NATIONAL INSTITUTE OF GENETICS

JAPAN

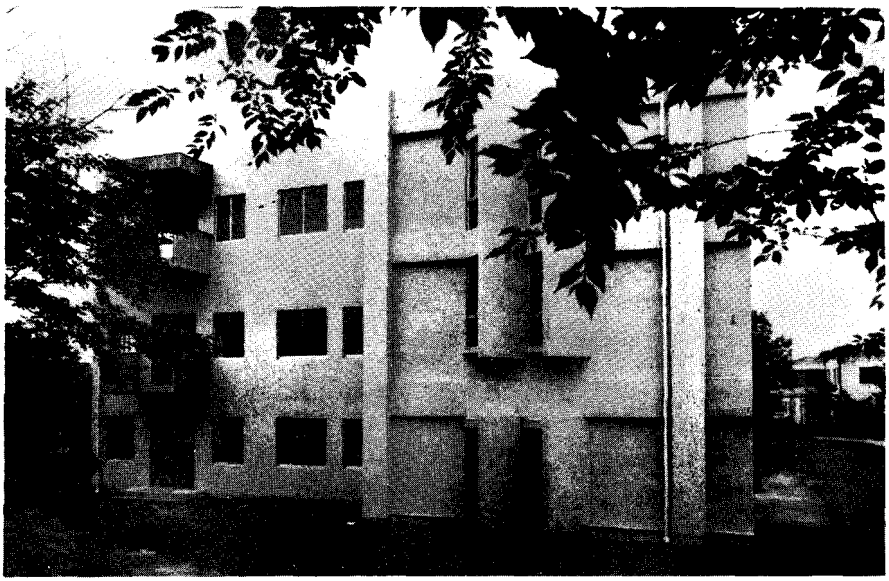
ANNUAL REPORT

No. 21 1970

Published by
THE NATIONAL INSTITUTE OF GENETICS
Misima, Sizuoka-ken, Japan
1971

Annual Report
of the
National Institute of Genetics

No. 21, 1970



Published by
The National Institute of Genetics, Japan
1971

CONTENTS

General statement 1
Staff 2
Council 5
Association for propagation of the knowledge of genetics 5
Projects of research for 1970 6
Researches carried out in 1970 11

I. Molecular Genetics

Terminal structure of double-stranded RNA of cytoplasmic polyhedrosis virus from silkworm (CPV). MIURA, K. and Furuichi, Y. 11
RNA polymerase associated with purified virion containing double-stranded RNA. SHIMOTONO, K. and MIURA, K. 12
Double-stranded RNA isolated from the CP virus of pine caterpillar. MIURA, K. and SHIBUYA, A. 12
Structure and function of transfer RNA. FURUICHI, Y. and MIURA, K. 13

II. Microbial Genetics

Further mapping of *fla* and *mot* cistrons closely linked to *H1* in *Salmonella abortus-equi* and its derivatives. YAMAGUCHI, S. and IINO, T. 14
Activity of glutamine dependent carbamyl phosphate synthetase in arginine sensitive mutants of *Salmonella typhimurium*. TOSHIMA, H. and ISHIDSU, J. 14
Activities of enzymes concerning uracil biosynthesis in 6-azauracil resistant mutants of *Salmonella typhimurium*. ISHIDSU, J. 16
Physiological and enzymological analysis of azide resistant mutants of *Salmonella typhimurium*. ISHIDSU, J. 17
Reconstitution of salmonella flagella attached to cell bodies. IINO, T., SUZUKI, H. and YAMAGUCHI, S. 18
Possible differential control of flagellin mRNA synthesis in *Bacillus pumilus* depending on cell age. SUZUKI, H. 20

Phage-induced alteration of colony type in *Xanthomonas citri.* WU, W. C. and IINO, T. 21
Genetic analyses of nonmotile double mutants in *Salmonella typhimurium.* ENOMOTO, M. 22
A new transducing phage related to P22 of *Salmonella typhimurium.* ENOMOTO, M. and ISHIWA, H. 22

III. Biochemical Genetics

Purification and properties of tryptophan pyrrolase in *Ephestia.* NAWA, S. 24
Characterization of DNA from eggs of *Ephestia.* YAMADA, M. A. 25
Hereditary change in silworm with DNA. NAWA, S., TSUJITA, M. and YAMADA, M. A. 26
Three allelic isozyme variants in rice peroxidase. PAI, C. and ENDO, T. 27
Lethal effects of a genetic abnromality in the pteridine metabolism of silkworm larvae. TSUJITA, M. and SAKURAI, S. 28
Genetic and biochemical studies of lethal albino larvae of the silkworm, *Bombyx mori.* TSUJITA, M. and SAKURAI, S. 29
The phenotype of double recessive lethal (lem^1/lem^1; *al/al*) larvae of the silkworm. TSUJITA, M. 30
Genetic variation observed in membrane proteins isolated from pteridine granules of several oily mutant strains of the silkworm. SAKURAI, S. and TSUJITA, M. 31
Immunochemical studies of structural membrane proteins of pteridine granules in hypodermal cells of silkworm larvae. SAKURAI, S. 33
Differences in amino acid composition of serum transferrin among various species of *Rattus.* MORIWAKI, K., SATO, T. and TSUCHIYA, K. 34
Serum transferrin polymorphism in oceanian black rat, *Rattus rattus rattus.* MORIWAKI, K. and SATO, T. 36
Serum transferrin patterns of *Rattus rattus* obtained from Southern India and Italy. MORIWAKI, K., SATO, T. and TSUCHIYA, K. .. 37
Studies on the transferrin of the Mongolian gerbil. MAYEDA, K. and MORIWAKI, K. 37

Comparative studies of the transferrins of rodents. MORIWAKI, K. and MAYEDA, K. 38

IV. Developmental Genetics

Specific inhibition by glucosamine of aggregate formation of rat hepatoma cells in rotation culture. KURODA, Y..... 41
Growth-stimulating effect of peptone on *Drosophila* ovarian cells in culture. KURODA, Y..... 42
Evidence for the restitution of sorting-out activity of HeLa cells following fractionated X-irradiation. KURODA, Y..... 43
Further study of relationship between molting and cell cycle in insect epidermis. MINATO, K..... 44
Effects of culture media on the survival of embryonic quail liver-specific cells. MINATO, K..... 45

V. Cytogenetics

Karyotype evolution in *Rattus* species from black rat, *R. rattus*. 1. Species with a basic chromosome number, but with structural chromosome difference. YOSIDA, T. H. and TSUCHIYA, K. 46
Karyotype evolution in *Rattus* species. 2. Species with fewer chromosome numbers than $2n=42$ by Robertsonian chromosome fusion. YOSIDA, T. H. and TSUCHIYA, K..... 47
Karyotype of Indian black rats, *Rattus rattus rufesens*. YOSIDA, T. H. and TSUCHIYA, K..... 48
Studies of aneusomic clones isolated from a Chinese hamster cell line following induction of nondisjunction. KATO, H..... 49
Distribution of two chromosomal types of Japanese wood mouse, *Apodemus speciosus*. TSUCHIYA, K. and YOSIDA, T. H..... 49
Differences in biochemical characteristics between two sibling forms of Japanese wood mouse, *Apodemus speciosus*. TSUCHIYA, K. and MORIWAKI, K..... 50
Spontaneous sarcomas in black rats, *Rattus rattus*, bred in laboratory, and chromosome observations. YOSIDA, T. H. and KATO, H. 51
Preliminary note on karyotype of guppy and topminnow. YOSIDA, T. H. and HAYASHI, M..... 52

Chromosome survey of small mammals in Japan. TSUCHIYA, K. and YOSIDA, T. H..... 54
Frequency of chromosome polymorphism in pair Nos. 1, 3, 9 and 13 in black rats, collected in Misima. YOSIDA, T. H..... 55
In Vivo proliferation of two sublines of MSPC–1 mouse myeloma in mixed inoculation. MORIWAKI, K. and SATO, T..... 56
An estimation of the number of definitive spermatogonial cell divisions in the silkworm. MURAKAMI, A..... 57

VI. Radiation genetics in animals

Effect of the moulting hormone on mutation induction and development of spermatogenic cells in the silkworm. TAZIMA, Y. and FUKASE, Y..... 60
Studies on strain differences in radiosensitivity in the silkworm. XI Sensitivity to dominant lethal induction. TAZIMA, Y. and FUKASE, Y. 61
Studies on strain differences in radiosensitivity in the silkworm. XII Different modes of dominance observed in F_1 with regard to radiosensitivity at various levels of biological reaction. TAZIMA, Y. 62
Studies on strain differences in radiosensitivity in the silkworm. XIII Radiation-induced breakage and rejoining of single strand DNA. TAZIMA, Y., ONIMARU, K. and KATO, H..... 63
Relative biological effectiveness of 14 MeV neutrons for recessive visible mutations in meiotic spermatocytes of the silkworm. MURAKAMI, A. 64
Changes in ultraviolet light sensitivity to embryonic killing during early developing egg of the silkworm. MURAKAMI, A..... 65
Sex differences in X-ray sensitivity to embryonic killing of dormant silkworm egg. MURAKAMI, A..... 66
Differential nuclease activities in radiation-sensitive and radiation resistant silkworms. SAKURAI, S., KADA, T. and ANDO, T. 67

VII. Radiation genetics and chemical mutagenesis in microorganisms and plants

Biochemical factors involved in the rapair of γ-ray-induced

damage in transforming DNA of *Bacillus subtilis*. NOGUTI, T. and KADA, T. 69
Spores of rec⁻ strains of *Bacillus subtilis* are sensitive to UV or γ-ray. SADAIE, Y. and KADA, T. 70
Mutagenic action of phloxine. KADA, T., SADAIE, Y. and TUTIKAWA, K. 72
Host-mediated "rec-test" for chemical mutagens. TUTIKAWA, K. and KADA, T. 73
Efficiencies of some mutagens in maize. AMANO, E. 74
Mutation experiment in *Capsicum annuum*. AMANO, E. 75
Effect of temperature during the split dose interval of gamma-rays in maize pollen. FUJII, T. 76
Tissue culture from wheat anthers and roots. FUJII, T. 77

VIII. Population genetics (theoretical)

The length of time required for a selectively neutral mutant to reach fixation through random frequency drift in a finite population. KIMURA, M. 79
Probability of fixation of a mutant gene in a finite population when selective advantage decreases with time. KIMURA, M. and OHTA, T. 79
Genetic loads at a polymorphic locus which is maintained by frequency-dependent selection. KIMURA, M. and OHTA, T. ... 80
Statistical analysis of the base composition of genes using data on the amino acid composition of proteins. OHTA, T. and KIMURA, M. 81
Development of associative overdominance through linkage disequilibrium in finite populations. OHTA, T. and KIMURA, M. 81
The rate of decay of genetic variability in a two-dimensionally structured finite population. MARUYAMA, T. 82

IX. Population genetics (experimental)

Deleterious genes in natural summer and autumn populations of *Drosophila melanogaster*. OSHIMA, C., WATANABE, T. K. and CHOO, J. K. 88
Sterility genes in natural summer and autumn populations of

D. melanogaster. OSHIMA, C., WATANABE, T. K. and KAWANISHI, M. 90
Correlation between productivity and viability of *D. melanogaster*. WATANABE, T. K. 92
Fecundity and longevity of *D. melanogaster* under constant and fluctuating temperature environments. OSHIMA, C. and KAWANISHI, M. 92
Effect of light on oviposition of *D. virilis*. OSHIMA, C. and INOUE, K. 94
Relationship between phototaxis and oviposition of *D. virilis*. OSHIMA, C. and INOUE, K. 95

X. Evolutionary genetics

An intergeneric hybrid between *Agropyron tsukushiense* and *Elymus mollis*. SAKAMOTO, S. 97
Collection of wheats and various other species of the tribe Triticeae in Iraq, Turkey and Iran. YAMASHITA, K., TANAKA, M., SAKAMOTO, S. and ISHII, H. 98
Dynamics of plant domestication: Cultivation experiments with *Oryza perennis* and its hybrid with *O. sativa*. OKA, H. I. and MORISHIMA, H. 99
Partial self-incompatibility in *Oryza perennis*. MORISHIMA, H. and OKA, H. I. 100

XI. Human genetics

Inheritance of total finger ridge count. MATSUDA, E. and MATSUNAGA, E. 101
Polymorphic red cell enzymes among Japanese. SHINODA, T. 102
Structure of human macroglobulin. SHINODA, T. 102
Birth weight in Down's syndrome: Comparison of interchange trisomics and G trisomics. MATSUNAGA, E. 103
Parental ages in translocation Down's syndrome. MATSUNAGA, E. 103
Distribution of fast renaturining DNA in human chromosomes. NAKAGOME, Y. 104
A Cp+mat chromosome found in a malformed boy. OISHI, H., MATSUDA, E. and KIKUCHI, Y. 105

Chromosome studies in congenitally abnormal children. NAKAGOME, Y. and MATSUMOTO, H. 106
Clinical conditions of patients with apparently normal chromosomes, VII. OISHI, H. 107

XII. Apllied genetics

Geographic variation on the isoenzyme level of Abies sachalinensis in Hokkaido. SAKAI, K. I. and MATSUURA, T. 109
Genetic differentiation within a natural forest of *Cryptomeria japonica D. Don.* SAKAI, K. I. and PARK, Y. G. 110
Genetic studies of peroxidase isozymes of the Japanese red pine, Pinus densiflora Sieb. and Zucc. PARK, Y. G. and SAKAI, K. I. 110
An analysis of sexual dimorphism in body size in the Japanese quail. KAWAHARA, T. 111
Monte Carlo studies on the relationship between the system of reproduction and inbreeding. IYAMA, S. 112
Selection in rice: An experiment. IYAMA, S. 115
Growth pattern and its plasticity in wild and cultivated types of rice. MORISHIMA, H. and OKA, H. I. 116
Inter-genotypic interaction in rice. HOSHINO, T., MORISHIMA, H. and OKA, H. I. 117
Evaluation of an electronic instrument for estimation of plant weight. MORISHIMA, H., OKA, H. I. and HOSHINO, T. 117

Books and papers published in 1970 by research members 119
Abstracts of diary for 1970 123
Foreign visitors in 1970 124
Acknowledgement 125
Author index 126

GENERAL STATEMENT

A new concrete building, 811 m² in floor dimensions, designed for a new library is now under construction and will be completed in the spring of 1971. The library in our institute has been expanding year by year with new periodicals, monographs and reprints and was in need of a new installation equipped with versatile functions. The first plan to erect a three-storied building had soon to be reduced to two-stories according to the reduced budget. Fortunately, at this juncture, Dr. H. Kihara stepped in, and the mutilation of our plan was prevented. His Institute for Biological Research offered us a large sum of assistance, allowing us to execute the original plan. We are deeply grateful for the generous help.

In July we have organized for the 3rd time a three day seminar on "Population Genetics and Evolution" as an advanced course in special fields of genetics. Luckily at that time Dr. J. F. Crow, Professor of Wisconsin University, has been on a visit of three months here as a Visiting Member. He was one of the speakers at the seminar and gave us an excellent lecture. As another Visiting Researcher, Dr. Kazutoshi Mayeda, Associate Professor of Wayne State University of Michigan, is staying here since this summer. He takes part in a US-Japan Cooperative Science Programme of one year at the Department of Cytogenetics.

Dr. M. Kimura, Head of the Department of Population Genetics, was given an award from the Japanese Society of Human Genetics in November 1970, and presented at the general assembly a prize lecture "Population genetics and Human genetics."

Three members of our institute, Drs. Tazima, Kada, and Fujii, went to Evian, France, to attend the 4th International Congress of Radiation Research. After the Congress, Dr. Tazima, Head of the Department of Morphological Genetics visited USSR for 10 days. He was invited by the USSR Academy of Sciences, and was surveying the recent advances in genetics in that country. After a short time he went again to Europe and attended in Geneva as a Member of National Delegations from 18th to 27th September the 20th Session of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). During the summer session Dr. T. Iino, Head of the Department of Microbial Genetics, gave lectures at Purdue University, U.S.A., and then attended the 10th International Congress of Microbiology held in Mexico City in August. Dr. H. I. Oka, Head of the 3rd Laboratory of the Department of Applied Genetics is on leave of two years since November 22, 1970, staying at Central Luzon State University, Philippines, as a UNESCO Specialist in Crop Improvement and Seed Production.

Speaking of personnel changes, Mr. T. Oyauchi, who had been Head of the Department of Administration was transferred to Tokyo Educational University and replaced by Mr. M. Kudo.

STAFF

Director

MORIWAKI, Daigoro, D. Sc., Emeritus Professor of Tokyo Metropolitan University

Members

1. Department of Morphological Genetics

TAZIMA, Yataro, D. Ag., Head of the Department

The 1st Laboratory

TAZIMA, Yataro, D. Ag., Head of the Laboratory
MURAKAMI, Akio, D. Ag.
ONIMARU, Kimiharu

The 2nd Laboratory

KURODA, Yukiaki, D. Sc., Head of the Laboratory
MINATO, Kiyoshi

2. Department of Cytogenetics

YOSIDA, Tosihide H., D. Sc., Head of the Department

The 1st Laboratory

YOSIDA, Tosihide H., D. Sc., Head of the Laboratory
KATO, Hatao, D. Sc., TSUCHIYA, Kimiyuki

The 2nd Laboratory

MORIWAKI, Kazuo, D. Sc., Head of the Laboratory
IMAI, Hirotami, D. Sc.

3. Department of Physiological Genetics

OSHIMA, Chozo, D. Sc., Head of the Department

The 1st Laboratory

OSHIMA, Chozo, D. Sc., Head of the Laboratory
WATANABE, Takao K., D. Sc.
OISHI,* Kugao, Ph. D., CHOO,* Jong-Kil, M. S.

The 2nd Laboratory

OSHIMA, Chozo, D. Sc., Head of the Laboratory
SAKAMOTO, Sadao, D. Ag.

4. Department of Biochemical Genetics

TSUJITA, Mitsuo, D. Ag., Head of the Department

The 1st Laboratory

NAWA, Saburo, D. Sc., Head of the Laboratory
YAMADA, Masa-Aki

The 2nd Laboratory

OGAWA, Yoshito, D. Med., Head of the Laboratory
ENDO, Toru, D. Ag.

The 3rd Laboratory

TSUJITA, Mitsuo, D. Ag., Head of the Laboratory
SAKURAI, Susumu, D. Med.

5. Department of Applied Genetics

SAKAI, Kan-Ichi, D. Ag., Head of the Department

The 1st Laboratory

SAKAI, Kan-Ichi, D. Ag., Head of the Laboratory
KAWAHARA, Takatada, D. Ag.; FUJISHIMA, Tohru, D. Ag.

The 2nd Laboratory

IYAMA, Shin-ya, D. Ag., Head of the Laboratory
BAGCHI,* Sujit; IWAGAMI,* Seiro; PARK,* Y. G.

The 3rd Laboratory

OKA, Hiko-Ichi, D. Ag., Head of the Laboratory
MORISHIMA-OKINO, Hiroko, D. Ag.
HOSHINO,* Tsuguhiro; PAI,* Chiang

6. Department of Induced Mutation

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

The 1st Laboratory

TUTIKAWA, Kiyosi, Acting Head of the Laboratory
NOGUTI, Takehiko, D. Sc.

The 2nd Laboratory

KADA, Tsuneo, D. Sc., Head of the Laboratory
FUJII, Taro, D. Ag.

The 3rd Laboratory

KADA, Tsuneo, D. Sc., Head of the Laboratory
AMANO, Etsuo, D. Ag.; SADAIE, Yoshito

* Research members under grant from other organization or visiting researchers.

7. Department of Human Genetics

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

The 1st Laboratory

MATSUNAGA, Ei, D. Med., D. Sc., Head of the Laboratory
SHINODA, Tomotaka, D. Sc.; MATSUDA, Ei.

The 2nd Laboratory

MATSUNAGA, Ei, D. Med., D. Sc., Head of the Laboratory
NAKAGOME, Yasuo, D. Med.; OISHI, Hidetsune, D. Sc.

8. Department of Microbial Genetics

IINO, Tetsuo, Ph. D., D. Sc., Head of the Department

The 1st Laboratory

IINO, Tetsuo, Ph. D., D. Sc., Head of the Laboratory
ENOMOTO, Masatoshi, D. Sc.; YAMAGUCHI,* Shigeru, D. Sc.; ISHIWA,* Hiromi

The 2nd Laboratory

IINO, Tetsuo, Ph. D., D. Sc., Head of the Laboratory
SUZUKI, Hideho, D. Sc.; ISHIDSU, Jun-ichi; WU,* Wen Chuan

9. Department of Population Genetics

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

The 1st Laboratory

KIMURA, Motoo, Ph. D., D. Sc., Head of the Laboratory
OHTA, Tomoko, Ph. D.

