare should encourage and promote the regional registration of childhood malignancies on the population basis.

Mitochondrial DNA Polymorphism in Japanese I. Analysis with Restriction Enzymes of Six Base Pair Recognition

Satoshi HORAI, Takashi GOJOBORI and Ei MATSUNAGA

Since Potter *et al.* (1975) showed that a restriction enzyme can detect variability in mammalian mitochondrial DNA (mtDNA) sequences, restriction enzyme analysis has become a powerful tool for detecting genetic variations in human (Brown and Goodman 1979; Brown, 1980). This paper deals with the results of analysis of 120 mtDNAs from Japanese digested with 15 restriction enzymes that recognize six base pairs.

The digestion patterns with HincII and HaeII are highly polymorphic. Seven distinct cleavage patterns were observed for the HincII digestion and five different patterns were found for the HaeII digestion. In addition EcoRV was found useful to detect polymorphism in our sample, because 5% of individuals showed an atypical cleavage pattern. In digestions with StuI, HindIII, ScaI and XhoI, three different cleavage patterns were observed for each enzyme respectively. While StuI and ScaI were not examined in previous studies, digestion patterns with HindIII, XhoI, SacI, EcoRI and PvuII were reported to be monomorphic (Brown, 1980). In the present samples, an atypical cleavage pattern was observed for each of SacI, EcoRI and PvuII. The analysis with four additional restriction enzymes, BamHI, XbaI, KpnI and DraI showed monomorphic patterns in all samples.

Based on the combination restriction enzyme morphs observed for each of the 120 individuals, we found 22 distinct mtDNA restriction types. Seventy-two percent of the individuals showed the cleavage pattern identical to that of the published mtDNA sequence except the HincII and StuI digestions (type 1). Although other 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 comparisons (Nei and Li, 1979). In the present study, an average of 62 sites (equivalent to 372 base pairs, or 2.2% of the

whole mtDNA sequence) could be compared. Estimating the number (δ) of nucleotide substitutions per nucleotide site between any two types, we obtained the mean value of 0.0042. Because we used restriction enzymes that recognize six base pairs, a nucleotide substitution by site gain can be easily deduced from the published sequence. In the present study 11 site gains were observed, of which 8 occurred in the protein coding regions, resulting in amino acid substitution in at least 4 cases. Of the 11, 7 were transitions and 4 were transversions. The apparent ratio of transitions to transversions if 1.75: 1, which is slightly less than the ratio (2.5: 1) obtained by Cann *et al.* (1984). These are, however, significantly lower than the ratio obtained by sequence comparisons among higher primates mtDNAs (11: 1, Brown *et al.* 1982) and among a 900 base pair segment of the non-coding region from several human mtDNAs (32: 1, Aquadro and Greenberg 1983). A phylogenetic tree was constructed by genetic distances among the 22 restriction types, suggesting that the Japanese population conceals a considerably high degree of mtDNA diversity. For details, see Human Genetics **68**: 324–332.

High-resolution Studies in Patients with Aniridia-Wilms Tumor Association

Y. NAKAGOME[1,2] and N. NAGAHARA[3]

In a recent issue of Human Genetics, we described results of high-resolution studies in patients with aniridia-Wilms tumor association and a few related conditions (Nakagome *et al.* Hum. Genet. **67**: 245, 1984). It included a boy with aniridia-Wilms tumor association (case 2545) with 11p deletion. The mother (case 2580) also had an apparently identical 11p deletion and no insertion could be detected at that time. Both the father and maternal grand mother had a normal karyotype.

Recently, we have had an opportunity to examine phenotypically normal sister as well as to re-examine the mother by the acridine-orange high-resolution technique. Both were balanced carriers of an insertion between a no. 11 and a no. 12, karyotype being 46, XX, ins (12; 11) (p. 11. 2; p. 12 p. 14.2) (Nakagome & Nagahara: Hum. Genet. in press).

[1] Dept. Hum. Genet., Nat. Inst. Genet., Mishima.
[2] Dept. Cong. Abn. Res., Nat. Child. Med. Res. Ctr., Tokyo.
[3] Dept. Surgery, Osaka Child. Med. Ctr., Osaka.

High-resolution Study in Patients with Prader-Willi Syndrome

Y. NAKAGOME[1,2], T. TAKANO[3], S. NAGAHUCHI[4], I. HIBI[4],
F. TANAKA[5], Y. NAKAMURA[6] and A. TANAE[4]

For many years, Prader-Willi syndrome has remained to be a typical example of malformation syndrome with unknown etiology. In increasing number of cases chromosome abnormalities involving proximal 15q have been described. However, in still considerable number of cases chromosome abnormality remains undetected. The lack of resolution in cytogenetic analysis is one possibility. The other is variation of criteria in the clinical diagnosis of the syndrome.

In the present study, 27 clinically diagnosed cases of Prader-Willi syndrome were studied by the technique of acridine-orange high-resolution banding (Matsubara and Nakagome, Cytogenet. Cell Genet. **35**: 148, 1983). In one case, number of mitotic cells was not satisfactory. In 6 cases, karyotype analysis was possible at or lower than 400-band per haploid-set level. In the remaining 20 cases, chromosome analysis was carried out at about 550-band per haploid set or higher. In 14 of them, small deletion involving proximal 15q is detected. In the remaing 6, no delection was detected. Clinical evaluation of patients in the two groups is in the progress to detect difference, if any, between them.

Origin of Restriction Sites in a Simple Repeated DNA Cloned from Human Y Chromosome

Yutaka NAKAHORI* and Yasuo NAKAGOME*

A DNA fragment was cloned from EcoRI-digested and gel-electrophoresed DNA from a normal man. It was 3.5 kb in length, highly repeated and male specific (Nakahori and Nakagome, this report, No. 34). Restriction analysis revealed that there were enzymes which cleaved it at many sites

1) Dept. Cong. Abn. Res., Nat. Child. Med. Res. Ctr., Tokyo.
2) Dept. Hum. Genet. Nat. Inst. Genet., Mishima.
3) Dept. Hum. Genet., Tsukuba Univ.
4) Nat. Child. Hosp., Tokyo.
5) Dept. Pathol., Univ. Tokyo.
6) Dept. Pediat., Metropolitan Huchu Hosp., Tokyo.
* Dept. Hum. Genet., Nat. Inst. Genet., Mishima and Dept. Cong. Abn. Res., Nat. Child. Med. Res. Center, Tokyo

Table 1. Number of restriction sites in pHY6

4bp[1]		5bp		6bp	
TaqI[2]	many (1)	Hinf I	many (1)	EcoRI	2 (2)
Rsa I	4 (2)			BanIII	1 (2)
Sau3AI	2 (2)				
HaeIII	1 (2)	DdeI	1 (3)	BamHI	0 (3)
HpaII	0 (2)			SalI	0 (3)
				AatI	0 (4)
AluI	0 (3)			BanII	0 (4)

1) Length of recognition sequence.

2) For each enzyme, observed number of sites in pHY6 and number of base substitutions needed to generate them (in parenthesis), are shown. There is no EcoRI site within the fragment but both ends of it are marked with them.

(Taq I and Hinf I), 4 sites (Rsa I), two sites (Sau 3AI and Eco RI; at both ends) and one site (Hae III, Dde I and Ban III). No site was detected as to Hpa II, Alu I, Bam HI, Sal I, Aat I and Ban II (Table 1).

A small segment of the fragment was sequenced by the dideoxy method using M13 phage. It was basically composed of repeat of a 5-base-pair unit TTCCA.

Assuming (TTCCA)n to be the original sequence of the entire fragment, number of base substitution(s) necessary to generate each type of site in the table 1 was counted and was also included in the table (in parenthesis). Only two types of restriction sites, TCGA (Taq I) and GANTC (Hinf I), were possible to occur through a single base change. They can be generated in an overlapped form within the (TTCCA)n tract by a single base substitution; from TCCATTC to TCGATTC. It is noteworthy that they were frequently detected on the fragment. There are other 4- or 5-base pair sequences which can be generated by a single base change, however, none of them represented to recognition sites of known restriction enzymes. Five of six restriction sites that can be generated by the substitution of two base pairs were actually detected in the fragment (Table 1). None of the available enzymes that needed three or more base substitution actually cut the fragment with the exception of Dde I.

Possible mechanisms for all observed restriction sites are summarized in the Table 2. Among the two-base change sites, it is expected that either Rsa I or Sau 3AI has better chance of cutting it than either of Hae III or Hpa II as the former two can be generated through two different mechanisms.

Table 2. Possible restriction sites in a (TTCCA)n tract by one- or two-base substitutions

Original sequences	Generated restriction sites	Enzymes	Base changes needed		
			transversion I	transition	transversion II
TCCA[1]	TCgA[2]	Taq I	C—G[3]		
CATTC	gATTC	Hinf I	C—G		
TTCC	gTaC	Rsa I[4]			T—G, C—A
ATTC	gTaC	Rsa I[4]	T—A	A—G	
ATTC	gaTC	Sau3AI[4]	T—A	A—G	
CATT	gATc	Sau3AI[4]	C—G	T—C	
TTCC	ggCC	Hae III			T—G, T—G
CCAT	CCgg	Hpa II		A—G	T—G
CCATTC	gaATTC	EcoRI	C—G		C—A
TTCCAT	aTCgAT	Ban III	T—A, C—G		
CCATTC	gCATgC	Sph I	C—G		T—G

[1] A segment of a tract (TTCCA)n. [2] Eleven cleavage sites for 9 different enzymes are shown. Substituted bases are represented in a small letters. [3] C—G denotes a C in the original sequence was replaced with a G. [4] An identical site can be generated by 2 different mechanisms.

This was the case as shown in the Table 1. Out of three Rsa I sites sequenced, two were due to the one shown on the 3rd line and the other was on the 4th (Table 2).

As a whole, observed number of sites is in agreement with expectations if we assume that the fragment started as (TTCCA)n and they have been generated more or less randomly. Further sequencing of the fragment is in progress.

X. BEHAVIORAL GENETICS

Effects of Feeding Experience before Weaning on Food Preference in Mice

Tohru FUJISHIMA

Clarifying the mechanism controlling the food preference of animals will be meaningful not only for animal husbandry but for human beings. The study has been carried out to investigate the roles of genetical and environmental factors in determining the food preference of mice. The present study was conducted to determine which time period in development (if any) was most effective in controlling the food preference of adult mice.

Three groups of male inbred mice, each having been reared with one of three conventional foods (A, B and C), were divided into three sub-groups at weaning time of 21 days of age, respectively. Animals in each sub-group were fed one of the three foods from weaning to the food preference test. Individual food preferences were assessed with the cafeteria feeding test of the three foods for over 60-day-old mice. In the preference test the weekly amounts of each consumption of the three foods were measured during the 4-week test period.