The 2nd Laboratory

MARUYAMA, Takeo, Ph. D., Head of the Laboratory

10. Department of Molecular Genetics

MIURA, Kin-ichiro, D. Sc., Head of the Department
FURUICHI, Yasuhiro, D. Med.

11. Experimental Farm

SAKAI, Kan-Ichi, D. Ag., Head of the Farm
MIYAZAWA, Akira

12. Department of Administration

KUDO, Masaaki, Head of the Department
MURAMATSU, Masanori, Chief of the General Affairs Section
KATO, Shigeo, Chief of the Finance Section

Honorary Members

KIHARA, Hitoshi, D. Sc., Director of the Kihara Institute for Biological Research, Member of Japan Academy, Emeritus Professor of Kyoto University

KOMAI, Taku, D. Sc., Member of Japan Academy, Emeritus Professor of Kyoto University

KUWADA, Yoshinari, D. Sc., Member of Japan Academy, Emeritus Professor of Kyoto University

LILIENFELD, Flora A., Ph. D.

OGUMA, Kan, D. Ag., Emeritus Professor of Hokkaido University

TANAKA, Yoshimaro, D. Ag., D. Sc., Member of Japan Academy, Emeritus Professor of Kyushu University

Visiting Member

CROW, James F., Ph. D., Member of the National Academy of Science U. S., Professor of the University of Wisconsin. (June 15~Sept. 1)

COUNCIL

KIKKAWA, Hideo, Chairman, Professor of Osaka University

FUJII, Takashi, Vice Chairman, Professor of Shizuoka University

BABA, Isamu, Director of National Institute of Agricultural Sciences

IZEKI, Shoei, Professor of Gunma University

KAYA, Seiji, Emeritus Professor of University of Tokyo

KIHARA, Hitoshi, Emeritus Professor of Kyoto University

MAKINO, Sajiro, Professor of Hokkaido University

MISONOO, Keisuke, Director of National Institute of Radiological Sciences

OCHI, Yuichi, President of Azabu University of Veterinary Science

SAKATA, Takeo, President of T. Sakata Company

TACHI, Minoru, Director of Institute of Population Problems

TAKAHASHI, Ryuhei, Professor of Okayama University

TAKEYAMA, Yutaro, Governor of Shizuoka Prefecture

TANAKA, Nobunori, Professor of University of Tokyo

YANAGIDA, Tomomichi, Director of Institute of Applied Microbiology, University of Tokyo

ASSOCIATION FOR PROPAGATION OF THE KNOWLEDGE OF GENETICS

MORIWAKI, Daigoro, President, Director of the National Institute of Genetics
TAZIMA, Yataro, Managing Director, Head of the Morphological Genetics Department
OSHIMA, Chozo, Managing Director, Head of the Physiological Genetics Department
KIHARA, Hitoshi, Manager, Emeritus Professor of Kyoto University
MATSUNAGA, Ei, Manager, Head of the Human Genetics Department
SINOTO, Yosito, Manager, Professor of International Christian University
WADA, Bungo, Manager, Emeritus Professor of University of Tokyo

PROJECTS OF RESEARCH FOR 1970

Department of Morphological Genetics

Genetics of the silkworm (TAZIMA and ONIMARU)
Repair processes in radiation mutagenesis (TAZIMA and ONIMARU)
Genetic studies of radiosensitivity in the silkworm (TAZIMA and MURAKAMI)
Chemical mutagenesis in the silkworm (TAZIMA and ONIMARU)
Genetic studies on insect cells in tissue culture (KURODA and MINATO)
Developmental genetic studies on carcinogenesis in tissue culture (KURODA)
Effects of radiation on cells in tissue culture (KURODA)

Department of Cytogenetics

Studies on chromosomal polymorphism of rodents (YOSIDA, TSUCHIYA and KATO)
Chromosome alteration and development of tumor (YOSIDA and MATSUSHIMA)
Study on incorporation of isolated chromosomes into cultured mammalian cells (KATO, SEKIYA and YOSIDA)
Cytogenetical study on monosomic and trisomic cultured mammalian cells (KATO)

Cytogenetical and biochemical studies on mouse plasma cell tumor (MORIWAKI, YAMASHITA and SATO)
Biochemical studies on serum transferrin variations in rodents (MORIWAKI, SATO, TSUCHIYA and MAYEDA)
Experimental breeding and genetics of mice, rats and other wild rodents (YOSIDA, MORIWAKI, TSUCHIYA, SAKAKIBARA and SONODA)
Cytogenetical study of ants (IMAI)

Department of Physiological Genetics

Population genetics of deterious genes in natural populations of *Drosophila melanogaster* (OSHIMA, WATANABE and CHOO)
Mechanisms of peristence of some lethel genes (OSHIMA and WATANABE)
Physiological and population genetics of sterility genes in natural populations of *Drosophila melanogaster* (OSHIMA and WATANABE)
Analysis of fitness in a fluctuating environment (OSHIMA, WATANABE, INOUE and CHOO)
Analysis of inversion chromosome in natural populations of *Drosophila melanogaster* (OSHIMA and WATANABE)
Cytoplasmic inheritance in *Drosophila* (OISHI)
Nucleus substitution in wheat and related species (KIHARA, SAKAMOTO OHTSUKA and YOSHINO)
Cytogenetic studies in the tribe Triticeae (SAKAMOTO)
Studies on ecotypic differentiation in Japanese *Agropyron* (SAKAMOTO)

Department of Biochemical Genetics

Studies on transformation in higher organisms (NAWA, YAMADA and TSUJITA)
Genetical and biochemical studies of pteridine metabolisms in insects (NAWA and TSUJITA)
Studies on a gene for retarded moulting (*rm*) in the silkworm (TSUJITA)
Studies on the pteridine granule formation in larval hypodermal cells of the silkworm (TSUJITA and SAKURAI)
Analysis of gene action on cell differentiation in higher organisms (TSUJITA and NAWA)
Biochemical studies on the differentiation of muscle proteins in animals (OGAWA)

Genetical and biochemical studies of human serum proteins (OGAWA)
Genetical and biochemical studies on Japanese middle size dog (OGAWA)
Genetical and biochemical studies of the membrane protein of pteridine granules in the silkworm (SAKURAI and TSUJITA)
Genetics of isozymes in plants (ENDO and PAI)
Effects of exogenous DNA on plant seed formation (ENDO)

Department of Applied Genetics

Quantitative genetic studies in poultry (SAKAI and KAWAHARA)
Genetic studies in wild population of Japanese quails (KAWAHARA)
Theoretical studies on breeding techniques (SAKAI and IYAMA)
Studies on competition in plants (SAKAI and IYAMA)
Genetic studies in natural stands of forest tree species (SAKAI, IWAGAMI and PARK)
Zymographic studies in forest trees (SAKAI, IWAGAMI and PARK)
Simulation studies on artificial selection (IYAMA)
Evolutionary studies in wild and cultivated rice species (OKA and MORISHIMA)
Analysis of genetic variations in plant type and growth pattern in rice varieties (MORISHIMA and OKA)
Analysis of sterility genes in *Oryza sativa* (OKA)
Intergenotypic competition and synergism in rice (HOSHINO and MORISHIMA)
Genic analysis of isozymes in rice plants (PAI and ENDO)

Department of Induced Mutation

Radiation genetics in mice (TUTIKAWA)
RBE and dose rate effects in higher plants (FUJII and AMANO)
Radiation genetics in *Arabidopsis* (FUJII)
Fine sturcture analysis in maize (AMANO)
Biological effects of ultraviolet radiation (KADA, FUJII and AMANO)
Radiation-induced and chemical mutagenesis in microorganisms (SADAIE and KADA)
Studies on biochemical factors involved in induced mutagenesis on the cellular level (NOGUTI, TUTIKAWA and KADA)

Department of Human Genetics

Genetic consequences of population trends (MATSUNAGA)
Dermatoglyphics (MATSUNAGA and MATSUDA)
Down's syndrome in Japan (MATSUNAGA, OISHI and KIKUCHI)
Cytogenetics in man (OISHI, KIKUCHI and NAKAGOME)
DNA replication in human chromosomes (KIKUCHI and OISHI)
Molecular hybridization studies of human chromosomes (NAKAGOME)
Polymorphic red-cell enzymes among Japanese (SHINODA)
Studies on structure of human immunoglobulins (SHINODA)

Department of Microbial Genetics

Genetic fine structure analysis on microorganisms (IINO, ISHIDSU and YAMAGUCHI)
Genetics of cellular regulatory mechanisms (SUZUKI and ISHIDSU)
Genetics of bacterial flagella (IINO, ENOMOTO and SUZUKI)
Genetics of motility in bacteria (ENOMOTO)
Genetics of host range in bacteriophages (ENOMOTO)
Transduction mechanism of phage P22 (ENOMOTO and ISHIWA)
Genetics of phytopathogenic bacteria (WU and IINO)

Department of Population Genetics

Theoretical studies of population genetics (KIMURA)
Studies on molecular evolution from the standpoint of population genetics (KIMURA and OHTA)
Mathematical studies on the genetics of structured populations (MARUYAMA)
Linkage disequilibrium in finite populations (OHTA and KIMURA)

Department of Molecular Genetics

Studies on the chemical structure of genome of viruses containing double-stranded RNA (MIURA, FURUICHI, SHIMOTONO)
RNA polymerase in a virion containing double-stranded RNA (MIURA, FURUICHI, SHIMOTONO)
Structure and function of transfer ribonucleic acid (FURUICHI, MIURA)

RESEARCHES CARRIED OUT IN 1970

I. MOLECULAR GENETICS

Terminal structure of double-stranded RNA of cytoplasmic polyhedrosis virus from silkworm (CPV)

Kin-ichiro MIURA and Yasuhiro FURUICHI

Genome RNA of CPV is double-stranded and consist of ten segments, which are detected by electrophoresis on acrylamide gel (I. Fujii-Kawata, K. Miura, M. Fuke: J. Mol. Biol. 51 (1970) 247). As we have not succeeded to obtain these segments in a preparative scale, mixed segments were analyzed. The 5′-termini of the RNA were phosphorylated by polynucleotide kinase using γ-^{32}P-labeled ATP or GTP only after removal of the pre-existent phosphate group by phosphomonoesterase treatment. Phosphorylation proceeded much faster for the single-stranded state of RNA than for the double-stranded state. ^{32}P-labeled terminal nucleoside was isolated as ^{32}pNp [nucleoside-5′, 3′-diphosphate] by alkaline (or RNase T_2) digestion. Preliminary result indicates that purine nucleosides are predominant at 5′-termini of CPV–RNA segments.

The 3′-termini of these RNA segments were analysed by ^{3}H-labeling the terminal ribose moiety with periodate oxidation and successive reduction using tritiated borohydride. None of the 3′-terminus of ten RNA segments was phosphorylated. All ten RNA segments were labeled in the same extent. The 3′-terminal nucleosides were determined as 50% cytidine (ten termini) and 50% uridine (ten termini) by analysis of nucleoside trialcohols obtained from the ribonuclease T_2 (or alkali) digest of the ^{3}H-labeled RNA. Since the 3′-terminal of a viral single-stranded RNA was adenosine, or cytosine for a exceptional case so far analyzed, the termini of the double-stranded CPV–RNA segments are unique.

In the course of the latter experiments, a ^{3}H-labeled polysaccharide-protein complex was found in a ^{3}H-labeled RNA preparation.

RNA polymerase associated with purified virion containing double-stranded RNA

Kunitada SHIMOTONO and Kin-ichiro MIURA

It is shown that the reovirus contains RNA polymerase activity in a purified virus particle itself. Purified CP virus particle also revealed RNA polymerase activity in vitro without any pretreatment of the virus preparation. Optimum condition for this RNA polymerase reaction was seeked. If the product has a specific character, the template must be a double-stranded RNA genome in a virion. The characterization of the reaction product is now under investigation. Preliminary results show that the product is a single-stranded RNA and has the large molecular size corresponding to segments of genome RNA.

Double-stranded RNA isolated from the CP virus of pine caterpillar

Kin-ichiro MIURA and Akiko SHIBUYA

As the size and character of disease of the cytoplasmic polyhedrosis virus of pine caterpillar is similar to the CP virus of silkworm, the genome nucleic acid of the former virus was characterized and compared with the latter. Midgut of the infected pine caterpillar was gathered by Dr. Katagiri at Asakawa Forestry Experimental Station of the Ministry of Agriculture. The CP virus was isolated as a single pure component from the diseased midgut through purification of polyhedra. It is established from many kinds of chemical experiments that the nucleic acid prepared by phenol treatment for this virus is RNA and double-stranded. The base composition is quite similar to the RNA of CP virus from silkworm. Genome RNA of pine caterpillar CP virus is reproducibly separable into ten segments as silkworm CP virus by polyacrylamide gel electrophoresis. Size distribution of these segments of both the virus RNAs is not distinguishable. In the case of reovirus some differences in size distribution of genome RNA segments are detected among different strains. Therefore, CP virus from pine caterpillar is very similar to silkworm CP virus. Origin of both the viruses may be same. Analysis of the size distribution of the segments of genome nucleic acid would be useful technique for the com-

parison of biological species.

Structure and function of transfer RNA

Yasuhiro FURUICHI and Kin-ichiro MIURA

(As Miura and Furuichi had engaged in the work on the structure and function of transfer RNA in the former laboratories, some works on this line were also carried out this year.)

Furuichi performed the modification of tyrosine transfer RNA by sulfite to seek functional site with Prof. Ukita's group in the University of Tokyo. Uracil residue and isopentenyladenine located in either side of the looped-out anticodon sequence were modified. Although amino acid acceptability is not changed by the modification, the binding ability of the tryosine transfer RNA to the messenger RNA-ribosome complex was damaged.

Miura studied the nucleic acid of a living fossil, Brachiopod Lingula, with Dr. N. Shimizu of Nagoya University. DNA of this organism showed small GC content (35%). Several species of RNA molecules were identified. 4s RNA can accept an amino acid by yeast enzyme. This molecule has a similar structure to transfer RNA of the evolved present organisms, for example bacteria and rat, in its molecular size, base components and partial nucleotide sequences.

II. MICROBIAL GENETICS

Further Mapping of *fla* and *mot* Cistrons Closely Linked to *H1* in *Salmonella abortus-equi* and Its Derivatives

Shigeru YAMAGUCHI and Tetsuo IINO

Spontaneous non-flagellate or paralyzed mutants were isolated from a phase-1 stable strain of *Salmonella abortus-equi* and its *H1*-replaced derivatives. Taking trail production on transduction as the criterion, complementation tests were carried out with these mutants. Besides already known cistrons, that is, *flaA, flaB, flaC, flaD, flaE, flaF, flaK, flaL, flaM, motA,* and *motB*, three cistrons *flaN, flaP,* and *flaQ* were recognized, all co-transduced with *H1*. A group of non-flagellate mutants was shown to be $H1^-$ or $ah1^-$. *FlaA* mutants were classified into three subgroups, *flaAI, flaAII,* and *flaAIII*. A group of paralyzed mutants corresponding to *motC* was found to belong to the same group as the non-flagellate mutants of *flaAII*, and their average flagellar numbers per bacterium varied by the strains from almost the same as that of the wild type strain to nearly 0.

By deletion mapping supplemented with three-factor cross tests, the relative positions of *fla* and *mot* cistrons closely linked to *H1* were mapped. They were found to form two clusters. The order of cistrons in one of the clusters was established to be: *-flaD-flaB-flaQ-flaN-flaAIII-flaAII-flaAI-H1(ah1)-flaL-*, and that of the other cluster was partly determined as follows: *(flaE, flaK)-motA-mot-B-flaB-flaC-flaM-*. (The details were submitted to J. gen. Microbiol.)

Activity of glutamine dependent carbamyl phosphate synthetase in arginine sensitive mutants of *Salmonella typhimurium*

Hiro-o TOSHIMA and Jun-ichi ISHIDSU

It has been reported that the sites of arginine sensitive (*ars*) and arginine-uracil sensitive (*aus*) mutations in *S. typhimurium* are closely linked to the *pyrA* locus, the structural gene for glutamine dependent carbamyl phosphate synthetase (carbamyl-P synthetase), by P22 phage mediated transduction (Ishidsu, J., 1970. Ann. Rep. Nat'l. Inst. Genet. 20: 95). It has

been also known that both activity and repressibility of ornithine transcarbamylase and aspartate transcarbamylase in these strains are quite normal.

On the basis of the above finidings, the activity of carbamyl-P synthetase and its regulation pattern were compared between the wild type and the sensitive mutants using crude cell extracts. Unexpectedly, very low activity was found in *ars* and *aus* comparable with that found in *pyrA81*, a deletion mutant requiring arginine and uracil for growth, even in extracts obtained from fully derepressed cells grown in minimal medium. This confirms on one hand the results of transductional analysis which showed that these mutations have occurred in the *pyrA* locus, but on the other hand, it remains to be explained, since these mutants can grow in minimal medium even though more slowly than the wild type, how they are able to prepare carbamyl phosphate necessary for biosyntheses both of arginine and uracil. The answer might be either that even a very low level of carbamyl-P synthetase, if any, is sufficient to support growth, or that the carbamyl-P synthetase of the mutants is active in intact cells but is easily destroyed or inactivated during the procedure of extraction, or that some other pathway is utilized by the mutants to synthesize this compound. Activity of carbamate kinase, which is also known to exist in microorganisms and to catalyze the reaction of carbamyl phosphate synthesis from NH_4^+, CO_2 and ATP, was found to exist at the wild type level in extracts of mutant cells.

With a wild type strain LT2, the synthesis of carbamyl-P synthetase is partially repressed by arginine or uracil and their effect is cumulative, The activity of the enzyme is greatly inhibited by UMP, stimulated by ornithine and, when UMP and ornithine are given together, the inhibitory effect of the former is neutralized to quite an extent by the latter. These findings are in good agreement with the results reported before (Abd-El-Al, A. and J. L. Ingraham, 1969. J. Biol. Chem. 244: 4033). Among mutations occurring in the *pyrA* locus, several phenotypes have been reported. They are, in addition to arginine-uracil auxotrophy which is the most frequent one, arginine sensitivity and arginine-uracil sensitivity described above, arginine auxotrophy, cold sensitive arginine auxotrophy and uracil sensitivity. To explain the regulation pattern of the enzyme activity and the occurrence of various phenotypes, a model of an allosteric structure of the enzyme was drawn. Arginine, by binding to the modified ornithine site on the enzyme, is supposed to interfere with, or contrariwise to stimulate,

the binding of ATP in *ars* and *aus*, or in arginine auxotrophic strains, respectively. In *aus* strains, uracil, after being transformed to UMP, also prevents glutamine from binding to its own site completely because of the modification of UMP site by a mutation. This hindrance is only partial in the wild type strain. A detailed report is in preparation.

Activities of enzymes concerning uracil biosynthesis in 6-azauracil resistant mutants of *Salmonella typhimurium*

Jun-ichi ISHIDSU

It has been known that 6-azauracil (6-AU), an analogue of uracil, can act as a co-repressor in place of the latter repressing the pyrimidine synthesizing pathway. However, since it cannot replace uracil or cytosine in active nucleic acid molecules, growth of a wild type strain is inhibited under the presence of 6-AU unless uracil or cytosine is supplemented in addition. This means reversely that some, if not all, of the 6-AU resistant mutants can synthesize uracil even in the presence of this drug; that is, they are consititutive for uracil biosynthesis. Many such mutants, supposed to have some alteration in genetic regulatory systems of pyrimidine biosynsynthesis, were isolated and their properties were examined.

About 10^8 washed cells of a wild type strain LT2 of *S. typhimurium* were spread over minimal agar medium containing 1.5 mg/ml 6–AU. After about 50 hour incubation at 37°, 100 to 200 large and 10 to 50 minute colonies were formed on each plate. Thirty-eight small (*pyr-1074—pyr-1111*) and 6 large (*pyr-1112～pyr-1117*) colonies were picked up at random and the level of aspartate transcarbamylase (ATCase) activity was measured with toluenized cells of each mutant grown under repressed as well as derepressed conditions. The level of the enzyme in small colony formers, either repressed or derepressed, was found to be as high as 6 to 70 times the level in the repressed wild type strain. In large colony formers, it was also higher than that of the wild type, but the difference was not as significant as in the case of small colony formers. These evidently synthesize ATCase constitutively.

The activities of two other enzymes in the pyrimidine biosynthetic pathway, dihydroorotase (DHOase) and dihydroorotic acid dehydrogenase (DHO dehydrogenase), were also examined with toluenized cells. The

synthesis of DHOase was found to be also constitutive in many of the 6-AU resistant mutants. The derepression effect in the absence of uracil on the synthesis of DHO dehydrogenase was not apparent, though one of the small colony formers, *pyr-1082*, which showed the highest level of ATCase, also had a very high level of this enzyme even under repressed conditions ($\times 6.5$ of the wild type). The regulation of the synthesis of ornithine transcarbamylase (OTCase), which belongs to the arginine synthesizing pathway, was quite normal in these mutants; namely, its synthesis was not constitutive and was repressed by arginine. The derepressed level of glutamine dependent carbamyl phosphate synthetase in *pyr-1082* was slightly higher than that of the wild type and the extent of the partial repression by uracil of its synthesis was narrower when compared to the wild type.