Food consumption in the preference test was highest in B food and lowest in C food in all the sub-groups, no matter what food mice had been fed until the test time. This may be suggestive of the fact that mice used in the present experiment genetically prefer B food best of the three foods. However, the ratio of each food consumption to the total amounts of food ingested during the test period was highest in the food of all the groups with which mice had been reared before weaning, suggesting that feeding experience before weaning, or nursing period, plays the most important environmental role in determining the food preference of adult mice. The extents of genetical and environmental factors involved in the determination of the food preference of mice were also estimated with the analysis of variance technique. They were 29.3% for genetical and 70.7% for environmental factors. In the environmental factors the factor due to before-weaning feeding was 60.7%.

Effects of Wave Length of Light on Sexual Maturity in Female Japanese Quails

Tohru FUJISHIMA and Masami SAITO

Environmental light influences a number of physiological mechanisms in birds, including reproductive development, growth and endocrine function. The mechanism by which light acts on the reproductive system has not been resolved. It has been only demonstrated that the long wave length of light accelerates sexual maturity in birds, while the short wave length has no such an effect. This study has been conducted to genetically approach resolving the mechanism of light affecting sexual maturity through the nervous system in the Japanese quail.

Four replicate experiments with 250 Japanese quails in total were conducted to obtain the preliminary information on the selective experiment for the sexual maturity of quails under different colored-light conditions. Female birds were housed in battery brooders under a white light condition for 5 weeks after hatching and then randomly allotted to three light-controlled chambers. The light environments were 16L: 8D white (Toshiba white fluorescent lamp, FL10D-EDL-56K), 16L: 8D red (National pure-red colored fluorescent lamp, FL40S R-F, 9 lux, 590–700 nm) and 16L: 8D blue (National pure-blue colored fluorescent lamp FL40S S-F, 9 lux, 380–540 nm). During the experimental period, the age in days at first egg was measured for each bird in each group.

The age at first egg was 55.3 for the white, 55.7 for the red and 72.5 for the blue light groups. Female quails kept under blue light had significantly lower sexual maturity than birds kept under red or white light ($P<.01$). There were no familial correlations of the age at first egg between different colored-light groups, suggesting that the mechanism of light affecting sexual maturity in quails differs with different wave lengths of light. Significant differences in sexual maturity among families were found in the white and the red light groups, but not in the blue light group.

XI. ECOLOGICAL GENETICS

Monitoring of the Wild-Rice Populations in Thailand

Hiroko MORISHIMA, Yoshiya SHIMAMOTO*, Yoshio SANO
and Yo-Ichiro SATO

Effect of environmental stress on population dynamics has been a central theme of population biology. Little is known about wild-rice populations. Environmental constraints for survival of the Asian common wild rice *Oryza perennis* (=*O. rufipogon*) might be water stress, habitat disturbance and gene flow from cultivated rice growing nearby. To make a long-term observation for a demographic-genetic study, seven study-sites of wild rice populations were designated in the suburb of Bangkok during our study-tour in 1983, some of which were repeatedly observed since 1973. Those sites were revisited in June and August in 1984, and January in 1985. Various items regarding life-history characters and habitat conditions were recorded. Preliminary results on plant demography and population flux are summarized below. Genetic change in population structure associated with demographic process is reported elsewhere in this issue (Barbier and Morishima).

a) By checking juvenile plants, it was confirmed that four populations propagate mainly by seeds (annual type, sexual reproduction), one population by ratoons (perennial type, asexual reproduction), and two populations both by seeds and ratoons (intermediate or mixed type). b) Annual types are essentially inhabitants of shallow swamps which are parched in the dry season, while perennial types are found in the sites which retain soil moisture during the dry season. The sites inhabited by the intermediate types are characterized by strong habitat disturbance and introgression from cultivated rice. c) June and August census indicated that high mortality occurred at the juvenile stage in seedlings as well as in ratooned tillers. d) Annual populations were fugitive. During our observation period, one population became almost extinct showing decrease in genic diversity possibly due to change in water regime in the area. Another population rapidly expanded on a huge area after destruction of its vegetation. e) In the inter-

* Hokkaido University.

mediate populations, a trend of habitat segregation between seedlings and ratoons was often recognized on a fine scale. This could be explained by environmental heterogeneity within a site.

Together with the results of our general survey on wild and cultivated rices, details of the above study-sites are given in our Trip Report "Observation on Wild and Cultivated Rices in Thailand for Ecological-Genetic Study" pp. 82, 1984).

Comparison between Potential and Realized Genetic Variability in the Wild-Rice Populations with Different Life-Histories

Pascale BARBIER* and Hiroko MORISHIMA

Populations of Asian wild rice *Oryza perennis* (=*O. rufipogon*) show a continuous variation in the degree of perenniality and associated characters. Seven populations (4 annual, 1 perennial and 2 intermediate types) found in the suburb of Bangkok are continually studied to monitor their demography, genetic structure and population flux. In the present study, plants collected from these populations were examined to compare genetic structure of population at different stages of life cycle. In each population, the plants examined consisted of two groups sampled in the different seasons: The first group was raised from seeds collected at maturity stage (December 1983, early dry season), and the second group from seedlings and/or ratoons collected at juvenile stage (June 1984, early rainy season). The former is considered to represent potentially available variability and the latter as actually realized variability.

Genotypes at 6 isozyme loci (*Pox-1*, *Sdh-1*, *Est-2*, *Pgi-1*, *Pgi-2*, *Pgd-1*) and several life-history characters (reproductive allocation, regenerating ability, anther length, awn length, flowering time) were recorded on an individual basis. Average genic diversity was calculated for each sample using allozyme data. In the populations raised from seeds, the annual types showed a low genic diversity mainly due to fixation of alleles at many loci, while the perennial and intermediate type showed higher genic diversity with high heterozygosity. When compared with this potential variability, significant drops in the level of genic diversity were found in realized variability observed in juvenile-derived populations for the perennial and some

* Université des Sciences et Techniques du Langudoc, Montpellier.

annual types. This might be because the perennial types propagate mainly by vegetative means though they are highly allogamous, while the annual types are predominantly selfing and propagate by seeds. The intermediate types showed high genic diversity in realized as well as in potential variability. This can be explained by their partial allogamy combined with mixed mode of sexual and vegetative reproduction. Such intermediate type could have a high evolutionary potential.

XII. APPLIED GENETICS

Genetic Diversity in Indonesian Native Rice Cultivars with Reference to the Indica-Japonica Differentiation

L. U. GADRINAB*, Y. I. SATO and H. MORISHIMA

Asian rice cultivars are known to be differentiated into the Indica and Japonica types. However, there is very meager information on the Indonesian rices in this regard. Two-hundred thirty seven accessions collected in Java, Kalimantan and Sumatra were investigated to examine whether or not they were differentiated into these two types. Three diagnostic characters, $KClO_3$ resistance, phenol reaction and apiculus hair length, were examined for each accession. A bimodal distribution of the discriminant scores combining the above three characters showed a variation ranging from the Indica to Japonica types, though accessions with Japonica-specific characteristics were more frequent. Based on this score, 160 accessions were classified as the Japonica type and 77 as the Indica type. Relative frequencies of the two types did not differ much according to the water regime in the fields; 68% of upland and 59% of lowland varieties were Japonica types. A trend of differentiation into two types was observed similarly in the materials collected in different islands, although number of accessions collected in Sumatra and Kalimantan was not many.

An electrophoretic survey of 6 isozyme loci of 5 enzyme species was carried out. All loci assayed were polymorphic and their allelic frequencies significantly differed between the Indica and Japonica types except for one locus (*Est-2*). Among 96 possible genotypes expected from the allelic combinations at 6 loci, 33 genotypes were found. A genotype with alleles 2 (*Cat-1*), +9 (*Acp-1*), 2 (*Pgi-1*), 0 (*Pox-2*) and 1 (*Pgi-2*) was most predominant in the present materials (68%). Most accessions classified as the Japonica type were found to have this or its similar genotype. Among those classified as the Indica type, in addition to this same genotype, contrasting genotypes with different alleles at most loci were frequently found. It was found, further, that in allozyme variation lowland rices showed higher varietal diversity than upland rices, and that in lowland as well as in upland rices

* BIOTROP, Indonesia.

the Indicas were more diverse than the Japonicas.

Frequency Changes in Isozyme Genes Observed in a Hybrid Population Derived from a Cross between an Indica and a Japonica Rice Varieties

Reiko SANO and Hiroko MORISHIMA

Studies of multilocus polymorphism for isozymes in Asian rice cultivars revealed that two major varietal groups, the Indica and Japonica types, tend to have different allelic combinations for several loci. It is not known, however, how such non-random association in isozymes was established in Asian rices. To look into this problem, genotypes of 6 isozyme loci whose gene frequencies significantly differ between the Indica and Japonica types were examined in a hybrid population derived from a cross between an Indica (130, Taiwan) and a Japonica (221, the Philippines) varieties. Plants examined consisted of two F_7 populations raised from F_6 populations which were established in F_3 and thereafter separately maintained in bulk without conscious selection in Japan as well as in the Philippines. The loci assayed were *Cat-1*, *Acp-1*, *Pgi-1*, *Pgi-2*, *Pox-2* and *Est-2*, and the parents carry different alleles at all these loci. Among these 6 loci, *Est-2* and *Pgi-2* are linked with 14% recombination and *Acp-1* and *Pox-2* with 31%, and *Cat-1* and *Pgi-1* seem to be independent of each other as well as of the above 4 genes.

The results obtained are summarized as follows: 1) When compared with F_3, frequencies of alleles derived from an Indica parent increased consistently at all loci examined. 2) Nonrandom associations between independent loci (parental as well as recombined types) were detected in some cases. 3) Increasing trend of parental genotypes at the cost of recombined genotypes was not observed. 4) Two populations maintained in Japan and the Philippines showed similar trends. Thus, an increasing trend of allelic frequencies of genes derived from the Indica parent, which is often observed in the Indica-Japonica crosses, was clearly demonstrated. But, mechanisms which brought about differentiation into the Indica- and Japonica-specific isozyme genotypes could not be detected in the scope of this experiment.