Pyr-1082, transferred to a glucose minimal medium containing arginine and uracil from the same medium with no supplementation, showed a transient growth stop when the level of OTCase dropped to the repressed level. In glycerol medium where generation time is longer, however, such interruption of growth was not observed. Unbalanced growth caused by the difference in the amount of ATCase and OTCase might be one reason for such arrested growth.

Genetical analysis of these mutants yet remains as the next step.

Physiological and enzymological analysis of azide resistant mutants of *Salmonella typhimurium*

Jun-ichi ISHIDSU

As has been reported before (Ishidsu, J., 1970. Ann. Rep. Nat'l. Inst. Genet. 20: 97), usual isolates of spontaneous sodium axide (NaN_3) resistant mutants of *S. typhimurium* on nutrient agar medium cannot grow on minimal medium, but require cysteine or some other sulfur containing compounds in the presence of this drug. In liquid basal medium containing 3 mM NaN_3, it was found that 20 mM NaSH, 10 mM $Na_2S_2O_3$, 20 mM Na_2SO_3 or 1 mM cysteine could support well the growth of the mutants. Methionine, at the concentration of 1 mM, was less effective. One mM L-djenkolic acid, which is known to allow the growth of the wild type as a sole sulfur source but not to repress the formation of enzymes concerning the cysteine synthesizing pathway (Dreyfuss, J. and K. J. Monty, 1963.

J. Biol. Chem. 238: 3781), was much less effective.

Isolation of azide resistant mutants, not dependent on sulfur compounds under the presence of NaN_3, from a wild type strain LT2, *pyrA81* and their cysteine dependent azide resistant derivatives, was tried by spreading cells on minimal agar medium containing 4 mM NaN_3. *PyrA81*, a deletion mutant derived from LT2 damaged in the structural gene for glutamine dependent carbamyl phosphate synthetase, was used because the gene maps very close to one of the known *azi* loci, *aziA* (Sanderson, K. E., 1970. Bacteriol. Rev. 34: 176). In total 7 such mutants were obtained either from azide sensitive parents directly, or from cysteine dependent azide resistant derivatives, showing that the cysteine dependency is not necessarily a character accompanying azide resistancy.

Activities of one of the enzymes entering the cysteine biosynthetic pathway, sulfite reductase, were measured with crude extracts. It was found that NaN_3 does not repress the formation of this enzyme either in sensitive or in cysteine dependent azide resistant strains. Neither does NaN_3 in reaction mixtures inhibit the activity of this enzyme. As it has been reported that NaN_3 severely inhibits the activity of nitrate reductase (Nason, A. and H. J. Evans, 1953. J. Biol. Chem. 202: 655), activities of this enzyme in crude extracts were also examined. NaN_3 induced the synthesis of the enzyme quite considerably, especially in young cultures, in any sensitive, cysteine dependent resistant or independent resistant strains. The last group had a tendency to possess more nitrate reductase than the former two groups. Since NaN_3 was found to inhibit color yielding up to 90% or more in the color exhibiting reaction mixture also used by the above authors, it was not possible to elucidate its inhibition of enzyme activity by the method used.

Examination of effects of NaN_3 on earlier enzymes of the cysteine synthesizing pathway and genetical analysis by an Hfr-F^- conjugation experiment are being carried out.

Reconsitituion of Salmonella Flagella Attached to Cell Bodies

Tetsuo IINO, Hideho SUZUKI and Shigeru YAMAGUCHI

Studies in Salmonella of *in vitro* reconstitution of flagellar filaments from their component flagellin monomers have shown that the flagellar filaments have structural polarity and that they grow at the end distal to the cell body

(Asakura, S. *et al.* 1968. J. Mol. Biol. 35: 227). Growth of flagella *in vivo* was also demonstrated to take place at their tips (Iino, T. 1969. J. gen. Microbiol. 56: 227). These informations suggested a possibility that even when the flagella are attached to living cells, reconstitution of flagellar filaments from exogeneous flagellin monomers occurs at their tips under appropriate conditions. An experiment was carried out to examine this possibility.

The reaction system used was a mixture of cells of a straight flagellar mutant, SJ814, of *S. typhimurium* and flagellin monomers of the wild strain TM2 which produced normal flagella. The cells of SJ814 are entirely non-motile. Failure of their movement was attributed to the loss of spiral structure of their flagellar filaments (Iino, T. and M. Mitani. 1967. J. gen. Microbiol. 49: 81). The cells of SJ814 starved for 1 hour carried 3.2 flagella per cell on the average. The length of the flagella ranged up to the size corresponding to 6 normal wave units. After blending the cells in an electric mixer (Micro-Mixer TM101) for 3 min, most of the flagella longer than 3 normal wave units were broken to less than 2 normal wave units and 55% were detached from the cells at or near the base. The cell suspension was then washed twice with 0.03 M phosphate buffer. To the flagellin solution, which was prepared from the flagella of TM2 by acid dissociation, 0.3 M final concentration of 1 M phosphate buffer (pH 6.9) was added, and mixed with an equal volume of bacterial suspension. Finally 1/25 volume of 2 M K_3-citrate was added to the flagellin-cell mixture which was incubated at 26°C.

During the 4 hours of incubation at the concentration of 5×10^{10} cells and 10 mg flagellin per ml, neither the number of cells in the mixture nor the distribution of number and length of straight flagella was significantly changed. Among the straight flagella longer than a half normal wave unit, 18% grew normal filaments at their tips and formed heteromorphous filaments. In addition to these straight and heteromorphous flagella, such consisting entirely of normal waves appeared. Their number was much higher than that of heteromorphous flagella, and was equivalent to 82% of the sum of fully detached flagella and such that were broken less than a half normal wave unit upon blending. Distribution of the lengths of reconstituted normal flagellar filaments was quite different from that of the native flagella. Long flagella of more than 7 normal wave units, which never appear among native flagella, were seen among the reconstituted ones. The maximum length observed was 20 normal wave units, which is about

3 times as much as the length of the maximum native flagella. In the mixture after 4 hours, 0.8% of the cells were wobbling and wriggling. When the cells of the mixture were inoculated into broth and incubated at 37°C, the fraction of motile cells increased rapidly, reaching 13% within 20 min without appreciable increase in cell number. Thereafter, the fraction of motile cells decreased in inverse ratio with increasing cell number.

These results indicate that exogenous flagellin monomers can be reconstituted to flagellar filaments at the tip of flagella attached to the living cells, and that the reconstituted flagella on the bacterial body can exert their locomotive function. (The details were submitted to Nature)

Possible Differential Control of Flagellin mRNA Synthesis in *Bacillus pumilus* Depending on Cell Age

Hideho SUZUKI

The cell-free flagellin synthesizing system, composed of RNA from *B. pumilus* and preincubated S-30 from *E. coli* Q13 was developed, whereby incorporation of ^{14}C-amino acids into the flagellin fraction represented about 0.2% of total incorporation (Suzuki, H. and H. Koffler, 1970. Bacteriol. Proceedings 70:23). However, in some reproduced experiments, the RNA preparation, highly active in stimulating amino acid incorporation into general protein, was not always active for amino acid incorporation into the flagellin fraction. Amino acid incorporating activity of an RNA preparation could be affected by the age of the cells from which the RNA was extracted. Therefore, incorporation into the flagellin fraction was examined by using RNA preparations extracted from cells either at an early or at a late logarithmic growth phase.

B. pumilus cells were grown in a Penassay broth medium. RNA was extracted by the phenol method from lysozyme-lysate. Thirty O.D. units/ml of the 2.5 M sodium acetate-insoluble RNA fraction served as the source of mRNA in the reaction mixture of protein synthesis, which contained 2.5 μc/ml of ^{14}C-fifteen amino acid mixture. Newly synthesized flagellin was recovered with carrier flagellin from pH 2 soluble fraction, purified through reaggregation and chromatographed on a DE-32 (Whatman) column.

The RNA extracted from aged cells stimulated amino acid incorporation

into the flagellin fraction at least several times as strongly as that extracted from young cells, even when incorporation into total TCA-precipitable materials by the former fell to a low level. This suggests that flagellin synthesizing ability of the cells varies with their age. Messenger RNA for flagellin may be produced at a higher rate or readily accumulated upon aging. Glucose effect might offer another explanation. Glucose contained in the Penassay medium may repress the synthesis of mRNA for flagellin. Upon exhaustion of glucose, cells may produce mRNA for flagellin at a maximum rate. RNA prepared from cells grown in the absence of glucose exhibited moderately higher activity with respect to incorporation into the flagellin fraction. However, trials to demonstrate the *in vivo* repression of flagellin synthesis by glucose were unsuccessful. Even in an enriched medium, growth of *B. pumilus* was worse in the absence of glucose than in its presence. An omission of glucose from the medium may produce somewhat unfavorable growth condition for the cells, e.g. leading to rapid aging.

Apart from those explanations, it may be expected that extraction of RNA from the cells at an appropriate growth phase could produce an RNA preparation enriched with flagellin mRNA.

Phage-induced Alteration of Colony Type in *Xanthomonas citri*

Wen C. WU and Tetsuo IINO

Xanthomonas citri XCJ19, infected with temperate phage PXC7, had its smooth colonies altered to dwarf colonies. The altered dwarf-colony clones were lysogenic. After incubation in nutrient broth for 72 hr, each of them reverted in 0.1–6% of cells to the production of smooth colonies, among which 60% were lysogenic, 25% resistant, and 15% sensitive. The lysogenic dwarf-colony clones could not be recovered to produce smooth colonies by supplements of amino acids, purines, pyrimidines, vitamins or nucleic acid bases; they did not change qualitatively in sensitivity to phages CP_1 and CP_2, fermentation of mannitol and lactose, starch hydrolysis, catalase activity, and somatic antigens; however, they lysed and liberated spontaneously phage PXC7 at a considerably higher frequency, and decreased their growth rate and the number of bacterial cells contained in their colonies, as compared with either their lysogenic smooth revertants or their

smooth original strain. These results indicate that *Xanthomonas citri* XCJ19, when lysogenized with phage PXC7, underwent spontaneous lysis and liberation of the phage at a considerably high frequency that resulted in the alteration from smooth to dwarf colonies.

Genetic Analyses of Nonmotile Double Mutants in *Salmonella typhimurium*

Masatoshi ENOMOTO

A number of nonmotile-double mutants, *mot*$^-$*fla*$^-$, *mot*$^-$*mot*$^-$ and *mot*$^-$*ahl*$^-$, have been isolated from three parental *mot* mutants, *motA*$^-$, *motB*$^-$ and *motC*$^-$, of *Salmonella typhimurium* and used for mapping relative positions of *mot*, *fla* and *H1* genes. The inferred order is as follows: *his*-(*flaB*, *flaD*) -*flaA*$_3$-*motC*-*flaA*$_2$-(*flaA*$_2$, *flaA*$_1$)-(*H1*, *ah1*)-(*flaK*, *flaE*, *flaJ*)-(*motA*, *motB*)-*flaC*-*trp*. The number of abortive trnasductants issuing from the double mutants in transduction with the wild-type strain is in inverse proportion to the distance between two mutations. In this case one mutation should be located between *flaB* and *ah1* (left side) and the other between *flaK* and *flaC* (right side), and the number of abortive transductants is very few as compared with that produced by the double mutants whose two mutations are together located in either the left side or the right side. This phenomenon is useful for genetic mapping as a new tool and for studying the mechanism how the transducing fragments arise from the bacterial chromosome. Details will be published in Genetics.

A New Transducing Phage Related to P22 of *Salmonella typhimurium*

Masatoshi ENOMOTO and Hiromi ISHIWA

Properties of a new general transducing phage, PSA68, isolated from a commercial digestive are studied in comparison with other general transducing phages of Salmonella, L, MG40 and P22. The morphology, the latent period and average burst size of PSA68 are indistinguishable from those of P22. This phage has most resemblance to L serologically and to MG40 in immunity properties; PSA 68 and MG 40 are dominant over L, and P22 is dominant over the other three phages. The buoyant density of PSA68

is 1.510 g/cm^3 identical to L and MG40, that of P22 being 1.520 g/cm^3. The relative number of abortive transductants produced by nonmotile-double mutants are not significantly different among the four phages, though the transduction frequencies per p.f.u. are different from each other. This means that these transducing phages carry the genetically identical length of chromosome fragments. Details will be published in J. gen. Virology.

III. BIOCHEMICAL GENETICS

Purification and properties of tryptophan pyrrolase in *Ephestia*

Saburo NAWA

The mutant *a* suffers from the inability to convert tryptophan to formylkynurenine, which is the precursor of ommochromes, the brown eye pigments. It is known that this conversion is catalyzed by an enzyme, tryptophan pyrrolase. As reported previously, we obtained individuals of wild-type eyes in experiments where larvae or eggs, having the recessive genotype *a*, had been treated with DNA from the wild type. Therefore, it is of interest to examine whether the transformants produce the enzyme normally. Although tryptophan pyrrolase has been assayed in unfractionated homogenates, purification may be necessary to examine the properties of the enzyme in detail.

Newly hatched adults of wild type were homogenized in the cold with phosphate buffer. After centrifugation, the protein was precipitated from the clear supernatant fraction by the addition of solid ammonium sulfate to 45% saturation. The resulting pellet was redissolved, dialyzed, and then treated with calcium phosphate gel by step-wise elution. The most active fractions were applied to DEAE-Sephadex column and developed with a linear gradient of NaCl, 0.05 to 0.3 M, in phosphate buffer. Activity was routinely eluted at about 0.25 M NaCl. Approximately 80-fold purification was achieved with an over-all yield of about 10%. The enzyme can be assayed by measuring kynurenine formation from tryptophan on condition that the added kynurenine formamidase, the enzyme which converts formylkynurenine to kynurenine, is not rate limiting. The purified enzyme was unstable, gradually losing activity upon storage at −20°C: about 10% of activity remained after one week and no activity was observed after one month. In conditions used for the assay, kynurenine formation was shown to be proportional to time only for one hours at 37°C and two hours at 25°C. The absolute requirement for methylene blue was observed with the purified enzyme. Thus, there seems to be a fundamental difference between *Ephestia* tryptophan pyrrolase and the enzymes from other organisms such as rat,

Drosophila or *Pseudomonas* which do not require methylene blue. Furthermore, unlike *Drosophila* tryptophan pyrrolase, no stimulation by ascorbate was observed for the purified enzyme. Tryptophan pyrrolase was also purified from transformants. It was observed that the enzymes had the same properties as the wild-type enzyme.

Characterization of DNA from eggs of *Ephestia*

Masa-Aki YAMADA

It has been reported that the DNA from eggs at an early developmental stage was heterogeneous and consisted of a major component and a minor component (Annual report No. 20). In order to ascertain the localization of these components, the homogenates of eggs were fractionated into two parts, one containing the cell and nucleus fraction (CN), and the other containing the mitochondria and yolk grain fraction (MY). Eggs ranging from 0 to 18 hours after they were laid were gently homogenized in 0.03 M Tris-HCl buffer containing 1 mM EDTA and 0.25 M sucrose, filtered through gauze, and centrifuged at 1,500 rpm for 20 minutes. It was found that the precipitate (CN fraction) was contaminated with a small amount of mitochondria and yolk grains. MY fraction was precipitated from the supernatant at 12,000 rpm for 20 minutes. DNA was extracted from each of those fractions with Tris-EDTA-phenol method, treated with RNase and α-amylase, followed by deproteinization with chloroform. The density of DNA was analyzed by using Spinco E.

It was found that the CN fraction contained only one component ($\rho=$ 1.699) having the same density as adult DNA. DNA from MY fraction consisted of a major ($\rho=1.698$) and a minor component ($\rho=1.680$). It seems that the minor component corresponds to that of whole egg DNA, indicating that it is specifically located in MY fraction. In addition to those components, two weak bands having densities of 1.677 and 1.668 were detected in MY fraction. Their properties are at present unknown.

There seems to be a difference between the density of DNA from CN (1.699) and that of the main component from MY (1.698). Though the difference is quite small, it was always observed in repeated experiments. Thus, it is probable that those are different DNA s. Moreover, it has been previously observed that the distribution of the main 1.698 fraction of

whole egg DNA was asymmetrical in density gradient, indicating that the fraction is heterogeneous. Therefore, it is reasonable to assume that the main fraction of whole egg DNA consists of two components, one from CN and the other from MY.

Hereditary change in silkworm with DNA

Saburo NAWA, Mitsuo TSUJITA and Masa-Aki YAMADA

Previous communications have reported that treatment of silkworm larvae of w_1 genotype with DNA from wild-type produced wild-type individuals (Nawa, *et al.* 1971. Genetics 67: 221). In order to know whether other loci are responding to such treatment, a triple recessive genotype, *lem*; *oc*; *pe* was used for the same experiment. In addition, the use of this mutant was valuable for the examination of a possible contaminant.

Using several samples of wild-type DNA and *lem*; *oc*; *pe* DNA prepared separately, a series of independent treatments of *lem*/*lem*; *oc*/*oc*; *pe*/*pe* larvae was carried out. Out of 5,726 larvae injected with wild-type DNA, 819 individuals were raised to adults. No eye color mutant was obtained. From the crosses of the treated adults, either female or male, to untreated *lem*; *oc*; *pe*, eggs from 187 batches on the whole were examined. No pigmented egg was obtained. However, when some of the white eggs were raised to adults, two black-eyed moths were obtained among 3,365 adults (BF_1). They behaved as if they had the genotype *lem*/*lem*; *oc*/*oc*; pe^+/*pe*, since they produced *lem*/*lem*; *oc*/*oc*; pe^+/*pe* and *lem*/*lem*; *oc*/*oc*; *pe*/*pe* in the proportion of 1:1 when crossed with *lem*; *oc*; *pe*. The appearance of the heterozygote in respect of the *pe* locus may be explained by transformation of the gene by incorporated DNA. The possibility of contamination in this case can be excluded by the fact that they were homozygotes for both *lem* and *oc* loci. If there had been an accidental genetic contamination produced from the cross of *lem*/*lem*; *oc*/*oc*; *pe*/*pe* to wild type, they would have had the genotype lem^+/*lem*; oc^+/*oc*; pe^+/*pe*. The line which could be suspected of being a source of contamination in this case should have been *lem*/*lem*; *oc*/*oc*; pe^+/pe^+. However, no such line was reared in our laboratory.

Some BF_1 animals, which were pink eyed, were crossed to untreated *lem*; *oc*; *pe*. In this 2nd backcross generation, one egg batch which contained

two black eggs among 345 white eggs was obtained. This gives a very low frequency, since a total of 1,418 batches were examined. Although one of the black eggs did not hatch, another black egg developed into a black-eyed moth which behaved as if its genotype was *lem*/*lem*; *oc*/*oc*; pe^+/*pe*. The white eggs from the same batch developed into pink-eyed moths. However, a cross (9 pairs) between the pink-eyed moths again produced one egg batch which contained one black egg among 445 white eggs. This may represent the persistence of introduced pe^+ information in unexpressed form in some cell lineages. Similar results to those have been observed in the experiments using w_1. No black egg or black-eyed moth was obtained so far in the experiments where DNA from *lem*; *oc*; *pe* was injected, though the number of treatments was rather small. It should be noted that no instance of a change in respect of the genes *lem* and *oc* was obtained so far.

Three allelic isozyme variants in rice peroxidase

Chiang PAI and Toru ENDO

Genetic studies on peroxidase isozyme variants have been reported for several plants, *e.g.*, morning glory, corn, oats and wild rice, *Oryza perennis*. With the exception of *O. perennis* the genetic behavior of those isozyme variants has shown the absence of hybrid bands in the heterozygotes. In the previous papers (Shahi *et al.* 1969. Japan. J. Genet. 44: 321, Endo 1971. *ibid.* 46: 1), two co-dominant alleles, Pe^{2A} and Pe^{4A} at the *Pe* locus which specify rice peroxidase isozymes, were reported for two rice strains, W107 and W1294. Also, it was noted that the intensity ratio of the three isozyme bands deviated from the expected distribution pattern in the lemmas and paleas of the F_1 plants and most of the F_2 heterozygous segregants.

Now we have detected a third alle, Pe^{OC} specifying the band OC. The three parental strains, W593, T65 and W120–4′, each had one major band, cathodic OC, anodic 2A or 4A, respectively, in the lemmas and paleas. The three F_1 plants among them, *i.e.*, T65×W593, T65×W120–4′ and W593×W120–4′. Each produced not only the bands characteristic for both parents but also a hybrid band having intermediate mobility. It was noted, however, that the intensity ratios of the three bands in those F_1 plants almost followed the distribution expected from random association of the monomers, in contrast to that of the F_1 plant between W107 and W1294.