Cytoplasm Substitution between an Indica Strain of *Oryza sativa* and *O. glaberrima*

Yoshio SANO

To look into differential nucleus-cytoplasm interactions between the two cultivated rice species, *Oryza sativa* and *O. glaberrima*, cytoplasmic substitution lines have been made by using a *glaberrima* strain (G) and an Indica strain of *sativa* (B). The G cytoplasm had no adverse effect on pollen development when combined with the nucleus of S. On the other hand, when the S cytoplasm was combined with the G nucleus, the substitution line showed no seed set due to male sterility although the pollen grains were normally stained with I_2-KI solution. A dominant gene derived from S strain seemed to cause anther indehiscence in the substitution line. Further, a restorer gene (Rf_j) from Akebono of Japonica type was effective on pollen restoration in the male sterile line, suggesting that the S cytoplasm is the same as those of Japonica type in terms of a fertility-restoring system. (Euphytica in press)

Correlations between the Amounts of Amylose and *Wx* Protein in Rice Endosperm

Yoshio SANO, Mitsuko KATSUMATA and Etsuo AMANO

Amylose content and *Wx* protein level were analyzed in 25 nonwaxy rice cultivars and in F_2 endosperms of a cross of 74–5 (dull)×T65*wx* (glutinous) in order to look into the relationship between the two traits. Regression analysis indicated that 69% (among 25 cultivars) and 77% (in F_2) of the variation in amylose content were associated with the variation in the *Wx* protein level. This implied that in rice endosperm amylose content increases as the *Wx* protein increases. Further, the difference in amylose content between Indica and Japonica types appeared to be well explained by the allelic difference at the waxy locus. (SABRAO J. in press)

Varietal Variations in Basic Vegetative Phase and Photosensitive Phase, and Genetic Control of Basic Vegetative Phase in Japanese Native Cultivars

Yo-Ichiro SATO

Heading time of rice plant is an important character from evolutionary

as well as from agronomic standpoints. The number of days from seeding to heading is considered to be determined mainly by the lengths of the photosensitive phase (PSP) and basic vegetative phase (BVP). In order to look into the origin and dissemination of early-maturing cultivars in Japan, the variations in the above two phases were examined among 93 native cultivars collected from various localities in Japan, and allelism test of the genes controlling the length of basic vegetative phase was carried out. They were grown under a shortday (30°C phytotoron in winter) and a longday (a paddy field, in summer) conditions. The lengths of BVP and PSP were estimated for each cultivar by the number of days to heading in shortday condition and by the difference in the days to heading between longday and shortday conditions, respectively. The cultivars tested were divisible into 2 groups with a long (75–95 days) and a short (38–75 days) BVP. Among the cultivars with short BVPs, the distribution of PSP, varying from 0 to 72 days, was trimodal. Among those collected from areas north of 32°N in latitude, PSP decreased with increasing latitude of collection site. But no correlation between PSP and latitude was found among cultivars from the southern islands. The early-heading cultivars (with short BVP and PSP) were found throughout Japan although they were infrequent. The pattern of distribution of native rice cultivars with varying BVP and PSP periods seems to reflect the variations in natural environments and cropping system under which they have been cultivated so far.

The early-heading cultivars were divisible into 2 groups, early-A (extremely early) and early-B (about 10 days later than early-A). To test allelic relationship of genes carried by Japanese early cultivars with a known earliness gene which shortens BVP (*Ef-1*^{b}), twelve early cultivars were each crossed with a test-strain carrying *Ef-1*^{b}, and a modifier (*m*) emphasizing its effect. Segregation patterns of heading date in F_2 showed that the early-A types carried *Ef-1*^{b}, and *m* and/or another modifier with stronger effect than *m*. Many of the early-B types possessed the same genes as of the test-strain (*Ef-1*^{b} *m*), but a few others seemed to carry a different earliness gene. The *Ef-1*^{b} gene was found in many of early-maturing cultivars distributed in different localities of Japan. This suggests that those cultivars were differentiated in an early period of diversification of rice in Japan. (Japan. J. Breed. **35**: 72–75, and **35**: 160–166).

Genetic Control of Apiculus Hair Length in Rice

Yo-Ichiro SATO

The length of apiculus hair is known to serve as an Indica-Japonica discriminating character as it is longer in Japonica than in Indica. No major genes have been yet reported for this character except for *gl-1* and *gl-2* for the absence of hair or glabrousness. The mode of inheritance of this character was studied in randomly selected F_3 plants and their derivative lines from an Indica×Japonica cross. The parents used are f. 145 (Indica, black hull, apiculus hair 0.32 mm) and T65 (Japonica, non-black hull, apiculus hair 0.90 mm).

The F_4 plants derived from 15 F_3 with apiculus hairs shorter than 0.7 mm showed an apiculus hair length ranging from 0.1 to 0.7 mm. Almost all F_4 plants derived from 14 F_3 plants with apiculus hair longer than 0.7 mm had the same range of hair length as of the F_3 plants. The F_4 plants derived from other 7 F_3 plants with similarly long apiculus hair segregated into 2 classes of apiculus hair length, shorter and longer than 0.7 mm. The numbers of plants with short and long apiculus hair were 37: 96, which fitted the 1: 3 ratio expected when monogenic segregation was assumed (χ^2=0.56, non-significant). The numbers of F_4 lines with all long, all short and segregating apiculus hair were 14, 15 and 7, respectively, which appeared to fit a 3: 3: 2 ratio (χ^2=0.63, non-significant). These data suggested that the apiculus hair length is controlled by a major gene, although there are other modifier genes. Tentatively this gene is symbolized as *Aph*/*aph* (t).

Of 21 F_3 plants with long (longer than 0.7 mm) apiculus hair, only one had the black hull, whereas 8 out of 15 F_3 plants with short (shorter than 0.7 mm) apiculus hair showed black hull. Since the black hull is controlled by 3 genes, *Ph*, *Bh-a* and *Bh-b* gene, *aph* would be linked with one of the three genes. Among 26 F_3 plants with yellow hull, 18 had *Ph* and 9 of them had apiculus hairs longer than 0.7 mm and the other 9 had shorter apiculus hairs. Therefore, the *aph* gene does not seem to be linked with *Ph*. It would linked with either *Bh-a* or *Bh-b*.

Two-dimensional Gel Electrophoresis of Four Kinds of Fractionated Reserve Proteins in Rice Endosperm

Toru ENDO

Four kinds of protein molecular species, i.e., albumin, globulin, prolamin and glutelin, were sequentially extracted from rice endosperm, where their yields for 10 g of the powdered endosperm (150 mesh) were, in one case, 14 mg, 202 mg, 19 mg and 393 mg, respectively. Extraction of glutelin was carried out as follows: endosperm was macerated with ten volume of 0.1 M NaOH for 15 min. and centrifuged. The supernatant was neutralized with 1 M ascorbic acid and 0.1 M 2-mercaptoethanol to about pH 6.5. Most of glutelin fraction was obtained as precipitate. The dry precipitate was found to be soluble in 8 M urea solution. Other kinds of neutralization procedures produced an insoluble form in urea solution.

One or two hundred micrograms of each protein fraction were applied to O'Farrell's system (1975 and 1977), where albumin and glutelin were analysed with equilibrated pH gradient IEF method, because most polypeptide components of both the fractions were in acidic isoelectric points. Also, globulin and prolamin were applied to non-equilibrated method, because of their alkalinity in their major components. All polypeptide maps were obtained by silver staining method due to Oakley *et al.* (1980).

Although the number and the configuration of polypeptide spots in the maps are not completely reproducible at present, there are detected more than 40 spots in albumin fraction, 30 in globulin, 20 in prolamin and 15 in glutelin. It was noted that the albumin fraction showed the maximum number of spots though the total amount extracted was the least and, in contrast, the glutelin fraction produced the minimum number despite being the most abundant in rice endosperm.

Sample Size and Number of Loci to Investigate for Estimating Effective Population Number

Shin-ya IYAMA

Based on the well-known theoretical relationship between genetic drift and population size, effective population number of fish populations in hatchery was estimated from the amount of genetic drift based on the frequencies of various isozyme loci (p. 88, Ann. Rep. 1982).

For the estimation, frequencies of various isozyme markers were estimated on the basis of N_s individuals sampled from the population and the variance of gene frequency (V_d) was calculated on the basis of those of n_1 loci (x_i) after standardized with the initial gene frequencies (p_i):

$$d_i=(x_i-p_i)/p_i(1-p_i) .$$

The range of confidence at 95% probability for estimated N_e was calculated using the confidence intervals of estimated variance V_d'

$$Sd_i^2/\chi^2_{.025}<V_d<Sd_i^2/\chi^2_{.975} ,$$

Numerical calculation of the intervals with various N_s and n_1 revealed that width of the range is dependent on the sample size N_s and number of loci examined (n_1). Results of computation showed that more than ten loci at least may be desired for estimating the variance of genetic drift and that estimated N_e becomes erroneous if N_s is as small as N_e.

Surveys on the Experimental Biological Stocks

Shin-ya IYAMA

Survey on the various experimental biological stocks including animals, plants, microorganisms and cultured cells maintained in the universities and other related institutes was done in cooperation with other staffs in Genetic Stocks Research Center. The purpose of this survey is to collect the informations about experimental biological stocks maintained in Japan and construct a useful database for biological research. So far the replies to the inquiry have been obtained from 187 universities and institutes and they are under data-processing. Preliminary results showed that the number of stocks maintained at various locations were 6,800 animal stocks at 316 locations, 80,000 plant stocks at 73 locations, 205,000 microbial stocks at 310 locations and 3,200 cultured cell stocks at 240 locations.

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ABSTRACTS OF DIARY FOR 1984

Biological Symposium

215th	meeting	Jan. 11	Restriction endonuclease study of the mitochondrial DNA of closely related frogs and fishes (David L. Jameson)
216th	〃	Mar. 10	Chromosome polymorphism of a few mammals in South India (N. V. Aswathanarayana)
217th	〃	Mar. 26	Mouse genetics; the last ten years (A. G. Searle)
218th	〃	Mar. 28	Developmental genetics: present and future prospects (R. S. Edgar)
219th	〃	Mar. 29	No title of his talk was previously announced, but he talked on his past research activities which were related to genetics (Linus Pauling)
220th	〃	Apr. 4	RNA-dependent RNA polymerases in plant cells (H. Fraenkel-Conrat)
221st	〃	Apr. 13	Generation of two different H-2K histocompatibility antigens through different splicing of a unique H-$2K^d$ gene (G. Gachelin)
222nd	〃	May 10	Studies of the mutation process in Drosophila by means of MR-mediated P-transposition and employing repair-deficient mutants. (F. H. Sobels)
223rd	〃	May 21	DNA sequences and a primeval genetic code (John C. W. Shepherd)
224th	〃	May 28	Molecular analysis of an ecdysone regulated gene from *D. melanogaster* (Kenneth C. Burtis)
225th	〃	Jun. 25	Epigenetics of early development: biochemical regulation of sperm behavior before and after fertilization (sea urchin) (Bennett M. Shapiro)
226th	〃	Aug. 10	Evidence for heterogeneity in the nerve cell populations and neuronal plasticity in hydra (Hans R. Bode)
227th	〃	Aug. 18	Isolated muscle cells of hydromedusae can

undergo pluripotent transdifferentiation and form a complex regenerate (Volker Schmid)