As previously suggested (Endo 1969. this report, 20: 45), it could be assumed to be some factor or factors affecting the alleclic expression of the *Pe* locus, resulting in a deviated distribution isozyme pattern in the latter strains.

Lethal effects of a genetic abnormality in the pteridine metabolism of silworm larvae

Mitsuo TSUJITA and Susumu SAKURAI

It has been known that embryos homozygous for the *lem*^{l} gene produced from sib-mating individuals having the genotype $+^{lem}/lem^{l}$ develop normally to young black larvae which hatch by chewing up their egg chorion and grow normally until the 1st molting. Immediately after the 1st molting normal larvae become blackish due to melanin biosynthesis in their hypodermal cuticle. However, molted lethal lemon larvae (lem^{l}/lem^{l}) are distinctly yellow due to the presence of a large amount of sepiapterin in their hypodermal cells. In addition, they can not chew up mulberry leaves and, thus, starve to death. Yellow lethal larvae can also be recognized at the beginning of the 2nd instar from the cross $+^{lem}/lem^{l} \times lem/lem^{l}$. However, from the cross $lem/lem^{l} \times +^{lem}/lem^{l}$ or $lem/lem^{l} \times lem/lem^{l}$, all homozygous lem^{l}/lem^{l} embryos develop normally, but become partly yellowish and partly yellowish brown at the stage of body pigmentation. They can not chew up their egg chorion and die inside the eggs, which show the yellowish brown dying larvae through the semi-translucent shells.

Based on several biochemical experimental results, the inability to chew up mulberry leaves or egg chorion has been assumed to be due to incomplete hardening and imperfect differentiation of the cuticle, especially the mandibular cuticle of the mouth organ, which may be caused by abnormal phenylalanine and tyrosine metabolism.

Since it was found that in the YD_4 strain young homozygous lem^{l}/lem^{l} larvae can be discriminated from normal ($+^{lem}/lem^{l}$ and $+^{lem}/+^{lem}$) larvae by their body color at the stage immediately after hatching, the activity of phenol oxidase, *i.e.*, the rate of dopachrome production from dopa and from tyrosine (Tsujita and Sakurai 1971) was examined.

At the beginning of the 1st instar, normal and homozygous lem^{l}/lem^{l} larvae showed almost the same enzyme activity. However, in a later

stage of the 1st instar and the stage immeidately after the 1st molting a significantly weaker enzyme activity was detected in lethal lemon larvae than in normal larvae. Furthermore, it was shown that lethal lemon larvae have a weaker activity of phenylalanine hydroxylation than normal larvae.

It seems that the maternal effect of $+^{lem}$ gene on sepiapterin reductase within the homozygous *lem/lem* eggs laid down by the $+^{lem}/lem^{l}$ female moth (Tsujita 1961) has close relation to normal phenylalanine and tryosine metabolism by which homozygous embryos are able to finish their complete development and a sufficient differentiation of their cuticlular layer.

Genetic and biochemical studies of letahal albino larvae of the silkworm, *Bombyx mori*

Mitsuo TSUJITA and Susumu SAKURAI

Lethal albino larvae appear immediately after the 1st molt. Since *al* is a simple recessive gene, from sib-mating of $+^{al}/al$ individuals normal ($+^{al}/+^{al}$ and $+^{al}/al$) and lethal albino (*al/al*) larvae segregate in the ratio of 3:1. Doira *et al.* (1970) showed that the *al* gene locates on the 5th chrosome (20.5 or 42.9). The phenotype of lethal albino larvae resembles that of lethal lemon larvae. In both mutants a small amount of melanin is produced in the cuticle layer of the hypodermis and their mandibular cuticle does not develop sufficiently due to a defective phenylalanine metabolism, so that they can not chew up mulberry leaves and starve to death after the 1st molting. These two mutants differ only in body color, namely, the lethal albino larvae are partly light brown and partly white and their hypodermal cells contain a large amount of pteridine granules filled with pteridines and uric acid. However, they showed an abnormal pteridine metabolism.

It is well known that in normal larvae uric acid and isoxanthopterin are accumulated in their hypodermis. However, in lethal albino larvae the amount of uric acid is comparable with that found in normal larvae, while the amount of isoxanthopterin is much smaller in *al/al* larvae than in the normals and they show an accumulation of an unknown fluorescent substance. It is considered from several biochemical experimental results that in lethal albino larvae some step of sepiapterin formation is blocked in the main pteridine metabolism of the silkworm.

A spectrophotometric assay with L-tyrosine or L-dopa as the substrate revealed that *al/al* larval extracts have a significantly lower phenol oxidase

activity than $+^{al}/+^{al}$ larval extracts, and the activity of phenylalanine hydroxylase is weaker in *al/al* larval extracts than in $+^{al}/+^{al}$ extracts.

So far as our experimental results thus far obtained are concerned, we could not show an accurate evidence of direct control of phenol oxidase production by the $+^{al}$ gene. Therefore, it seems that in lethal albino larvae abnormal phenylalanine and tyrosine metabolisms which seriously effect the differentiation of the hypodermal cuticle are secondary phenomena caused by the primary defective pteridine metabolism quite like in lethal lemon larvae.

The phenotype of double recessive lethal (*lem^l/lem^l; al/al*) larvae of the silkworm

Mitsuo TSUJITA

In order to elucidate the phenotype of double recessive (*lem^l/lem^l; al/al*) homozygotes, segregation from the cross $+^{lem}/lem^l$; $+^{al}/+^{al} \times +^{lem}/+^{lem}$; $+^{al}/al$ was examined in the F_2, F_3 and F_4 generation. Experimental results are given in Table 1.

Table 1. Segregation from the cross $+^{lem}/lem^l \times +^{al}/al$

Filial generation	No.	No. hatched larvae	Hatching rate	Phenotype of larvae			Total
				Normal	*lem^l/lem^l*	*al/al*	
F_2	2	434	97.0	238	72	98	408
	18	460	94.3	225	69	99	393
F_3	1	301	95.3	159	53	70	282
	3	276	95.3	150	49	63	262
	15	373	93.8	200	63	83	346
	16	342	97.1	187	60	83	330
	19	446	98.7	245	81	110	436
F_4	3	417	96.9	210	66	89	365
	5	203	84.2	116	35	47	198
	12	348	85.6	161	50	66	277
Total				1891	598	808	3297
Theoretical				(*al/al*+*lem^l/lem^l*; *al/al*)			Total
segregation				1855	618	824	3297
ratio				9	: 3	: 3	: 1
				9	: 3	: 4	
						$\chi^2=1.66$	
						$0.5>P>0.25$	

Table 1 shows segregation in normal, lethal lemon and lethal albino larvae in the ratio 9:3:4. It is clear that the double recessive homozygous larvae have lethal albino phenotype, indicating the accumulation of an unknown pteridine compound in their hypodermal cells.

Furthermore, it was confirmed that double recessive (*lem*/*lem*; *al*/*al*) larvae which segregated from the cross *lem*/*lem*; $+^{al}/+^{al} \times +^{lem}/+^{lem}$; $+^{al}/al$ had also lethal albino phenotype, showing no accumulation of sepiapterin in their hypodermal cells.

These experimental results present evidence in support of the idea that in lethal albino larvae the formation of sepiapterin is blocked in their main pteridine metabolic pathway and that they lack the function of tetrahydropteridine quite like in lethal lemon larvae (Fig. 1).

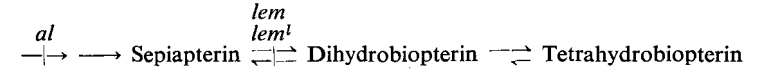


Fig. 1. A possible main pteridine metabolic pathway from sepiapterin to isoxanthopterin in the silkworm.

Genetic variation observed in membrane proteins isolated from pteridine granules of several oily mutant strains of the silkworm

Susumu SAKURAI and Mitsuo TSUJITA

W–C, C124 and E-lemon were used as normal strains with non-transparent larval skin, and w^a, w^b, w^{ol}, w^{oa}, w^{oh}, *oa* and *oc* were used as oily mutant strains with semi-transparent or transparent larval skin. Pteridine granules were isolated from the hypodermal cells of normal strains and oily mutants on the 4th day of 5th instar. The cytoplasm of the hypodermal cells which contains the granules were homogenized with teflon-glass homogenizer in 8.56% sucrose containing 1 mM $NaHCO_3$ and 5 mM EDTA, pH 7.5 ($NaHCO_3$–EDTA-sucrose). After homogenization in $NaHCO_3$–EDTA-sucrose, unbroken cells and floating cell components of the hypodermis were removed by filtration through a nylon cloth. The resultant filtrate was layered over discontinuous density gradients consisting of 20, 25, 30, 35, 40, 45, 50, and 80% (w/v) sucrose and was centrifuged in the Tominaga No. 7 S W rotor at 3,000 rpm for 2 hrs at 0–4°C.

Using the above method, the normal pteridine granules formed a compact zone at the 50%/80% interface, while the abnormal ones of w^{ol} and w^{oa} oily mutants did the same at the 40%/45% interface. On the other hand in the case of w^{a} oily mutant the specimen was collected from the upper layer of 8.56% sucrose. After washing once with 0.01 M sodium phosphate buffer, pH 7.0, containing 5 mM EDTA and 5 mM 2-mercaptoethanol, the pellet was resuspended in a solution containing 0.8% sodium dodecyl sulfate (SDS), 0.025 M Tris, 0.3 M NaCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, pH 8.5. The suspension was incubated at 37°C for 2 hrs to solubilize the membranes. Insoluble material was then removed by centifugation. The soluble sample was dialyzed overnight at room temperature against 0.1% SDS, 0.1 M NaCl, 1 mM 2-mercaptoethanol (SDS-NaCl-EDTA-2-mercaptoethanol) pH 8.5 and the dialysed solution was chromatographed at room temperature through a 2.4×50 cm column of Sephadex G 75 equilibrated with SDS-NaCl-EDTA-2-mercaptoethanol. The protein fraction eluted at the void volume was then applied to a 4.0×55 cm column of Sephadex G 200 equilibrated with same buffer at room temperature. The effluent protein peak was collected and dialysed overnight against 0.025 M Tris, 0.1 M NaCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, pH 8.5. The pH of dialysed solution was lowered to 5.5–5.7 by adding 1% acetic acid and the resulting precipitate after standing overnight at 4°C was collected by centrifugation at $9,000 \times g$, freeze-dried and stored at −20°C. The membrane protein fraction of pteridine granules consisted of equal shares of lipid and protein. Protein, lipid and carbohydrate content as well as its amino acid composition of the delipidated membrane proteins obtained from normal pteridine granules are very similar to those obtained from the abnormal pteridine granules of oily mutants. Freeze-dried membrane protein obtained from a normal strain was dissolved in 1% SDS in 0.05 M Tris–0.38 M glycine buffer containing 8 M urea, 10 mM EDTA and 10 mM 2-mercaptoethanol, pH 8.8 and incubated at 37°C for 1 hr.

The solution was analyzed by 7.5% polyacrylamide gel in 0.05 M Tris-0.38 M glycine buffer containing 0.1 % SDS, 5 M urea, 1 mM EDTA, 1 mM 2-mercaptoethanol, pH 8.8 (SDS-urea polyacrylamide).

Electrophoresis was carried out at 5 ma/gel at 5–10°C. A single band was observed, indicating that the membrane protein of normal pteridine granules was composed of an electrophoretically homogeneous protein component. However, it was revealed by SDS-urea polyacrylamide gel

electrophoresis that the SDS-urea treated pteridine granule membrane proteins of oily mutants were dissociated into multiple protein bands. Two or seven separate bands were observed by the following multiple gel analysis. These results show that the electrophoretic patterns of proteins obtained from several oily mutant larvae not only differ from that of the protein obtained from the strain with non-transparent skin, but also differ from each other among oily mutant strains studied. It has been known (Tsujita and Sakurai 1967) that the size and shape of pteridine granules produced in hypodermal cells of oily mutant larvae are abnormal, and the amount as well as the density of the mature granules are much smaller than in the normal strain. Thereby, it is considered that the transparency of larval skin of w^3 multi-allelic oily mutants and the complexity of the electrophoretic pattern of the membrane protein obtained from these oily mutants are both due to an alteration of structural protein components resulting in an abnormal structure or assembly of those pteridine granule membranes.

Immunochemical studies of structural membrane proteins of pteridine granules in hypodermal cells of silkworm larvae

Susumu SAKURAI

Oily mutant strains, w^3, w^a, w^b, w^{oa} and *oc* with semi-transparent or transparent larval skin and normal larval strains, W–C, C124 and E-lemon with normal granules and non-transparent skin were used. It has been known that the mutated loci of these strains, w^a, w^b and w^{oa} belonging to w^3 multiple allelic series are located on chromosome 10, and that of *oa* on chromosome 14.

Pteridine granules were isolated from the hypodermal cells of normal strains and oily mutants using discontinuous sucrose density gradients as described in the preceding report. The pellet was dissolved in a solution with PH 9.0, containing 1% sodium deoxycholate, 0.01 M $NaHCO_3$, 0.3 M NaCl, 1 mM EDTA, 1 mM 2-mercaptoethanol and 0.01 M ammonium formate and allowed to stand at 37°C for 2 hrs to solubilize the membranes. The protein fraction of the membrane was purified by several procedures such as precipitation at isoelectric point and gel filtration in Sephadex.

Antisera against the membrane proteins obtained from normal and abnormal pteridine granules were prepared by immunizing rabbits with them.

The immunization was done by intraperitoneal and intravenous injection of the antigens. Ouchterlony double diffusion test was performed in Petri dishes with 0.8% agar in 0.01 M ammonium formate buffer, PH 7.3, containing 0.01 M NaHCOi, 0.9% NaCl, 1 mM EDTA and 1 mM 2-mercaptoethanol, added with 0.01% sodium ethylmercurithiosalicylate for preservation. At immunodiffusion, the antiserum to E-lemon membrane protein produced a single precipitin band with normal membrane proteins obtained from C124 and W–C as well as with the homologous antigen. The membrane proteins of E-lemon and *oa* oily mutant gave a complete identical precipitin line with normal membrane antiserum. Antiserum to w^a abnormal membrane protein reacted with two precipitin bands against the homologous antigen, one of which was revealed to be partially in common with the band between anti-E-lemon serum and E-lemon protein. A similar common line was also seen at immunodiffusion between antiserum to E-lemon membrane protein and the membrane proteins of w^3, w^b, and w^{oa}. Anti-w^a membrane protein antiserum reacted against abnormal membrane proteins of w^3, w^a and w^b all with two precipitin bands but of slightly different pattern from one another.

It is therefore proposed that the $+w_3$ gene located on chromosome 10 is the structural gene for a membrane protein subunit. The results of immunodiffusion test suggest that immunological differences of membrane proteins of w^3, w^a and w^b are due to a defect of the primary structure of membrane protein subunits.

Differences in Amino Acid Composition of Serum Transferrin among various Species of *Rattus*

Kazuo MORIWAKI, Tamiko SATO and Kimiyuki TSUCHIYA

Amino acid composition of serum transferrin was analyzed in various species of *Rattus* obtained from South East Asia and Oceania. Transferrin was purified through several steps; acrinol treatment of the serum followed by DEAE-cellulose column chromatography of the soluble fraction, starch block electrophoresis of DEAE-separated β-globulin fraction and finally gel-filtration through Sephadex G–200 column. Purified transferrin was hydrolyzed and analyzed as to the amino acid composition using automatic amino acid analyzer (JEOL Model JLC–5AH and Hitachi Model KLA–3B).

Residue number of each amino acid per mole was computed by assuming that the molecular weight of transferrin is approximately 67,000. Difference in the number of each amino acid residue among various species is summarized in Table 1. These differences may represent, to a considerable extent, the differences in amino acid sequence among these species. In order to support this notion, composition difference and sequence difference in a single species of protein were compared among various kinds of higher organisms. If we use the data on cytochrome-c as a typical example (Dayhoff, M. O.: Atlas of protein sequence and structure, vol. 4, 1969), almost a linear relationship between composition difference and sequence difference can be observed in the range less than 40 amino acid differences. This rpobably indicates that differences in amino acid composition of certain specific

Table 1. Total difference in the number of each amino acid residue of serum transferrin among various species of *Rattus*

	R. rattus argentiventer (M)	*R. muelleri*	*R. rattus argentiventer* (C)	*R. norvegicus*	*R. rattus tanezumi* TfN	*R. rattus mindanensis*	*R. bowersi*	*R. rattus diardii*	*R. rattus tanezumi* TfR	*R. conatus*	*R. legatus*	*R. rattus rattus* (N.G.)	*R. exulans*
R. rattus argentiventer (M)		29	17	20	24	22	30	24	30	34	44	54	44
R. muelleri	29		24	24	26	24	20	28	34	30	42	50	62
R. rattus argentiventer (C)	17	24		22	30	22	32	32	38	28	44	49	59
R. norvegicus	20	24	22		22	24	28	32	32	44	48	60	42
R. rattus tanezumi TfN	24	26	30	22		30	20	34	18	58	44	60	48
R. rattus mindanensis	22	24	22	24	30		28	34	30	29	52	54	56
R. bowersi	30	20	32	28	20	28		28	34	48	48	56	56
R. rattus diardii	24	28	32	32	34	34	28		38	42	52	60	37
R. rattus tanezumi TfR	30	34	38	32	18	30	34	38		53	36	62	62
R. conatus	34	30	28	44	48	29	48	42	53		36	46	76
R. legatus	44	42	44	48	44	52	48	52	36	36		38	84
R. rattus rattus (N.G.)	54	50	49	60	60	54	56	60	62	46	38		88
R. exulans	44	62	59	42	48	56	46	37	62	76	84	88	
Total difference	372	392	397	398	404	405	418	441	467	514	568	677	704

Abbreviation. (M): Malaysia, (C): Celebes, (N.G.): New Guinea, TfR: R-type transferrin, TfN: N-type transferrin.

protein between two species can be used as a substantially meaningful tool for indicating distance index.

Serum Transferrin Polymorphism in Oceanian Black Rat, *Rattus rattus rattus*

Kazuo MORIWAKI and Tamiko SATO

Starch gel electrophoretic survey was carried out on serum specimens of 62 black rats collected from 4 localities in Oceania. Transferrin bands on the gel was identified by Fe^{59} labelling and autoradiography. Detailed analytical procedure has been reported by us (Moriwaki, K. *et al.*, Genetics 63: 193, 1969). Naming of transferrin phenotypes followed Malecha and Tamalin (Amer. Nat. 103: 664, 1969). Table 1 demonstrates the distribution of transferrin phenotypes in various localities of Oceania. That Oceanian *rattus* is markedly different from the Asian *rattus* in the karyotype has already been reported (Yosida, T. H. *et al.*, Jap. J. Genet. 44: 89, 1969): the former has 38 diploid chromosome numbers and the latter 42. In the present study, difference between both *rattus* was found also in the transferrin alleles. Oceanian *rattus* have Tf^C, Tf^D, Tf^E and Tf^F, whereas Asian *rattus* have Tf^R and Tf^N.

Frequencies of each allele in Oceanian materials studied were as follows. In Brisbane $Tf^C=0.33$, $Tf^D=0.60$, $Tf^E=0.03$ and $Tf^F=0.03$. In Cairns

Table 1. Distribution of transferrin phenotypes in Oceanian *Rattus rattus*.

Transferrin phenotype	Localities			
	Brisbane in Australia	Cairns in Australia	Wellington in New Zealand	Pt. Moresby in New Guinea
C	2	2	21	3
CD	4	5	2	2
CE	—	1	—	—
CF	2	—	—	—
D	8	—	—	8
DE	1	—	—	—
DF	1	—	—	—
EF	2	—	—	—
Total	18	8	23	13

Tf^C=0.83, Tf^D=0.08 and Tf^E=0.08. In Wellington Tf^C=0.96 and Tf^D= 0.04. In Port Moresby Tf^C=0.46 and Tf^E=0.54.

Serum Transferrin Patterns of *Rattus rattus* obtained from Southern India and Italy

Kazuo MORIWAKI, Tamiko SATO and Kimiyuki TSUCHIYA

Serum transferrin patterns of black rat, *Rattus rattus*, obtained from Mysore in southern India and from Rome, Italy were analyzed by starch gel electrophoresis. Identification of transferrin bands on the gel was carried out by Fe^{59} labelling and autoradiography. Detailed operational processes have already been reported (Moriwaki, K. *et al.*, Genetics 63: 193, 1969). In five *R. rattus*, kindly sent to us by Dr. T. Tachibana in India, two were *Tf-C* type and the remainder *Tf-CD* type. The nomenclature for the transferrin types is as described by Dr. Malecha for Hawaiian *R. rattus* (Malecha, S. R. and R. H. Tamalin, Amer. Nat. 103: 664, 1969). Two *R. rattus* obtained from Dr. Capanna in Italy contained one *Tf-C* and one *Tf-CD* types. These transferrin alleles, Tf^C and Tf^D, were also observed in the Oceanian *R. rattus* (Moriwaki, K. *et al.*, this Annual Report).