228th 〃 Aug. 30 Export of protein in *E. coli* (Linda L. Randall)

229th 〃 〃 Structure of chemotactic transducers, proteins conserved throughout bacterial evolution (Gerald L. Hazelbauer)

230th 〃 Sep. 6 DNA comparisons of immunoglobulin kappa genes of human, mouse and rabbit (Samuel Karlin)

231st 〃 Sep. 21 Evolution of antigen binding sites (Susumu Ohno)

232nd 〃 Nov. 27 Pheromone polymorphism in Drosophila (J. M. Jallon)

233rd 〃 Dec. 12 Genetic control of developmental pathway (Antonio Garcia-Bellido)

Mishima Geneticists' Club

289th meeting Feb. 2 Cloning of Y-specific repetitive DNA sequences (Yutaka Nakahori)

290th 〃 Feb. 23 Cellular skeleton of cultured muscle cells (Yutaka Shimada)

291st 〃 Mar. 12 Transposon Tn5: Variation and its regulation (Chihiro Sasagawa)

292nd 〃 Mar. 23 Numerical analysis of the experimental results about hydra morphogenesis (Hiroshi Shimizu)

293rd 〃 Mar. 29 Forty years of chromosome study—Retrospect and prospect—(Tosihide H. Yosida)

294th 〃 Sep. 11 Analysis of biological functions of *ras* oncogene (Toru Kamata)

FOREIGN VISITORS IN 1984

January 1–31	Jameson, David L., University of Houston, U.S.A.
January 15–June 20	Thipayathasana, Pairor, Chulalongkorn University, Thailand
January 14–29	Loresto, G. C., International Rice Research Institute, The Philippines
January 30	Zhang, Yihua; Jiang, Yuming; Si, Yiping; Qiu, Lingcang; China National Rice Research Institute, China
February 1	Yang, Han Chul, Korea
March 1	Sved, John, University of Sydney, Australia
February 29–March 13	Aswashanarayana, N. V., University of Mysore, India
March 5–6	Houba, Chris, University of Liège, Belgium
March 9–	Kim, Bong Ki, Don Kook University, Korea
March 13–14	Huh, Hong Wook, Pusan National University, Korea
March 26–27	Searle, Antony G., Medical Research Council's Radiobiology Unit, U. K.
March 28	Edgar, Robert S., University of California, U.S.A.
〃	Bailey, Donald W., The Jackson Laboratory, U.S.A.
March 29	Soerianegara, Ishemat, SEAMEO Regional Center for Tropical Biology, Indonesia
〃	Pauling, Linus; Zuckerkandl, Emile, Linus Pauling Institute of Science & Medicine, U.S.A.
April 1–	Houba, Nicole, University of Liège, Belgium
April 4	Frankel-Conrat, Heinz, University of California, U.S.A.
April 9–	Pascale, Barbier, Université des Sciences et

	Techniques du Languedoc, France
April 13	Gachelin, Gabriel, Pasteur Institute, France
April 23–24	Park, James T., Tufts University, U.S.A.
May 9	Inouye, M., State University of New York, U.S.A.
May 10	Sobels, F. H., Leiden University, The Netherlands
May 21	Shepherd, John C. W., Biocentre of the University of Basel, Switzerland
May 26	Wu, Weiguang; Tang Wei Liu; Wu, Peng Tuan; Huang, Guan Xian, Chen, Zuo Fu; Asia and Oceania Silkworm and Mulberry Training Center, China
May 28	Maas, Werner K., New York University Medical Center, U.S.A.
"	Burtis, Kenneth C., Stanford University School of Medicine, U.S.A.
June 17–18	Shankel, D. M., University of Kansas, U.S.A.
June 22–26	Shapiro, Bennett M., University of Washington, U.S.A.
July 12	Temtamy, Samia A., National Research Center, Cairo, Egypt
August 3	Chung, Yong-Jai, Ewha Women's University, Korea
August 8–20	Bode, Hans R., University of California, U.S.A.
August 17–20	Schmid, Volker, University of Basel, Switzerland
August 24	Macgregor, Herbert C., University of Leicester, England
August 24–25	Wongkaew, Wongchan C., South East Asian Regional Center for Tropical Biology, Indonesia
August 29–September 1	Hazelbauer, Gerald L., Washington State University, U.S.A.
"	Raldall, Linda L., Washington State Uni-

	versity, U.S.A.
August 30–31	Weir, Bruce S., North Carolina State University, U.S.A.
September 6–7	Karlin, Samuel, Stanford University, U.S.A.
September 10	Wu, J. S.; Shi, S. K.; Beijing Agricultural University, China
September 10–11	Martin, Malcolm A., National Institutes of Health, U.S.A.
September 11	Khush, G. S., International Rice Research Institute, The Philippines
September 14–16	Cockerham, Clark, North Carolina State University, U.S.A.
September 16–November 14	Baradjanegara, Abdal Aziz, National Atomic Energy Agency, Indonesia
September 17–18	Wurster-Hill, D. H., Dartmouth Medical School, U.S.A.
〃	Ward, O. G., University of Arizona, U.S.A.
September 21	Ohno, Susumu, City of Hope Beckman Research Institute, U.S.A.
September 29	Loekman, Irwansyah, National Atomic Energy Agency, Indonesia
〃	Gadrinab, Lilian Ungson, SEAMEO Regional Center for Tropical Biology, Indonesia
October 8	Kuliev, A. M., World Health Organization, Switzerland
October 9	Tjio, J. H., National Institutes of Health, U.S.A.
October 18	Ruffié, Jacques, Collège de France, France
October 25	Cui, Taishan, Academia Sinica, China
October 23	Eschbach, F., Stuckenschmidt, D., DAAD, Germany
October 31	Lederberg, J., The Rockefeller University, U.S.A.
November 6	Duda, George, U.S. Department of Energy, U.S.A.
November 13–16	Hartle, Daniel L., Washington University,