Thirty eight diploid chromosomes were observed in these Indian *R. rattus* (Yosida, T. H. and K. Tsuchiya, this Annual Report), and also in the Italian *R. rattus* (Capanna, E. *et al.*, Experientia, in press). The biochemical data obtained above seem to coincide with these cytogenetical findings in suggesting that European type *R. rattus* (*Tf*=*C*, *D*, *E* and/or *F*, $2n$=38) are distributed in southern India and Italy.

Studies on the transferrin of the Mongolian gerbil

Kazutoshi MAYEDA[1] and Kazuo MORIWAKI

The two dimensional electrophoresis of serum proteins of the Mongolian gerbil, *Meriones unguiculatus*, showed seven evenly spaced beta-globulin bands. In order to confirm that all seven bands were transferrin, autoradiographic study was carried out using radioactive $FeCl_3$. After tagging

[1] Supported by (N. S. F.) U. S.—Japan Cooperative Science Program; on leave from the Dept. of Biology, Wayne State Univ., Detroit, Michigan.

the transferrin with this substance, two dimensional electrophoresis showed all seven bands to be radioactive, indicating that they are transferrin.

Subsequently, the transferrin was purified by means of preparative electrophoresis and column chromatography. Physical characterization of the purified transferrin showed that the gerbil transferrin has a molecular weight of approximately 67,000, which corresponds well with other rodent transferrins such as the rat and the mouse.

The purified transferrin still maintained seven distinct bands upon starch gel electrophoresis and agar gel electrophoresis. In order to determine the effect of sialic acid on the electrophoretic mobilities, the purified gerbil transferrin was incubated in purified neuraminidase (Sigma Chemical Co.) for varying length of time. Subsequent electrophoresis showed that the mobility of all the bands were retarded, relatively, after such treatment. We were unable to obtain a single electrophoretic banding pattern for this protein even after prolonged incubation in neuraminidase.

In order to further characterize the observed seven bands, anti-serum to the purified gerbil transferrin was made by injecting it into rabbits. Subsequently, the purified gerbil transferrin was analyzed by the immuno-electrophoresis technique in agar gel. The precipitation pattern showed that all bands were antigenically identical.

Amino acid analysis of the purified transferrin was then carried out. As reported in the following article, the amino acid content of the gerbil transferrin does not seem to differ greatly when compared with transferrins of other rodents. The greatest difference seems to occur when the amino acid content of the gerbil transferrin is compared with those of the golden hamster. The least difference occurs between the gerbil transferrin and the mouse transferrin.

Comparative studies of the transferrins of rodents

Kazuo MORIWAKI and Kazutoshi MAYEDA[1)]

In a continuing study of the genetics of rodents, we have investigated the transferrin proteins obtained from several species of rodents. In order to make a thorough comparison, amino acid contents of the transferrins

1) Supported by NSF U. S.—Japan Cooperative Science Program; on leave from the Dept. of Biology, Wayne State Univ., Detroit, Michigan.

obtained from several different species of rodents were determined.

The molecular weight of the transferrins of all the rodents studied was approximately 70,000 by the technique of thin layer gel filtration analysis. This corresponds well with the published results of Gordon and Louis (Biochem. J. 88: 409, 1963) who reported that the molecular weight of the rat transferrin is 67,000. The method of purification of the transferrins is described in a previous article (Moriwaki, K. *et al.*, this Annual Report). Such purification process resulted in transferrins contaminated by other serum proteins by less than 5%. Amino acid analysis was carried out on Hitachi Model KLA 3B and JEOL Model JLC–5AH amino acid analyzers.

The results of the amino acid analyses are presented in Table 1. Generally speaking, there seems to be no great difference in the amino acid content of the transferrins from different rodents studied. In order to better visualize the extent to which differences exist, we have rearranged the data in Table 1 by making a pair-wise comparison as shown in Table 2. For example, comparing mouse with rat, the absolute number of amino acid difference between these two species is 20. Similarly, the number of amino acid difference between the transferrins of the golden hamster and gerbil is 74.

Table 1. Amino Acid Content of Various Transferrins

Amino Acid	Rat	Mouse	Apodemus	Peromyscus	Gerbil	Hamster
Lys	41	46	37	38	49	48
His	14	16	12	12	19	12
Arg	21	21	22	21	18	21
Asp	60	58	56	55	56	58
Thr	27	28	28	30	26	25
Ser	36	37	37	36	39	35
Glu	52	50	45	54	51	48
Pro	41	41	39	37	39	15
Gly	49	50	52	47	52	45
Ala	46	46	50	51	51	62
Cys	6	—*	—	—	—	9
Val	34	36	34	37	38	36
Met	5	3	5	6	2	7
Ileu	18	18	17	19	20	21
Leu	45	46	49	46	47	45
Tyr	20	16	26	27	18	27
Phe	26	28	25	25	22	28

* Not determined

Because of the technical errors involved with amino acid analyses, total amino acid difference of 20 is probably not significant. That is to say, the transferrins of the mouse and rat are probably identical. The mouse transferrin is relatively close to the transferrins of gerbil, peromyscus and apodemus but significantly different from the golden hamster transferrin.

Table 2. Number of Amino Acid Difference Between Species

	Rat	Mouse	Apodemus	Peromyscus	Gerbil	Hamster
Rat	—	20	40	36	40	78
Mouse	20	—	44	52	32	72
Apodemus	40	44	—	26	44	80
Peromyscus	36	52	26	—	52	60
Gerbil	40	32	44	52	—	74
Hamster	78	72	80	60	74	—
Total	214	220	234	226	242	364

IV. DEVELOPMENTAL GENETICS

Specific Inhibition by Glucosamine of Aggregate Formation of Rat Hepatoma Cells in Rotation Culture

Yukiaki KURODA

It is well known that malignant transformation of cells is accompanied by many biochemical, immunological, biological and morphological alterations of the surface cell membrane such as cellular adhesiveness, contact inhibition of movement and growth, electrophoretic cell mobility, also antigens and chemical compositions of the surface membrane. Recently some quantitative and qualitative changes in glycolipids or glycoproteins in the surface membrane were detected following malignant transformation. It has been previously found by the author that aggregate formation of dissociated cells was enhanced by the supernatant of the medium in which cells were cultured for a few hours and this supernatant contained proteins and hexosamines.

In the present experiment the effects of some hexosamines and their acetyl compounds on aggregation of DAB-induced rat hepatoma cells were examined in a rotation culture. Three rat hepatoma cell lines were used: dRLN–61, dRLa–74 and dRLh–84, which had low, moderate and high tumor-producing activity, respectively. The cell suspensions containing 3×10^5 cells in 3 ml culture medium were rotated under standard conditions in the presence of various hexosamines and their acetyl compounds at the concentrations of 1, 3, 10 and 30 mM.

Among the hexosamines tested D-glucosamine was most effective in reducing the diameter of cell aggregates from all three hepatoma lines. D-galactosamine and D-mannosamine had not any or a slight effect even at the concentration of 30 mM on the aggregate formation of hepatoma cells. Acetyl-D-glucosamine, acetyl-D-galactosamine and acetyl-D-mannosamine were also ineffective at the concentrations tested up to 30 mM. No differential effects were found of these hexosamines and their acetyl compounds on hepatoma cells having various tumor-producing activities.

Phytohemagglutinin Concanavalin A, isolated from the jack bean and known to agglutinate malignant cells, was also tested for affecting aggrega-

tion of rat hepatoma cells. No effect of Cancanavalin A was found at concentrations less than 0.1 mg/ml. At higher concentrations than 0.3 mg/ml Concanavalin A prevented completely aggregate formation of rat hepatoma cells in a rotation culture.

Growth-Stimulating Effect of Peptone on *Drosophila* Ovarian Cells in Culture

Yukiaki KURODA

In the series of experiments planned on a long-term cultivation of *Drosophila* cells, it was found that peptone had a pronounced growth-stimulating effect on *Drosophila* ovarian cells. Ovaries obtained from 48-hour pupae of the Oregon-R strain of *D. melanogaster,* which were reared under sterile conditions, were cut into several fragments and cultured in T–5 flasks with 0.8 ml of Kuroda's *Drosophila* medium K–6′ (Kuroda, Y. 1969. Japan. J. Genetics 44, Suppl. 1: 42; 1970. Exp. Cell Res. 59: 429), which was slightly modified and supplemented with 0.1 mg/ml fetuin and 15% fetal bovine serum.

Peptone (Wako Pure Chem. Ind. Ltd.; Pancreatic digest of casein) was tested at various concentrations for its growth-stimulating effect on ovarian cells. It was found that 5 mg/ml of peptone enhanced the outgrowth of fibroblastic cells from explanted ovarian fragments. At higher concentrations than 10 mg/ml, peptone had a slightly toxic effect on those cells.

In the presence of 5 mg/ml peptone fibroblastic cells initially issued from the cut end of ovarian fragments, stretched their cytoplasm on the surface of culture flasks, and formed a monolayer sheet around the original explants. Mitotic figures were frequently observed. Under a phase microscope some small granules were observed in the cytoplasm and one or two nuclei were found in the nucleus. From their morphology and behavior, these fibroblastic cells seem to be derived from the lumen cells in the ovariolar cavity.

Some insect cell lines established by Grace (1962. Nature 195: 788; 1966. Nature 216: 613) were also derived from ovarian tissues of *Antheraea eucalipti* and *Bombyx mori.* Although it is uncertain that Grace's cell lines originated from the same type of cells in the ovary as the fibroblastic cells observed in the present study, these ovarian cells may have some advantage in growing under *in vitro* culture conditions.

Evidence for the Restitution of Sorting-Out Activity of HeLa Cells Following Fractionated X-Irradiation

Yukiaki KURODA

HeLa cells have a characteristic sorting-out activity when intermixed with embryonic quail liver cells in a rotation culture. It has been previously found that the sorting-out activity of HeLa cells was quantitatively expressed by the number of quail liver cells intermingled in HeLa cell aggregates and that of HeLa cells in quail liver cell aggregates. These percentages of intermingled cells increased with gradually increasing doses of X-ray given to HeLa cells.

In the present experiment the presence of a restitution process in the loss of sorting-out activity of HeLa cells following X-irradiation appeared when HeLa cells were exposed to fractionated X-ray and the percentages of intermingled cells in aggregates of each type of cells were examined.

HeLa cells which were exposed to the first dose of 1,000 R X-ray were incubated for 2 or 4 hours, then exposed to a second dose of 1,000 R. These cells were intermixed with 7-day embryonic quail liver cells and allowed to form aggregates in a culture rotated for 24 or 48 hours. The harvested aggregates were examined for the number of both types of cells in the histological preparations. The results of such experiments are shown in Tables 1 and 2.

In the above tables it is indicated that the percentage of intermingled quail liver cells in HeLa cell aggregates and that of intermingled HeLa cells in quail liver cell aggregates markedly decreased when the irradiated

Table 1. Effect of fractionated X-irradiation expressed as the number of quail liver cells intermingled in 24-hour aggregates of irradiated HeLa cells

Dose	No. of total cells in aggregates*	No. of quail liver cells in aggregates*	Per cent of quail liver cells
0 R	52.3	4.3	8.2
1,000 R	77.0	13.3	17.3
2,000 R	50.0	17.3	34.6
1,000 R+1,000 R (Interval, 2 hr)	54.7	13.3	24.3
1,000 R+1,000 R (Interval, 4 hr)	56.7	16.0	28.2

Table 2. Effect of fractionated X-irradiation expressed as the number of irradiated HeLa cells intermingled in 24-hour aggregates of quail liver cells

Dose	No. of total cells in aggregates*	No. of HeLa cells in aggregates*	Per cent of HeLa cells
0 R	156.3	1.7	1.1
1,000 R	147.3	5.0	3.4
2,000 R	145.3	14.0	9.6
1,000 R+1,000 R (Interval, 2 hr)	150.7	7.3	4.8
1,000 R+1,000 R (Interval, 4 hr)	133.3	8.7	6.5

* The average numbers of total cells and intermingled cells in the largest sections through three different aggregates were calculated.

HeLa cells were incubated for 2 hours before the second X-ray exposure, followed by a slight increase when the cells were incubated for 4 hours.

Further Study of Relationship between Molting and Cell Cycle in Insect Epidermis

Kiyoshi MINATO

It has been previously found that the epidermal cells of larvae of *Philosamia cynthia ricini* may have a peculiar growth cycle: they do not divide after DNA synthesis in the same larval perid. DNA synthesis takes place synchronously prior to the period of the pause for molting of each larval instar, followed by cell division in the next larval instar after passing the relatively long G_2 period.

A previous autoradiographic analysis has ascertained the possibility of such a process. To confirm it further, a similar experiment was arranged. Fourth instar larvae were continuously labeled with H^3-thymidine and the incorporation into mitotic figures in various tissues was survayed during the same instar period.

As the result, mitotic figures of epidermal cells were scarcely labeled (1/115, 0.9%). On the contrary, the incorporation of H^3-thymidine into mitotic figures in the fat body, haemocytes, and trachea was frequently observed at the ratio of 85/226 (37.6%), 61/97 (63.0%) and 11/30 (36.7%), respectively.

These facts indicate that epidermal cells in mitotic stage were not labeled with H^3-thymidine when injected during the same instar period, although the incorporation of H^3-thymidine was observed in mitotic figures in other larval tissues. This result confirms the possibility that DNA synthesis takes place at the stage preceding the larval stage in which the cell division occurs.

Effects of Culture Media on the Survival of Embryonic Quail Liver-Specific Cells

Kiyoshi MINATO

It has been previously reported that tissue-specific cell colonies and non-specific colonies were isolated in the primary cultures of embryonic chick chondrocytes, retinal pigment cells and myogenic cells (Cahn, R. D. and Cahn, M. B. 1966. Proc. N. A. S. 55: 106; Coon, H. G. 1966. Proc. N. A. S. 55: 66). To isolate tissue-specific cells selectively, the following attempts were made.

Liver cell suspensions were prepared from 7-day or 10-day quail embryos by trypsinization and cultured in monolayer in TD-flasks or plastic petri dishes. Eagle's basal medium, minimum essential medium, Parker's TC-199 medium, and Ham's F-12 medium were tested with the supplement of 10% calf serum. Although small differences were found among the media tested, Eagle's minimum essential medium seemed to be most suitable for maintaining liver parenchymal cells. In this medium liver parenchymal cells became flattened after 1 or 2 days of cultivation and formed an epithelial cell layer. Giant cells with relatively transparent cytoplasm were frequently observed. No difference was found among the sera obtained from various manufactory sources. The adhesiveness of cells to glass or plastic surface was better in fetal bovine serum than in calf serum. In the former serum the parenchymal cells were not epithelial in shape and degenerated after 3 or 4 days of cultivation, although non-specific fibroblastic cells grew rapidly. For the maintenance of liver parenchymal cells calf serum seemed to be better than fetal bovine serum. Further investigations are carried out to find better culture conditions for the cells.

V. CYTOGENETICS

Karyotype evolution in *Rattus* species from black rat, *R. rattus*. 1. Species with a basic chromosome number, but with structural chromosome difference

Tosihide H. YOSIDA and Kimiyuki TSUCHIYA

Black rats, *R. rattus*, inhabiting wide area of East and Southeast Asia have $2n=42$ chromosomes, among which 13 pairs (No. 1 to 13) being acrocentrics of various sizes, 7 pairs being small metacentrics, and the rest pair being a large and a small acrocentric X and Y chromosomes. Among acrocentric group, No. 1, 9 and 13 pairs were found to be remarkably polymorphic, occurring in three chromosome types, namely an acrocentric homomorphic pair (A/A), a subtelocentric homomorphic pair (S/S) and an acrocentric and a subtelocentric heteromorphic pair (A/S). Based on the population survey of the frequency of chromosome polymorphism in the rat distributed in East and Southeast Asia, it is suggested that the acrocentric chromosomes in the three pairs are the basic karyotype, and that the subtelocentric chromosomes arose from the acrocentric ones by pericentric inversion. The following four species, namely the Norway rat, *Rattus norvegicus, Rattus exulans, Rattus annandalei* and *Rattus muelleri* have the same chromosome number ($2n=42$) and similar karyotype as the black rat. They have 13 acrocentric or subtelocentric autosome pairs and 7 small metacentric autosome pairs. External morphology of the Norway rat, *R. norvegicus*, is very similar to that of the black rat, and are distributed throughout the world. These animals are characterized by having No. 1, No. 9 and No. 13 subtelocentric homomorphic pairs. All *R. exulans* collected in Philippine, Indonesia, Thailand and Hawaii have $2n=42$ and similar karyotype to the black rat, but they are remarkable by having No. 1 and 9 subtelocentric pairs. *R. annandalei* collected in Malaysia is also characterized by having a similar karyotype to the black rat, but only No. 9 pair was subtelocentric. All 3 species have acrocentric X and Y chromosomes, but *R. muelleri* collected in Malaysia had a large metacentric X chromosome. This species had a basic *Rattus* karyotype with $2n=42$, but No. 1 and No. 9 pairs are usually subtelocentric. Based on the above comparative karyo-

type analysis in the four *Rattus* species, it is strongly suggested that the karyotypes in the above four species have arisen from chromosome polymorphism in the Asian black rat with $2n=42$.

Karyotype evolution in *Rattus* species, 2. Species with fewer chromosome numbers than $2n=42$ by Robertsonian chromosome fusion

Tosihide H. YOSIDA and Kimiyuki TSUCHIYA

It has been reported that *R. rattus* is classified into two geographical races (or subspecies) by chromosome numbers, $2n=42$ and 38, and further suggested that the latter evolved from the former by Robertsonian type of chromosome change. Those rats with 42 chromosomes are distributed in East and Southeast Asia, but those with 38 are found in Oceania, Europe and America. *Rattus fuscipes* which was collected in Brisbane, Australia, had $2n=38$ and the idiogram is very similar to that of *R. rattus* with $2n=38$. It is characterized by having two large metacentrics as found in Oceanian *R. rattus*. These chromosomes are suggested to have arisen by Robertsonian fusion of No. 4 and No. 7 and No. 11 and No. 12 acrocentrics numbered according to the basic karyotype with $2n=42$. Among six rats collected at Brisbane, two had $2n=39$ chromosomes which was remarkable by having one extra chromosome belonging to small metacentrics.

Five *Rattus* sp. collected in a jungle close to a suburb of Port Moresby, New Guinea, had $2n=34$ chromosomes. Four large metacentrics or submetacentrics which are suggested to have developed by Robertsonian fusion of Nos. 2 and 12, Nos. 3 and 6, Nos. 4 and 7, and Nos. 6 and 10 chromosomes observed in the basic karyotype of $2n=42$ are included. No. 9 pair is acrocentrics. Seven small metacentrics characterizing the basic karyotype of the black rat are observed in this species too. Four *Rattus conatus* collected in Cairns, Australia, had $2n=32$ chromosomes. They had five large metacentric or submetacentric elements. Biarms of those chromosomes seem to have arisen by Robertsonian fusion of Nos. 2 and 5, Nos. 3 and 6, Nos. 4 and 7, Nos. 8 and 11 and Nos. 10 and 12. No. 9 pair is submetacentrics. Seven small metacentric pairs quite similar to those of black rat are observed in these animals.

Both species, *R. fuscipes* and *R. rattus rattus*, have the same chromosome

number and similar karyotypes, but the former is native to Australia, the latter from Europe. They should have differentiated independently from the basic karyotype with $2n=42$ by the Robertsonian type of change. *R. conatus* and *Rattus* sp. are also suggested to have differentiated independently in Australia and New Guinea, because the constitution of the large biarmed chromosomes in the former is different from that of the latter. Interesting is the fact that No. 1 chromosomes which are characterized by acrocentric and subtelocentric polymorphism in the Asian black rats are usually subteleocentric in the above three species, but No. 9 pair is different depending on the species, namely, *R. fuscipes* and *Rattus* sp. showed acrocentric, but *R. conatus* subetlocentric.

Karyotype of Indian black rats, *Rattus rattus rufesens*

Tosihide H. YOSIDA and Kimiyuki TSUCHIYA

Black rats are roughly classified into two groups by chromosome constitution, namely Asian type with $2n=42$ and Oceanian type with $2n=38$. (Yosida *et al.*, 1969, Jap. J. Genet. 44: 89–91, Chromosoma 33: 30–40, 1971). The latter type *R. rattus rattus* is distributed in Oceania (Australia, New Zealand, New Guinea), North America, South America, Europe and Egypt. We suggested from data obtained by population survey and karyotype analyses that the black rats with $2n=38$ are derived from the Asian black rat with $2n=42$ by Robertsonian fusion of four acrocentric chromosomes. We also suggested that the black rats with the Oceanian type developed in the course of migration from Asia to Europe. Karyotypes of the black rats in India which is on the route of migration from Southeast Asia to Europe should be interesting to determine the karyotype evolution of this animal.

Three black rats were obtained by courtesy of Mr. Toshio Tachibana in Mysore of India. Chromosomes of these rats, named *Rattus rattus rufesens*, are clearly $2n=38$ chromosomes. Among them two pairs are large metacentrics as observed in Oceanian type rats. According to Ray-Chaudhudi and Pathak (1971) the animals collected in Varanasi located in Northern India had $2n=42$, but our material collected in Mysore located in Southern India had $2n=38$. From these observations, it is suggested that karyotype change in black rats occurred somewhere in the Indian district.

Studies of aneusomic clones isolated from a Chinese hamster cell line following induction of nondisjunction

Hatao KATO

A brief exposure of Chinese hamster cells to colcemid induced chromosomal nondisjunction. Although nondisjunction occurred randomly in every member of the chromosome complement, the viability of progeny cells differed markedly. Karyotype analyses of cells sampled at definite time intervals after induction of nondisjunction suggested strongly that cells with aneusomies of large chromosomes would die off or undergo karyotype remodeling by the time of the 3rd or 4th mitosis. The elimination of cells with unfavorable chromosome constitution appeared to begin as early as in the first cell cycle following the occurrence of nondisjunction, because we found no cell to lack the X chromosome in contrast to the frequent detection of those with two X's in 20-hour sample. On the other hand, cells with aneusomies of small chromosomes such as No. 9 or No. 10 (or No. 11) appeared to proliferate steadily.

Making use of this behavior, several trisomic as well as monosomic cell clones were isolated at a relatively high frequency from colcemid-treated cell population. Aneusomic lines now in our possession are as follows: No. 9 monosomy (one line); No. 10–11 monosomy (one line); No. 9 trisomy (2 lines); No. 10–11 trosomy (5 lines); No. 7–8 trisomy (2 lines); No. 4 trisomy (one line); No. 10–11 trisomy with two Y's (one line); No. 9 and No. 7–8 double trisomies lacking Y (one line).

Studies of differences in phenotypic expressions of these clonal lines are now in progress. Preliminary studies demonstrated a significant variations of UV sensitivity among clones with different doses of a specific chromosome type. (Details of this report will be published in Cytogenetics).

Distribution of two chromosomal types of Japanese wood mouse, *Apodemus speciosus*

Kimiyuki TSUCHIYA and Tosihide H. YOSIDA

Japanese wood mouse, *Apodemus speciosus*, which has a similar body size as the house mouse, is widely distributed in Japan. They are classified into two sibling forms by chromosome numbers, *i.e.*, $2n=46$ and 48 (Shimba *et al.*, 1969). The change is due to Robertsonian fusion of chromosomes.

In order to determine the distribution of these two forms, we collected 347 specimens in 66 different localities in Japan. The distributions of the two chromosomal types are strictly divided into two parts of northern and southern Japan. The borderline of the distribution is on a line connecting Toyama and Hamamatsu. Northern population from this borderline had $2n=48$, while southern population showed $2n=46$. Four animals collected in the Hamamatsu region, however, had $2n=47$ chromosomes which was determined to be a hybrid type between the two sibling forms.

The animals with $2n=46$ can not be distinguished from those with $2n=48$ by external morphology, blood transferrin pattern or other biochemical examinations, but the two forms are separated strictly into two geographic regions. To determine the ecology of the animals, we tried to breed them in captibity in laboratory cages. Although the animals survive in the cages for one or two years when fed commercial rat food, offspring could not be obtained. In a natural population room set up in the field, we succeeded in obtaining F_1 hybrids. They showed hybrid karyotypes with $2n=47$ chromosomes, similar to those obtained in Hamamatsu region.

Differences in Biochemical Characteristics between Two Sibling Forms of Japanese Wood Mouse, *Apodemus speciosus*

Kimiyuki TSUCHIYA and Kazuo MORIWAKI

As stated in a previous article in this Annual Report (Tsuchiya, K. and T. H. Yosida), two sibling forms of Japanese wood mouse, *Apodemus speciosus*, $2n=46$ and 48, are found in Japan. The former is distributed in the southern parts and the latter in the northern parts, both of which are indistinguishable from each other in morphological characters. In the present study we attempted to determine differences in biochemical characters between the two sibling forms, if any.

First, optimal temperature of liver acid phosphatase was compared. When the enzyme activity was assayed at pH 4.6 using para-nitrophenol phosphate as a substrate, the animals collected from 3 localities, Misima ($2n=48$, ♂), Tsushima ($2n=46$, ♂) and Kumamoto ($2n=46$, ♀) exhibited almost identical optimal temperature, 45°C.

Secondly, the whole serum proteins of the materials obtained from Kyoto ($2n=46$, ♂), Misima ($2n=48$, ♂) and Nopporo ($2n=48$, ♀) were

compared by agar-gel immunoelectrophoresis using rabbit anti-wood mouse whole serum ($2n=48$) antibody. All of the animals equally showed at least 11 precipitation arcs on the gel and no visible difference was detected in the corresponding arcs among them.

Thirdly, comparison of isozyme patterns of serum α-esterase was made between the northern populations ($2n=48$, 17 individuals) and the southern populations ($2n=46$, 32 individuals). Though no electrophoretic patterns definitely characteristic of either population have been found, an apparent difference in the average number of esterase bands was observed as follows: northern populations=4.1 bands, southern populations=3.7 bands.

Bottom half of starch-gel, upper half of which was used for demonstration of esterase isozymes mentioned above, was stained with amido black 10B for detection of serum proteins. Polymorphic features were observed in slow-α_2-macroglobulin and in certain β-globulin which was not iron (Fe^{59}) binding protein (transferrin), but these polymorphisms were not associated with either of the two kinds of *Apodemus* populations.

Lastly, difference in erthrocytic antigens between the two groups was checked by determining agglutination titer of erythrocytes obtained from groups. Anti-serum was prepared in rabbits by immunizing with erythrocytes of one type, $2n=48$ in this case. Four batches of northern group ($2n=48$) gave an equal titer of 1/24 and 4 batches of southern group ($2n=46$) 1/12, equally. This difference, however, does not seem to be significant.

Recently it has been revealed that another *Apodemus* species, *Apodemus miyakensis*, collected from Miyake island has similar 48 chromosomes as those of the northern type. These animals showed different electrophoretic mobilities in serum transferrins and albumin. Immunoelectrophoretic analyses of the whole serum using anti "48" rabbit serum demonstrated that one of the post-albumin proteins which can be detected in *Apodemus speciosus* is missing in this species.

Spontaneous sarcomas in black rats, *Rattus rattus*, bred in laboratory, and chromosome observations

Tosihide H. YOSIDA and Hatao KATO

In black rats, *Rattus rattus*, bred in the laboratory, three sarcomas, two fibrosarcomas and one hemangiosarcoma developed spontaneously. One

of the fibrosarcomas developed in one female (RRJ-497a) in the fourth generation of sister-brother mating in the Japanese black rats, and the other fibrosarcomas developed in a male (RA–2A) of the F_1 hybrids obtained by mating an Australian female (A–6) and a Japanese male rat (RRJ–373A). In the above female (RRJ-497a), the tumor developed in the peritoneal cavity as a solid tumor, and in the male, developed in the hypoderm at the left ventral site as a large tumor (about $35 \times 66 \times 77$ mm). The Japanese rat (RRJ–497a) had $2n=42$ and No. 1 A/A chromosomes, but the F_1 hybrid rat showed $2n=40$, being a hybrid karyotype between Japanese ($2n=42$) and Australian rat ($2n=38$). Chromosomes could be observed in bone marrow cells of the rats, but could not be successfully studied in the tumor tissues.

One hemangiosarcoma developed in the hypoderm at dorsal site in one female (RRT–519) in the sixth generation of sister-brother mating of the black rats collected in Korea. This rat had $2n=42$ and No. 1 A/A chromosomes. The chromosomes in the tumor tissue were successfully observed *in situ* and in cultivated cells. Remarkable feature was that most tumor cells had hypotetraploid chromosome numbers. The chromosome number of 101 cells observed were distributed widely from 42 to 167, but about 80 percent of the cells were at the hypotetraploid level (74 to 83). Among the 101 cells, those with 83 chromosomes showed the highest frequency (22 cells), cells with 80 chromosomes ranked second (14 cells) and those with 82 chromosomes ranked third (10 cells). Only five tetraploid ($2n=84$) and one hypertetraploid cells (85) were observed. Remaining seven cells were hyperdiploid (42 to 44), three cells were near triploid (63,68 and 69) and two cells were hypooctoploid (149 and 167).

Preliminary note on karyotype of guppy and topminnow

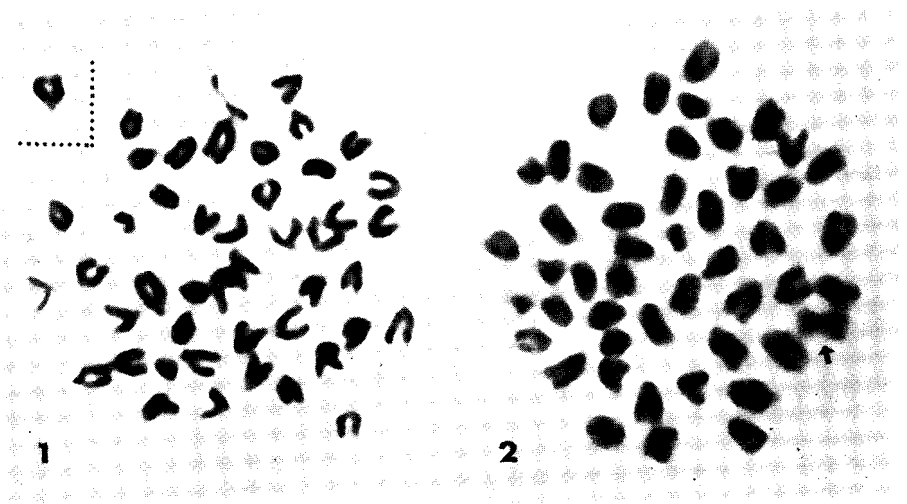
Tosihide H. Yosida and Makoto Hayashi[1)]

This paper is a preliminary note on the chromosome constitution of guppy, *Lepistes reticulatus* and topminnow, *Gambusia holbrook*. These two species belong to Cyprinodontidae, Teleostomi. Chromosomes of the guppy have been reported by Winge (1923) and Iriki (1932). According to these reports,

1) Department of Bioloby, Faculty of Science, Kwansei Gakuin University, Nishinomiya.

the diploid chromosome number in the fish is $2n=46$. Our observations carried out in female somatic cells agree with those observed by them. All 46 chromosomes are acrocentrics and the sex chromosomes are not recognizable in the somatic cells (Fig. 1).

Chromosomes of topminnow have been reported by Geiser (1924) who described that the fish has $2n=36$ chromosomes. The results of the present observation, however, differ markedly from that obtained by Geiser. Our observations revealed that the chromosome number in this fish is $2n=48$ in female somatic cells. Among 48 chromosomes, one large metacentric chromosome is remarkable in the female cell. The metacentric chromosome seems to be an X element (Fig. 2). Based on the above observations, it is suggested that the sex determining mechanism in the topminnow is a female heterogamety (XY). The sex chromosome mechanisms in these should be determined in more detail by further observations of male and female somatic cells.



Figs. 1 and 2. Metaphase chromosomes in female somatic cells.
1) Guppy, *Lepistes reticulatus*. 2) Topminnow, *Gambusia holbrook*. Arrow indicates the X-chromosome.

Chromosome survey of small mammals in Japan

Kimiyuki TSUCHIYA and Tosihide H. YOSIDA

Chromosomes in small mammals belonging to Insectivora, Chiroptera, Lagamorpha, Rodentia and Carnivora living in Japan were studied. The names of the species, diploid chromosome numbers, and the karyotypes are shown in the following table.

Species	2*n*	Karyotype*					
		M	SM	ST	A	(X)	(Y)
Insectivora							
Talpidae							
Dymecodon pilirostris	34	22	4	6		M	?
Urotrichus talpoides hondonis	34	22	4	6		M	m
Chiroptera							
Rhinolophidae							
Rhinolophus cornutus cornutus	62				60	ST	SM
R. ferrumequinum mikado	58	4			52	SM	?
Vespertilionidae							
Miniopterus schreibersi fuliginosus	46	6	2		36	SM	A
Lagamorpha							
Leporidae							
Lepus timidus ainu	48	10	6	10	20	M	A
L. brachyurus angustidens	48	10	6	10	20	M	A
Rodentia							
Sciuridae							
Petaurista leukogenys nikkonis	38	24	12			SM	A
Muridae							
Clethrionomys rutilus mikado	56	2			52	A	M
C. rufocanus bedfordiae	56	2			52	A	A
C. rex	56	2			52	A	ST
Aschizomys niigatae	56	2			52	ST	M
Eothenomys kageus	56	2			52	ST	SM
E. smithi smithi	56	2			52	A	SM
Microtus montebelli montebelli	30	12	12	2	2	SM	A
M. m. brevicorpus	30	12	12	2	2	SM	A
Ondatra zibethicus	54	2			50	A	A
Apodemus miyakensis	48	4	4		38	A	A
A. argenteus	46	4			40	ST	A
A. giliacus	55	4			48	A	A
Micromys minutus hondonis	68	6	4	38	18	A	A

M. m. japonicus	68	6	4	38	18	A	A
M. m. aokii	68	6	4	38	18	A	A
Mus musculus molossinus	40				38	A	A
M. m. yonakuniensis	40				38	A	A
Tokudaia osimensis muenninki	42	14	2	4	20	M	ST
Rattus legatus	44	8	2		34	?	?
Carnivora							
Canidae							
Nyceterutes procyonides viverrinus	42	14	12		14	A	ST
Mustelidae							
Mustela sibirica itatsi	38	10	6	8	12	SM	A

* M=metacentrics, SM=submetacentrics, ST=subtelocentrics, and A=acrocentrics.

Frequency of chromosome polymorphism in pair Nos. 1, 3, 9 and 13 in black rats, collected in Misima

Tosihide H. YOSIDA

The apearance of chromosome polymorphism in pair Nos. 1, 9 and 13 in balck rats, *Rattus rattus*, has been reported (Yosida *et al.* 1965, 1970, 1971). Among the chromosomes, frequency of No. 1 chromosome polymorphism has been reported in the rats collected in various localities in Japan. In the rats, polymorphism of pair No. 3 was newly found which has not been reported to date. This pair showed acrocentric and subtelocentric polymorphism as seen in the other chromosome polymorphism of Nos. 1, 9 and 13. In the present paper, frequencies of the acrocentric homomorphic pair (A/A), acrocentric and subtelocentric heteromorphic pair (A/S) and subtelocentric homomorphic pair (S/S) in the above four chromosomes in 16 animals collected in Misima are presented as a preliminary report. The frequencies are as shown in the following table (Table 1).

Table 1. Frequencies of three chromosome types in pair Nos. 1, 3, 9 and 13 in 16 animals collected in Misima

Pair No. / Pair type	No. 1	No. 3	No. 9	No. 13
A/A	8	9	5	10
A/S	7	5	3	1
S/S	1	2	8	0

As seen in the table, frequencies of three chromosome types (A/A, A/S and S/S) in the rats differ for the different chromosomes, but those of pair Nos. 3 and 13 are somewhat similar. Ratio of subtelocentric to acrocentric chromosomes in the three pairs (Nos. 1, 3 and 13) is 0.4, 0.4 and 0.2, respectively. In pair No. 9, however, the frequency of subtelocentric chromosome is markedly higher, and the ratio of A/S is 1.5. The frequency of acrocentric and subtelocentric chromosomes varies by colony of rats collected. For instance, among 16 black rats, 4 were collected in one house (colony A) and 8 rats collected in another house (colony B) which is about 5 km distant from colony A. The frequencies of subtelocentrics to acrocentrics in the four chromosome pairs are shown in the next table (Table 2).

Table 2. Frequency of subtelocentric to acrocentric in four chromosome pairs in two different colony

Colony \ Pair No.	No. 1	No. 3	No. 9	No. 13	No .of animals
A	1.6	0.3	0.3	0	4
B	0.1	0.3	4.3	0.8	8

The above table shows that the difference of frequency of chromosome polymorphism seems to be affected by random drift of the animals.

In Vivo Proliferation of Two Sublines of MSPC–1 Mouse Myeloma in Mixed Inoculation

Kazuo MORIWAKI and Tamiko SATO

Two sbulines of MSPC–1 mouse myeloma, P–40 and NP–39, were tested for their proliferating ability with the mixed or separated subcutaneous inoculation. P–40 subline derived from an early transplant generation of MSPC–1 tumor is a producer of myeloma protein and has 40 chromosomes without any marker. NP–39 subline derived from a later generation is a non-producer and has 39 chromosomes with 3 markers, A, B, and C.

When 10^6 P–40 cells was subcutaneously inoculated into the left abdomen and 5×10^3 NP–39 cells into the right abdomen separately, the population size of the former cells became 4.0×10^8 and that of the latter 1.4×10^8 after 2 weeks. In the mixed inoculation with the same cell doses as employed above, however, the resulting tumor, after 2 weeks, consisted of 1.5×10^8

P–40 cells and 4.8×10^8 NP–39 cells. The cell numbers for such experiments can be computed from the total number of cells in the grown tumor, the ratio of P–40/NP–39 in the metaphase figures and the mitotic index in each subline of tumors inoculated separately. The results, summarized in Table 1, clearly demonstrate that in the mixed inoculation, growth of P–40 cells is markedly retarded and growth of NP–39 cells is considerably enhanced. These interactions between the two sublines seem to occur only when they are in close contact with each other.

In order to determine the possibility of "allogeneic inhibition" like interaction between these two sublines of cells, intact P–40 cells were subcutaneously inoculated by mixing with NP–39 cells heavily irradiated with Cesium 139 (5000 r), treated with cold glycerine for one week or treated with cold acetone. No significant inhibition of the prolieferation of P–40 cells by the NP–39 cells treated in such manner has been observed thus far. Possibility of other kinds of immune mechanisms involved is now under investigation.

Table 1. Interaction between two sublines of MSPC–1 tumor in their *in vivo* proliferation.

Inoculation	Tumor line	Total cell number		
		Initial	Final	Expected
Separated	P–40	10^6	4.0×10^8	
	NP–39	5×10^3	1.4×10^8	
	Total		5.4×10^8	
Mixed	P–40	10^6	1.5×10^8	4.7×10^8
	NP–39	5×10^3	4.8×10^8	1.6×10^8
	Total		6.3×10^8	6.3×10^8

An estimation of the number of definitive spermatogonial cell divisions in the silkworm

Akio MURAKAMI

Since the first report of Kawaguchi (1928), there have been few papers describing the number of definitive (or secondary) spermatogonial divisions in the silkworm. According to Kawaguchi, six-definitive spermatogonial divisions occur resulting in the production of 64 primary spermatocy-

tes. In some insect orders, however, it has been shown that the number of successive definitive spermatogonial mitosis is not strictly constant, but fluctuates to some extent even within the some testes. To better understand the mechanism of spermatogenesis, it is worth while to study the number of spermatogonial divisions and other related subjects.

For these reasons, the present experiment was conducted to estimate the number of definitive spermatogonial mitosis using our usual experimental material, the *C108* silkworm strain. The testes used for observations were obtained either from the 4th or the 5th instrar larvae. The preparations were sampled by the squash method and were observed directly, without staining, under a phasecontrast microscope. If the total spermatocytes and spermatids within the cyst are counted, it would be easy to assume from what cell type of the definitive spermatogonial period the cyst arose before passing into the primary spermatocyte period.

As reported by other authors, synchronous cell divisions within the cyst either for the secondary spermatogonia or the meiotic spermatocytes was observed. The size of the cyst is proportional to the total number of cells within it, even in the rare event when the total number of cells vary from its theoretical number. It was also observed that there are at least two different types of spermatids per cyst: one of which is the type with 256 cells per cyst as reported by Kawaguchi and another is the type with 128 cells. These correspond to the six and five definitive spermatogonial divisions, respectively. According to a rough estimate of the frequency of these two types within an individual, the former type was found greater than 75 per cent and the latter type was observed less than 25 per cent. Although with low frequency, the type with 64 cells was also observed. In any case, it was concluded that the type with six definitive spermatogonial divisions is the main process in this material.

To confirm the findings, calculation of the number of primary spermatocytes (just prior to the first metaphase) per cyst was also made by using the same material in parallel. From this observation, the number of primary spermatocytes per cyst could be classified into three different types, among which the type with 64 cells which correspond to six definitive spermatogonial divisions was more frequently found than those for 32 and 16 cells which correspond, respectively, to five and four divisions. This finding is not inconsistent with that obtained from the estimate for the number of spermatids per cyst.