	U.S.A.
〃	Crow, James F., University of Wisconsin, U.S.A.
〃	Nei, Masatoshi, University of Texas, U.S.A.
〃	Milkman, R., University of Iowa, U.S.A.
〃	Selander, R. K., University of Rochester, U.S.A.
〃	Li, W.-H., University of Texas, U.S.A.
〃	Watterson, G. W., Monash University, Australia
〃	Charlesworth, B., University of Sussex, England
〃	Calder, N., England
November 23	Hu, Han, Institute of Genetics, Academia Sinica, China
November 27	Jallon, Jean-Marc, Laboratoire de Biologie Genetique Evolutives, CNRS, France
December 12	Garcia-Bellido, Antonio, Univerided Autonoind de Madrid, Spain
December 24–	Lee, Won Ho, Pusan National University, Korea

AUTHOR INDEX

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国立遺伝学研究所内
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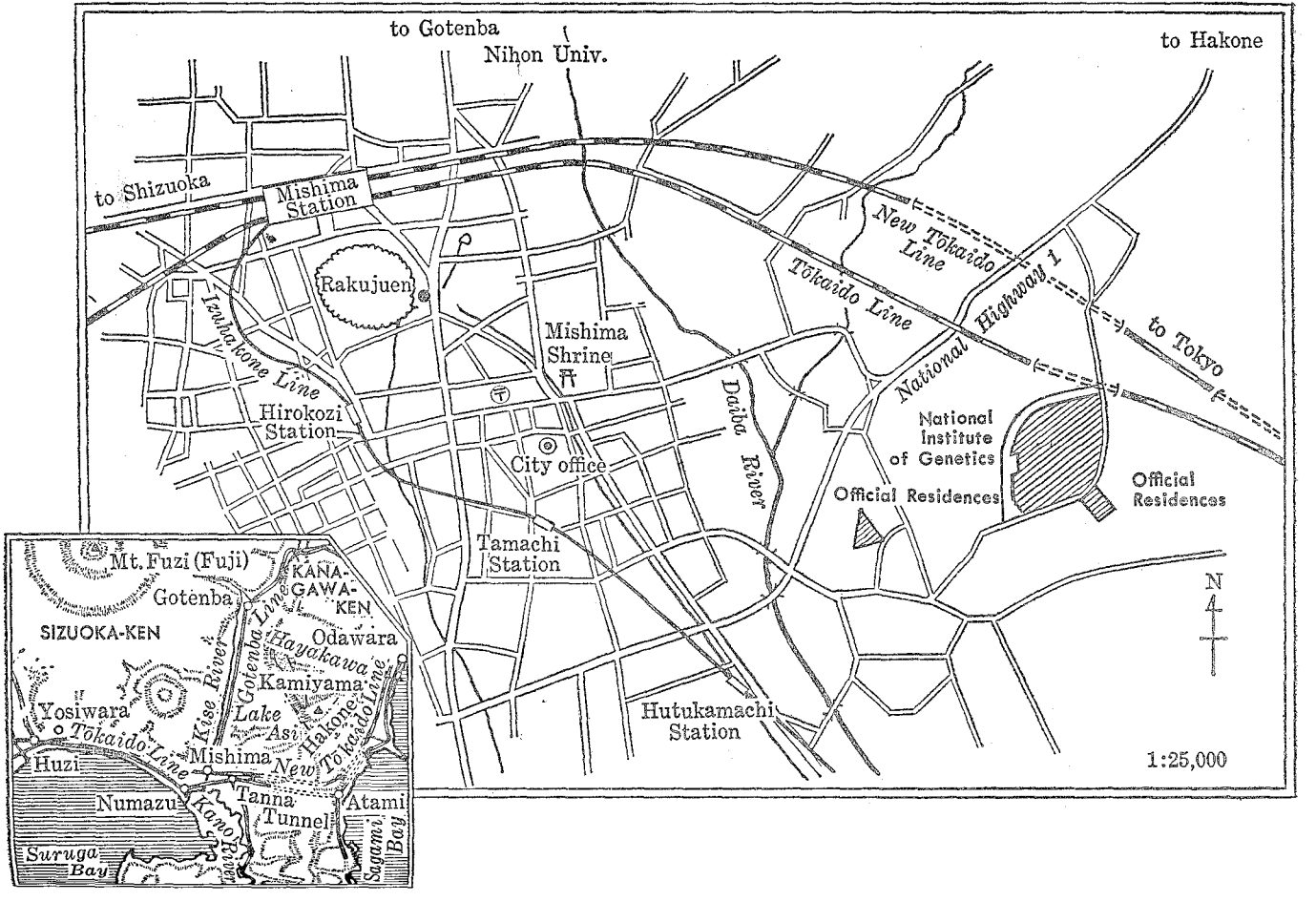
電　話 代表 (0559) (75) 0771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