Thus it can be concluded that, in the silkworm, the main type in the number of synchronous definitive spermatogonial divisions is six, but there are relatively low frequencies of other types. However, the question still remains as to which type takes part in the final fertilization.

VI. RADIATION GENETICS IN ANIMALS

Effect of the moulting hormone on mutation induction and development of spermatogenic cells in the silkworm

Y. TAZIMA and Y. FUKASE

It has been reported by Morohoshi *et al.* (1967, Jap. J. Genet. 42: 425) that the moulting hormone promotes pupation as well as moulting in the silkworm depending on the quantity of the co-existing juvenile hormone. These findings prompted us to carry out an experiment to find out whether the same hormone promotes the development of spermatogenic cells. We found some years ago that the frequency pattern of mosaic and whole body mutants was changing remarkably with the progress of spermiogenesis (Tazima and Onimaru, 1969, Mutation Res. 8: 177). Namely, until fifth instar day 4.5 relatively more completes than mosaics were produced, around V–4.5 the ratio of mosaics to completes reaching unity, and from this stage onward relatively more completes than mosaics were produced. By using this relation as an index we may assess the development of germ cells. The present experiment has, therefore, been attempted with two moulting hormones, ecdysterone and inokosterone, which were kindly provided by Prof. T. Takemoto of Tohoku University.

The hormones, dissolved in physiological saline solution, were injected into the male larvae of C108 at about the middle stage of the fifth instar. The injected doses per capita were 15 γ, 5 γ and 0 γ in 0.05 ml injection. The hormone-injected larvae attained maturity one or two days earlier than the non-injected control. The injected group with saline matured a half day earlier. All groups were irradiated simultaneously with 2000 R γ-rays when the hormone-injected groups had attained maturity. They were mated, after emergence, to *pe re* females for the detection of induced mutants and the proportion of mosaics to total number of mutants was calculated.

The results thus obtained were: in Expt. I 0.73 ± 0.05 (ecdy. 15 γ), 0.70 ± 0.05 (ecdy. 5 γ) and 0.67 ± 0.05 (saline only) and in Expt. II 0.62 ± 0.07 (ecdy. 15 γ) and 0.61 ± 0.05 (ecdy. 5 γ) and 0.55 ± 0.07 (saline only). The difference between the hormone injected group and the control was not sta-

tistically significant, but the regressions of the incidence of mosaics against the injected dose were in good agreement between two experimental series. From those results it can be concluded that ecdysterone has a promoting effect on the development of spermatogenic cells of the silkworm.

We have also examined whether the moulting hormone increases or decreases radiation induced mutation frequencies. Both ecdysterone and inokosterone were injected to male larvae at fifth instar days 5 and 6. Injected per capita were 5 γ ecdysterone, 5 γ and 10 γ inokosterone and no hormone in 0.05 ml injection. Six hours after injection the larvae were irradiated with 2000 R γ-rays. Injection of ecdysterone 5 γ increased clearly the mutation frequencies over the saline control, whereas inokosterone failed to show its effectiveness in increasing mutation frequency.

Studies on strain differences in radiosensitivity in the silkworm XI Sensitivity to dominant lethal induction

Y. TAZIMA and Y. FUKASE

Sensitivity to dominant lethal induction was compared among silkworm strains having different radiosensitivity to embryonic killing, by irradiating male germ cells at two developmental stages; mature sperm and young spermatogonia.

For the irradiation of mature sperm, the strains used were Kansen and Aojuku as the less sensitive and Sekko and rb as the highly sensitive strains. X-rays were administered to male moths of each strain in four dose groups, 0, 5, 10 and 15 kR. In the experiment with young spermatogonia Kansen, rb and their hybrid were used as materials. Doses administered were 0, 2 and 4 kR for both parental strains and additional 6 and 8 kR doses for the hybrid.

Irradiated males as well as males of the non-irradiated control were crossed to non-irradiated F_1 females of a hybrid between two commercial breeds, FxB$\times$95. The use of such hybrid females ensured the deposition of non-defective eggs so that the dying embryos due to dominant lethals were easily determined.

Results: After the irradiation of mature sperm, no clear difference was observed among the four strains. In contrast, irradiation of young spermatogonia revealed a clear diffeernce between strains, exhibiting higher

incidence of dominant lethals in the rb strain. In the F_1 the incidence of dominant lethals fell between the two parental strains. The results were in agreement with those obtained for recessive visible mutations, although the difference between the strains was much reduced for dominant lethals.

Studies on strain differences in radiosensitivity in the silkworm. XII Different modes of dominance observed in F_1 with regard to radiosensitivity at various levels of biological reaction

Y. TAZIMA

Using several radiosensitive and radioresistant strains of the silkworm, which were selected with regard to embryonic killing, we have carried out a comparative study in radiosensitivity at various reaction levels, *i.e.* sensitivity to embryonic killing (individual specimen level), cell killing (cell level), dominant lethals (chromosome level) and mutation induction at marked loci (gene level).

Among several findings obtained so far the most instructive was the contrasting mode of dominance observed between mutation induction and killing of embryos by radiation in F_1 hybrids produced between two sensitive strains. Namely, when F_1 hybrids, Kojiki×rb and Sekko×rb, were compared with the parental strains, both showed intermediate sensitivity with respect to mutation induction, but overdominance with respect to embryonic killing even in the same hybrid combination.

Incidentally, as to germ cell killing either type of intermediate sensitivity or overdominance was observed according to the combination of the hybrid. Kojiki×rb showed themselves to be intermediate, while Sekko×rb belonged to the overdominant type. Although the above two hybrids have not yet been examined, the sensitivity to dominant lethal induction was seen to be intermediate in the case of F_1 between Kansen and rb.

These results could roughly be interpreted as showing that radiosensitivity in F_1 represents the intermediate type at the gene level, the overdominant type at the individual specimen level, and a transitional type at the cellular level.

The intermediate type of dominance observed for mutation induction seems to suggest that a modification of gene action, responsible for pigment formation in this case, by several intracellular and/or extracellular con-

ditions, if any, is rather slight. Whereas, the possibility of such modification is fairly large in the case of individual specimen killing because the same F_1 crosses exhibited overdominance with regard to embryonic killing. The increased vigour of the F_1 for instance, seems to relieve the radiation lesions by an appreciable degree leading to an increased survival.

It can be concluded from the above results that overdominance is the outcome of the combined effect of several relevant factors even when each component allele acts additionally.

Studies on strain differences in radiosensitivity in the silkworm. XIII Radiation-induced breakage and rejoining of single strand DNA

Y. TAZIMA, K. ONIMARU and H. KATO

In order to have a better insight into strain differences in radiosensitivity, radiation-induced breakage and rejoining of single strand DNA have been compared among different sensitivity strains.

For the sedimentation analysis the alkaline sucrose density gradient method was used with a slight modification of the procedure as proposed by McGrath and Williams (1966) following Terashima and Tsuboi (1967). The cells used were spermatogonia and/or spermatocytes of one or two days old third instar larvae. Labelling of DNA was performed by in vitro culture of the testes in K6 medium containing ^{3}H-thymidine with a specific activity of 20 μc/ml for 5–6 hours.

The labelled testes were divided into three groups, each comprising 15, in cold K6 medium: two groups were for X-irradiation (10 kR) and one group for non-irradiated control. One of the two former groups was incubated after X-ray irradiation in a fresh K6 medium for 30 or 45 minutes at 25°C, so as to allow the rejoining of broken DNA strands. After appropriate treatment the testes in each group were torn to pieces in a hollow glass and drop of culture medium was added. The mixture was then subjected to filtration through tissue paper. The cell suspension (0.1 ml) was loaded gently on 0.2 ml of 2% solution of sodium dodecylsulfate, which was overlaid on 5–20% sucrose gradient at pH12 in 5 ml cellulose nitrate tubes. Gradients were centrifuged at 22000 rpm for 90 min. at room temperature in a swinging bucket rotor of a "Tominaga SS–500" centrifuge.

The contents were collected in about 30 fractions, to which cold 5% trichloroacetic acid was added. The precipitate was washed on a millipore disc with ethanol, and acetone-dried for liquid scintillation counting.

It was anticipated that the rejoining capacity of DNA strands might differ among the strains reflecting the different radiosensitivities. The results obtained so far are, however, contrary to this expectation. Either with respect to strand breakage or to rejoining, no clear difference was observed among the seven strains. Even such most sensitive strains as rb and Sekko have been found to have the capacity for rejoining single strand breaks in DNA as was observed for the resistant strain Kansen.

Those results indicate that the differential sensitivity among silkworm strains is not due to a differential capacity for rejoining single strand breaks in DNA.

Relative biological effectivenss of 14 MeV neutrons for recessive visible mutations in meiotic spermatocytes of the silkworm

Akio MURAKAMI

Meiosis is a biologically important cell process in the course of game togenesis. However, very little information is available as to radiation-induced mutagenesis in meiotically dividing cells, although there are numerous published reports for either pre- or post-meiotic germ cell in various organisms. This situation is more pronounced with densely ionizing (or high linear energy transfer: LET) radiations than with less densely ionizing (low LET) radiation. Such being the case, there are few reports as to the genetic RBE of fast neutrons in meiotic cells.

This communication presents the result of experiments on the RBE of 14 MeV fast neutrons as compared to ^{137}Cs γ-rays for recessive visible mutations in the early first meiotic cells at the first day of fifth instar larvae in the silkworm. The γ-ray doses given were three different doses given were three different doeses: 250, 500 and 1,000 R. For 14 MeV fast neutrons, irradiation was given at three different doses: 150, 250 and 540 rads. Mutations at both the *pe* and *re* loci were detected by the egg-color-specific locus method.

The cell at this stage showed extremely high radiosensitivity to cell de-

generation which was not found in post meiotic cells. With 1,000 R of γ-rays and 540 rads of neutrons, more than ninety-five per cent of the cell in meiosis resulted in sterility. However, these effects were negligible with other lower doses either for neutrons or γ-rays. Thus data presented come from the remaining two low doses for both radiations. The average mutation rate obtained on the two loci in this study was 10.9×10^{-7} for γ-rays. This rate was somewhat less than that of spermatids which have the highest rate throughout spermatogenesis as observed by Tazima (1961). The frequency of mutations increased non-linearly with dose for both radiations. Hence RBE was estimated tentatively by comparing the estimated doses for each radiation at an arbitary mutation frequency level. The RBE obtained in this manner is 1.8. This value is about one-third that in in post-meiotic cells or mature sperm and is the same as those in primordial germ cells and in spermatogonia. This finding could be explained reasonably as due to an interaction between effects of germinal selection and sensitivity on irradiated cells.

Changes in ultraviolet light sensitivity to embryonic killing during early developing egg of the silkworm

Akio MURAKAMI

To analyse the relationship between UV sensitivity and embryonic development, the present experiment was carried out using silkworm synchronized cell population during early embryogenesis. Embryos (or eggs) were obtained from crosses between C108 strain wild type females and *pe* and *re* marker strain males. This mating procedure has the advantage of permitting the estimation of both cell killing in terms of lowered hatchability and the measurement of the mutational incidence of the egg-color-specific loci of *pe* and *re*. The UV source was a germicidal lamp and the visible light as the photoreactivating source was obtained from the fluorescence light. These eggs were subjected to UV treatment with relevant time intervals from immediately after oviposition up to 16 hr old eggs.

A marked change of UV sensitivity was observed with the progress of embryonic development. Sensitivity to UV was relatively low in the first 10 hr after oviposition, and then it gradually increased with egg ages. Since 10 hr-old eggs, an extremely high sensitivity was observed. In the silkworm,

there is a well known fact that cleavage nuclei migrate somewhat into the inner part of the egg and those in the blastula stage move to the upper surface of the egg. Thus it can be concluded that these high sensitive stages correspond to the stage of blastula, while low sensitive stages correspond to the age of eggs from meiotic oocytes until the blastula stage.

It is of interest to note that photoreactivation to UV damage was more effective in eggs for UV sensitive stages than in those for resistant stages. In addition, no cyclic change of UV sensitivity in eggs during early cleavage stages (from 200 to 400 min after oviposition) was observed in accordance with the cleavage division cycle. But the cyclical change of sensitivity to ionizing radiation in this cleavage stage of the silkworm has been observed (Murakami, 1969). This suggests that the nuclei in the egg stage are not responsive to UV killing because the nucleus is shielded by cytoplasm. Although the cytoplasm was not so sensitive to UV, the death of cleaving egg may be attributable, in part, to cytoplasmic injury following exposure to UV. This finding is suggestive to the observation that the cytoplasm is able to repair the damage induced by ionizing radiation (Murakami, 1969).

In any case, the present study indicated that the effect of UV to embryonic killing is related to the event occurring within or governed by both nucleus and cytoplasm in the silkworm egg.

Sex differences in X-ray sensitivity to embryonic killing of dormant silkworm egg

Akio MURAKAMI

Since 1965, we have been studying the radiosensitivity to embryonic killing of the silkworm and the results of these experiments have been reported in various communications. On the occasion of the XII I.C.G., Dr. Oster asked whether there is a difference in the sensitivity of embryonic killing to radiation between sexes. At that time, we did not have detailed data to answer the question. However, it was empirically known that females are more sensitive to radiation killing than males as well as other biological damage in general. To answer this important question, experiments presented here were carried out, using X-irradiated dormant embryos of the *C108* silkworm strain. As this strain lacks the relevant sexual trait for sexing the egg stage, the sensitivity could not be estimated separately in

males and females on the day of egg hatching. However, by the fifth instar, the larvae could be sexed in the usual way by studying the ventral sex organs. Such being the case, the sensitivity in irradiated embryos was estimated, on the first day of the fifth instar, as being due to the reduction of their survival fractions as compared with the control.

The dose-response relations for the egg-hatchability decreased exponentially with dose as reported in earlier papers. Surviving larvae were kept until the fifth instar stage and their sexes determined to estimate the radiosensitivity in males and females. It was observed from this experiment that the female embryo is significantly more sensitive to X-ray killing than the male embryo. This difference in radiosensitivity may be interpreted as being due to 1) difference in the sex-chromosome constitution: the females of this insect have ZW heterozygous chromosome constitution, while the male has ZZ homozygous, 2) difference in the direct and/or indirect physiological function of the sex chromosome and 3) difference in whether recombination occurs or not as one of repair mechanisms in somatic cells as well as in germ cells: recombination in the female silkworm is absent in the germ cell; on the other hand this phenomenon is present in the male.

Fortunately, in the silkworm, there have been created various translocation strains for the purpose of establishing male-rearing method. Most of these strains have translocations between the W chromosome which has female determing factors and autosomal chromosomes which have either egg or body color mutant genes. By using these strains, it would be possible to estimate correctly the radiosensitivity between male and female in egg and/or early larval stages. We have carried out experiments with these strains. Similar but more pronounced difference in sensitivity between sexes to that found in the *C108* strain was observed in these strains. It can be said from this finding that strains with translocations are more sensitive to X-rays than those free of translocated chromosomes.

Differential Nuclease Activities in Radiation-sensitive and Radiation Resistant Silkworms

Susumu SAKURAI, Tsuneo KADA and Tadahiko ANDO*

Recent studies on recombinationless mutants of microorganisms suggest that they are lacking in certain nucleases. Because the increased radiation

* Institute of Physical and Chemical Research.

sensitivities of these mutants are due to the absence of recombination repair mechanisms involving functions of specific nucleases, it appears to be interesting to examine similar problems in higher organisms possessing different sensitivities to radiations. It is known that strains of silkworm remarkably differ as to their radiation sensitivities (Tazima and Murakami, Gamm-Field Symp. 8: 53, 1970). Therefore the activities of some typical strains were analyzed.

Ten g of 3rd instar larvae were homogenized with 10 ml of 0.01 M Tris-Hcl buffer (pH 7.0) containing 0.01 M 2-mercaptoethanol and the supernantant solution after centrifugation at 8000 g for 10 minutes was collected. Ten ml of crude extract were combined with 50 ml of 0.01 M Tris-HCl+ 0.01 M 2-mercaptoethanol (THM buffer, pH 7.0), then 12 ml of 5% streptomycin sulfate solution were added in the course of 30 minutes at cold. Into the supernantant solution obtained by centrifugation (8000 G, 10 minutes), 27 g of powdered ammonium sulfate were added slowly in the course of 30 minutes at cold. The supernatant obtained by centrifugation (8000 G, 10 minutes) was treated again with 21 g of ammonium sulfate. The precipitate obtained by centrifugation (8000 G, 10 minutes) was dissolved in 5 ml of THM buffer, and dialyzed overnight against 2 l of the same buffer at 4°C. The dialyzed enzyme solution was then charged on DEAE-cellulose column (2.5 g, equilibrated in THM buffer, 1.5 cm × 20 cm) and eluted with 300 ml of THM buffer and 300 ml of THM buffer containing 0.4 M NaCl by gradient solution. Nuclease activities of 5ml fractions were determined in transformation experiments with *Bacillus subtilis*. 0.1 ml fractions (previously diluted 10 times with phosphate buffer) were mixed with 0.1 ml solutions of transforming DNA (arg^+) containing 6 mM $MgSO_4$ and kept at 37°C for 10 minutes. The transforming activity (Arg^+) in 0.1 ml of each reaction mixture was determined using 0.9 ml of competent bacteria (H17, arg^- try^-).

Two typical strains, Aojuku and rb were studied in detail. The former is radiation-resistant while the latter is remarkably sensitive. Results obtained by fractionation experiments indicated that the strain Aojuku contained two nucleases (tentatively named A and B) respectively at 0.02 M at 0.10 M NaCl region of elutes, on the other hand, the strain rb contained a nuclease A′ at 0.02 M NaCl region. Because it is supposed that A and A′ may be similar enzymes, the strain rb lacks in B nuclease. Further studies are in progress to elucidate the characters and specificities of these enzymes.

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN MICROORGANISMS AND PLANTS

Biochemical Factors involved in the Repair of γ-ray-induced Damage in Transforming DNA of *Bacillus subtilis*

Takehiko NOGUTI and Tsuneo KADA

In an approach to understand the biochemical mechanisms involved in the repair of DNA damage induced by ionizing radiations, we have carried out experiments using toluene-treated cells of *B. subtilis*. Media, conditions of irradiation and technics of transformation experiments had been previously described (Kada, Noguti and Namiki, Int. J. Radiat. Biol. *17*: 407).

Cells of strain H17A (arg^+ try^-) were harvested at their growing phase in TF medium and were exposed to γ-ray (30 kR) of ^{137}Cs. Immediately after irradiation, the bacteria were shaken in 1% toluene-phosphate buffer (pH 7.4, 0.05 M) at 0°C for 10 minutes and were collected by centrifugation. The toluene-treated cells were incubated in phosphate buffer containing various supplements at 37°C for repair of DNA damage. The complete mixture contained 40 μM of each of four deoxynucleosides triphosphates (dXTP), 150 μM of NAD and 5 mM of $MgCl_2$. Cell lysates containing DNA were prepared as follows: Cells were first incubated at 0°C for 30 minutes in a lysozyme solution (1 mg/ml in 0.5 M NaCl–0.02 M EDTA–0.02 M Tris-Cl–25% sucrose), then incubated in the same solution supplemented with equal volume of Brij-58 in 0.01 M Tris-Cl (pH 7.2) at 37°C for 20 minutes; the cellular suspensions were mixed with two volumes of 1% SDS–0.1 M Tris-Cl (pH 9.0); the resulting lysates were heated to 60°C for 10 minutes and dialysed with a millipore filter (0.05 μ of pore size) chamber against 0.15 M NaCl–0.015 M sodium citrate, then against the above medium supplemented with 2 M NaCl for 2 hours. The dialysates were diluted and their transforming activities (Arg^+) were determined using H17 (arg^- try^-) as recipient.

The transforming capacity in the lysate was inactivated to approximately 1/10 by irradiation but rose higher more than several fold in the course of the 37°C incubation in the complete mixture. Such an increase was not found by removing any one of the components (Table 1).

Because 4dXTP are substrates for DNA synthesis by DNA-polymerase and NAD is a co-factor required for DNA-ligase function in *B. subtilis*, the above results strongly indicate that DNA-polymease (probably I) and DNA-ligase may be involved in the repair of γ-ray induced damage of DNA in *B. subtilis*.

Table 1. Requirements for repair of γ-ray-induced damage of transforming DNA in toluene-treated cells of *B. subtilis*. The complete mixture contained in phosphate buffer 40 μg of each of 4dXTP, 150 μM of NAD and 5 mM of $MgCl_2$

γ-ray dose (kR)	Incubation medium	Incubation in minutes	Transformants per ml of competent culture
0	Complete	0	11460
0	Complete	10	7320
0	-4dXTP	0	9820
0	-4dXTP	10	6300
30	Complete	0	1100
30	Complete	10	6440
30	-4dXTP	0	1270
30	-4dXTP	10	740
30	-NAD	0	1170
30	-NAD	10	1370
30	$-Mg^{++}$	0	1360
30	$-Mg^{++}$	10	980

Spores of rec$^-$ Strains of *Bacillus subtilis* are Sensitive to UV or γ-ray

Yoshito SADAIE and Tsuneo KADA

Since recombination repair is an efficient mechanism securing cellular resistance to irradiations, it may be interesting to ascertain, whether this kind of repair mechanism is also operating in the high radiation resistance of spores in which the conformation of DNA is modified so that they tolerate well radiations.

A number of γ-ray-sensitive mutants were isolated by treatment with N-methyl-nitroso-N′-nitrosoguanidine from a derivative named H17 (try$^-$ arg$^-$) of *Bacillus subtilis* Marburg possessing wild radiation sensitivity.

Two strains showing stable sensitivities to irradiations in the course of experiments over two years were named respectively M45 and L43. Cells of M45 were exposed to transforming DNA extracted from strain 168 possessing wild radiosensitivity and bacteria showing intermediate sensitivities between those of M45 and H17 were isolated. One of them was named R10. All these mutants carry the same growth requirements (arg^- try^-) as H17.

Bacteria grown exponentially in TF medium were harvested and their radiosensitivities were determined. Spores were prepared in Schaeffer's medium. γ-irradiations were carried out with γ-ray of ^{137}Cs.

Inactivation studies with γ-ray showed that strains M45, L43 and R10 were more radio-sensitive than their parental H17 (Table 1). Increased sensitivities of mutants were also found with UV. That these mutants have genetic recombination deficiencies was concluded on the basis of following additional experiments. First, they all possessed Hcr^+ capacities on UV-exposed phage M2. Secondly, frequencies of transformation and transduction (with phage PBS1) were reduced in comparison with those found in the wild strain.

We found that spores of rec^- strains were sensitive to both γ-ray and UV when compared to H17 which is rec^+ (Table 1).

The results given above show that the recombination repair is involved in DNA damage of spores. It is noted here that Munakata and Ikeda (Biochem. Biophy. Res. Comm. *33*: 3, 1968) have shown that spores of an UV-sensitive strain, not involving rec^- character, were sensitive to UV but not to γ-irradiation.

Table 1. Radiation sensitivities of rec^- mutants of *B. subtilis*

Strains	% of survivals			
	Vegetative cells		Spores	
	γ-ray (13.3 kR)	UV (32 ergs·mm^{-2})	γ-ray (160 kR)	UV (1776 ergs·mm^{-2})
H17	11.4	49.9	16.7	4.53
L43	0.118	0.240	0.158	0.240
M45	0.038	0.182	0.171	0.065
R10	1.23	26.9	0.576	1.39

Mutagenic Action of Phloxine

Tsuneo KADA, Yoshito SADAIE and Kiyoshi TUTIKAWA

Current hypothesis on radiation-induced mutagenesis suggests that mutagenic errors may be produced by recombination repair of DNA damage induced by irradiations. If this hypothesis is true, it is supposed that any chemical agent producing DNA damage subjected to recombination repair may be mutagenic. It is expected that the lethal action of such a chemical may be stronger in mutants deficient in genetic recomination capacity than in wild strains. We have recently isolated a number of rec^- mutants of *Bacillus subtilis* Marburg. A typical set of rec^+ and rec^- strains, 17A (arg^+ try^-) and 45T (arg^- try^+) were used in these experiments.

An easy method of testing lethal actions of different chemicals was devised. Papaer disks of 5 mm diameter were prepared by punching thick filter papers. A disk was soaked in a drug solution and then placed on the surface of a broth agar plate. The plate was stood overnight at room temperature. Cultures of strains 17A and 45T grown exponentially in TF medium were diluted 10 times and streaked separately from the edge of the disk. By incubation for 24 hours at 37°C, grown bacteria became visible except the inhibition zone in dependence on the strain and the drug used.

Studying a number of dyes, we found that phloxine, a red dye, inhibits more the growth of 45T (rec^-) than 17A (rec^+). Further studies were carried out using *E. coli* B/r WP2 try^- to examine if pholoxine is really mutagenic. Cells of the strain WP2 grown exponentially in broth were washed, resuspended in phosphate buffer (0.067 M, pH 7.0) containing phloxine at different concentrations and kept at 37°C for 20 minutes. After washing out the dye by centrifugations, the cells were plated, after appropriate dilutions, on synthetic minimal agar supplemented with 1% liquid broth to determine survivals and induced mutations. In a typical experiment, the number of viable cells per ml decreased from 4.8×10^8 to 1.3×10^8 by treatment with 30 μg/ml of phloxine. The absolute number of revertants approximately doubled; their frequency per 10^8 viable cells was 5.0 for the non-treated control and 33.3 for the treated sample. By repeating similar experiments, we concluded that the dye is an active mutagen.

Host-mediated "rec-test" for Chemical Mutagens

Kiyoshi TUTIKAWA and Tsuneo KADA

Because different chemicals may be modified by *in vivo* metabolisms, it is important to assess their mutagenecity in consideration of such modifications. Gabridge and Legator (Proc. Soc. Exptl. Biol. Med. *130*: 831, 1969) first deviced the host-mediated assay of mutagenes using strains of Salmonella. They injected intraperitoneally bacteria into mice that previously had received drugs through intramuscular injections and showed that bacteria taken out from the peritoneum were mutated by certain drug metabolities.

When experiments on mutation induction are carried out, contamination with other microorganisms must be avoided. Much more skill is required in a host-mediated assay of mutagenecity. On the other hand, studies on cellular sensitivities to chemicals are easy. In the previous report, we have shown that chemicals possessing mutagenic capacities have increased lethal actions on rec^- (strain 45T) over rec^+ (strain 17A) cells of *Bacillus subtilis*. This relationship was adopted to the host-mediated assay of potential mutagens.

A mixture (0.2 ml in volume) of rec^+ and rec^- bacteria were injected intraperitoneally into mice which had received a test drug through injection into a leg muscle. After 60 minutes, the peritoneum was washed with 3 ml portions of phosphate buffer and filled up to 10 ml. The number of survivals in this bacterial suspension was determined, by plating on basal medium containing tryptophane (for 17A, arg^+ try^-) or arginine (for 45T, arg^- try^+). Though the bacteria of both types were inactivated gradually even in the control mice without drug injection, the existence of chemical activity

Table 1. Inactivations of rec^+ and rec^- strains of *Bacillus subtilis* in the peritoneum of mice that had received muscle injections with drugs

Drug	Dose of drug (mg/kg of mouse)	% of survivals	
		17A (rec^+)	45T (rec^-)
Phloxine	0	23.2	13.1
	167	20.4	10.1
Butter yellow	0	32	38
	100	6.2	10.8

producing damage subjected to recombination repair could be detected by the difference of survival levels of rec^+ and rec^- cells.

Results obtained with phloxine and butter yellow are shown in Table 1. Phloxine is a red dye whose *in vitro* mutagenecity on bacteria has recently been confirmed by us. Butter yellow is a famous carcinogen. It is shown that phloxine inactivated rec^- and rec^+ cells to similar extents in mice, suggesting that this drug may not be an active mutagen *in vivo* at least at the high concentration examined. Butter yellow is inactive *in vivo* at least as to production of DNA damage subjected to recombination repair.

Efficiencies of some mutagens in maize

Etsuo Amano

The starch character, *wx* in maize is expressed in the pollen grain and wild type recombinant pollen grains between independently induced *wx* mutants are detectable, if present, by differential staining with iodine. By such pollen analysis it has been shown that most of the ethyl methanesulfonate (EMS) induced mutants produced wild type recombinants when crossed to standard *wx* stocks. The presence of many such recombinant producers, good transmission and high ratio of leaky mutants suggest that the chemical mutagen, EMS, is very likely to induce base exchange mutations in maize. To compare the mutagenic effects and the genetic alterations induced with those of EMS, ionizing and ultraviolet (UV) radiations were applied to maize as mutagen.

Since isolation of mutants as stocks were a primary requirement in this analytical study, experimental methods were modified to suit each mutagen. Ionizing radiations were applied to dry seeds of a homozygous multiple dominant marker stock (Yg_2 C^I *Sh Bz Wx*, *Y*), and test crosses were made with a recessive tester (Yg_2 *C sh bz wx*, *y*) to detect recessive mutation in endosperm characters. EMS soaking treatments were given to water presoaked seeds and the rest of the process was the same as in ionizing radiations. In both cases, treatment dosages were as high as to give 80% survival in the treated generation. In case of UV treatment, pollen of the dominant stock were irradiated in a single layer by germicidal lamps with dose of 1×10^4 ergs/mm^2 which was enough to decrease seed fertility to 50% or less under the dark condition used. The irradiated pollen were used to

pollinate the same dominant stock (sib pollination), and self pollinated ears of the following generation were examined for the presence of mutation in the endosperm or other mutation detectable in the seed. Thus in this case the recessive lethal mutation could be tested besides the above mentioned markers.

Since the experimental procedures were different among the mutagens used, results summarized in Table 1 cannot be compared directly with each other. Even with such reservations and also those on treatment dosage, EMS seems to induce more mutations than other mutagens. It has been reported (Amano and Smith, Mutation Res. 2 (1965) 344–351) that fission fast neutrons effectively induced mutations in loci *C*, *sh* and *wx*. However, recent endeavors to induce mutations in the *wx* locus with radiations were not successful except only one *wx* kernel found so far in a γ-ray treatment group. An other finding to be noted was the high incidence of recessive lethals induced by UV treatment. As the UV treatment and other procedures were carried out under dark condition (yellow sodium lamp in a darkroom and dim dry cell head lamp for night pollination in the field), photorepairable damage might be fixed as mutation. Photo-repairability of this mutation should be further investigated.

Table 1. Results of mutagen treatments in maize. Summing up of experiments for several years.

Mutagen	Fertile cobs examined	Mutations					*wx* mutation frequency (%)
		C	*sh*	*wx*	multiple	others	
X-, γ-rays	2203	0	1	1	0		0.045
Thermal neutrons	453	0	0	0	0	+*	0.00
UV	690	0	2	0	0	(53)**	0.00
EMS	2270	19	8	20	1		0.925

*: Abnormally shaped kernels were often found.
**: Out of 479 ears of 1970 harvest. Many of them were recessive lethals.

Mutation Experiment in *Capsicum annuum*

Etsuo Amano

Transfer or alteration of a genetic factor in a scion by grafting was often observed in *Solanaceae*. Recent studies on transformation in higher plants seem to give further support to these observations. To

study the basis of mutations obtained by grafting, mutants were newly induced in *Capsicum annuum* var. Sapporo Wase. Use of chlorophyll or morphologically mutated seedlings within a single variety should make easy the progeny test on a simple genetic background. Since structural alterations in such genetic markers may reveal the efficiency of graft mutation, two mutagens with different effects, γ-ray and ethyl methanesulfonate (EMS) were used. The chemical mutagen, EMS is a very effective mutagen and is known to induce point mutations in higher plants.

Seeds were presoaked in distilled and deionized water for 24 hours before soaking them in EMS solution (up to 0.1 M) for 5 hours. After soaking in water for 24 hours at the same 27°C as in other treatments, seeds were sown in sterilized soil in wooden flats. The seedlings were kept in the flats so that their growth was suppressed. Each plant gave only one fruit. To induce larger structural alterations, γ-ray irradiated samples were used. Fruits were collected from plants irradiated in a γ-greenhouse with a ^{137}Cs source. Chronic irradiation was used in expectation of accumulation of mutations.

Mutant seedlings were surveyed in wooden flats in the greenhouse. Each fruit was considered to represent a single mutational unit. Two morphological, four lethal and three viable chlorophyll mutations were found among 746 fruits from EMS treatments. Out of 531 fruits from γ-rayed materials tested, 210 gave more than four seedlings but none segregated the expected mutant. Chronic γ-ray doses used were more than 5 kR as measured by glass dosimeters.

Some preliminary graftings were tried on normal green plants. Graftings of two green seedlings (control) and three scions of each stock (self grafting) were successful but none of the four lethal chlorophyll mutants (12 graftings in total) gave a result.

Effect of Temperature during the Split Dose Interval of Gamma-Rays in Maize Pollen

Taro FUJII

Recovery of premutational damage in maize pollen grains by fractionated gamma-ray treatments with 2 hours interval have been already reported (Ann. Rep. Natl. Inst. Genet. 20, 86–88). In order to get more information

about the apparent loss of premutational damage in both whole and partial mutations, the effect of temperature in the interval period was investigated in the present experiment. Material was the same as in the previous experiment, namely, mutation from *Bz* to *bz* was examined. The interval time was settled as 2 hours since a significant decrease in mutation frequency was usually observed under this condition, and three different dosages, 0.5, 1 and 2kR in total, were applied to check the relation dose versus mutation frequency. Irradiated pollen grains in three single dosage lots were divided into two parts. Prior to pollination on the female side, one half of the irradiated pollen grains was kept under a low temperature (about 5°C) for 2 hours and the other part was kept under room temperature (about 28°C) for the same duration. Similar treatment was also applied to the fractionated lots, namely one half of pollen grains irradiated with one half of single dosage were kept under room temperature for a given interval, while the other half of the pollen grains were stored under low temperature before the second irradiation. Pollination was carried out after those treatments.

The total mutation frequency increased by increasing the dose from 0.5 to 2 kR, both in single and fractionation series, but it was always lower in fractional than in single treatment. Furthermore, the storage temperature of pollen grains did not affect considerably the mutation frequency; similar mutation frequencies were observed in room and low temperature lots of each dosage for single or fractionation series as well as in non-irradiated control lots. The results suggest that the reduction of gamma-ray induced mutation under fractionation conditions occurs merely for the whole mutation and that low temperature treatment does not prevent recovery. However it is very difficult to imagine at present that the supposed recovery would usually involve the enzymatic processus because no temperature dependency was observed at least in the range of 5–30°C.

Tissue Culture from Wheat Anthers and Roots

Taro Fujii

Callus induction from anthers was observed in di-, tetra- and hexa-polid species. Anthers containing pollen grains at tetrad stage were planted in

a test tube on modified White's medium supplemented with 20 mg/l 2,4-D and solidified with 9 g/l agar. Casein dehydrogenase, one of the known efficient substances for callus growth, was added to the medium at concentration of 1 g/l. Callus formation from the anthers of two wild species, *Triticum aegilopoides* (diploid) and *T. dicoccoides* (tetraploid), was significantly higher on this medium than when no casein was added. Furthermore, callus formation was observed in anthers of *T. durum*, while cultivated tetraploid species did not form any callus without casein. No callus was yet observed, so far examined, in three cultivated species, *T. monococcum* (diploid), *T. spelta* and *T. vulgare* (hexaploid) like in the previous experiment (Ann. Rep. Natl. Inst. Genet. 20, 91–92). The result suggests that the callus may be induced with certain chemicals specifically required.

To obtain an information on radiation sensitivity of cultured cells, calluses induced from dipolid wheat roots were irradiated with 0.5, 1, 2 and 5 kR of gamma-rays, because callus growth induced from pollen grains is very slow and is not produced in quantities is large enough for irradiation experiments. Irradiated calluses were inoculated again on the same medium (modified White's with 2,4-D), and the growth rate was measured by the increase of wet weight 70 days after the treatment. In the control lot, weight increase was about twice as large as the initial one, but about five times as large increase was observed in 0.5 and 1 kR lots. Growth rate in 2 kR lot was almost the same as that of the control lot, and 5 kR irradiation diminished growth rate amounted to 1.5 times of the initial weight (data from two repetitions). Although the growing plants or wet tissues are rather more sensitive to radiation than dry seeds, for instance LD_{50} of diploid wheat seedlings is less than 1 kR, stimulation of callus growth was observed in lower dose lots. Similar growth rate as that of the control lot was observed at 2 kR irradiation, a dose beyond LD_{100} for seedlings. Such resistance in cultured callus cells was reported for tobacco (Venketeswaran and Partanen 1966, Radiat. Bot. 6, 13–20). High radio-resistance in callus cells might be partly due to that the undifferentiated cell population which can grow by the growing point had substituted the undamaged cell for the damaged cell, whereas in the seedlings the growing point was already differentiated.

VIII. POPULATION GENETICS (THEORETICAL)

The length of time required for a selectively neutral mutant to reach fixation through random frequency drift in a finite population

Motoo KIMURA

Frequency distribution of the length of time until fixation (excluding the cases of eventual loss) of a selectively neutral mutant in a finite population was obtained based on the solution of Kimura (1955) on the process of random genetic drift. With $4N_e$ generations (N_e, effective population size) as the unit length of time, the distribution has mean $\mu_1=1$, standard deviation $\sigma=0.538$, skewness $\nu_1=1.67$ and kurtosis $\nu_2=4.51$ (Fig. 1). For details, see Kimura (1970), Genet. Res. Camb. 15: 131–133.

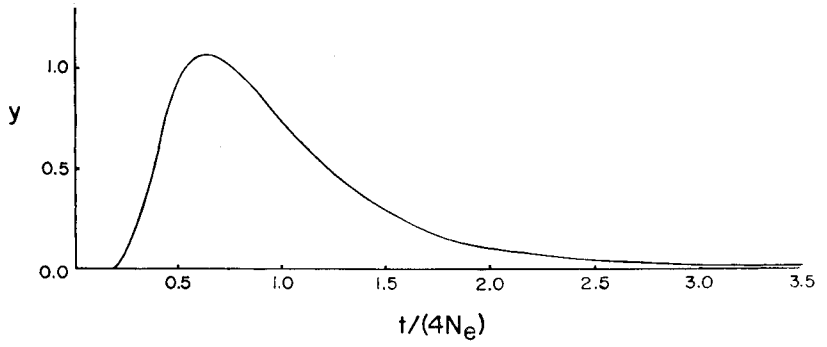


Fig. 1.

Probability of fixation of a mutant gene in a finite population when selective advantage decreases with time

Motoo KIMURA and Tomoko OHTA

A theory was developed which enables us, for the first time, to obtain the probability of fixation of a mutant in a finite population when its selective advantage decreases at a constant rate with time. The theory is based

on diffusion models and it can be extended to cover a more general case in which the selective advantage can be expressed as a function of the exponential function of time.

Let $u(p)$ be the probability of ultimate fixation of a mutant allele with initial frequency p and having selective advantage se^{-kt} at time t. Then, assuming that p is small, the fixation probability in a population of effective size N_e is given approximately by

$$u(p)=\{Y/(1-e^{-Y})\}\,p\,,$$

where Y is the solution of the differential equation

$$\frac{dY}{dS}=\frac{Y(S-Y)(1-e^{-Y})}{KS[1-(1+Y)e^{-Y}]}\,,$$

in which $S=4N_es$ and $K=4N_ek$. This equation was solved numerically and the values of Y are tabulated for various combinations of S and K. When S and K are large, the following approximation formulae were found useful to estimate Y: (i) $K\ll S$, we have $Y\approx S-K$, while (ii) if $S\leqq 2K$, we have $Y/(1-e^{-Y})\approx e^{S/(K+2)}$.

In order to check the validity of the theory, an extensive computation was carried out by using the method of multiplying the transition probability matrices, and the result turned out to be satisfactory.

As an application of the present theory, the probability of establishing a new inversion initially containing an exceptionally small number of deleterious genes was worked out. For details, see Kimura and Ohta (1970), Genetics 65: 525–534.

Genetic loads at a polymorphic locus which is maintained by frequency-dependent selection

Motoo KIMURA and Tomoko OHTA

If a polymorphic locus is maintained in finite populations by frequency-dependent selection with selective neutrality at equilibrium, it is generally accompanied by two genetic loads, that is, the dysmetric and the drift loads. The former arises because the fitness of the population may not be at a maximum at the equilibrium gene frequency and the latter because genetic drift in small populations displaces the gene frequency from its equilibrium value.

In some simple models of frequency-dependent selection considered, the drift load is independent of selection coefficients and is approximately equal to $(n-1)/(2N_e)$, where n is the number of alleles and N_e is the effective population size.

For details, see Kimura and Ohta (1970), Genet. Res. Camb. 16: 145–150.

Statistical analysis of the base composition of genes using data on the amino acid composition of proteins

Tomoko OHTA and Motoo KIMURA

A statistical method was developed to estimate base composition of genes (cistrons) from amino acid composition of proteins. The method enables us to obtain base frequencies in the first and second positions of codons through an iterative process with a computer. However, with respect to the second position of codons, the G–C (guanine and cytosine) content can be estimated directly and without error by a simple formula. Using this method, 17 vertebrate proteins were analyzed to obtain the distribution of base frequencies among genes in the vertebrate genome. The average G–C content turned out to be about 52% for the first position and 43% for the second position of codons. Their variances are higher than expected. It was also noticed that adenine content is almost always higher than uracil content, indicating different base composition between two complementary strands of vertebrate DNA. The problem of randomness in base arrangement in relation to molecular evolution by random drift and selection was discussed.

For details, see Ohta and Kimura (1970), Genetics 64: 387–395.

Development of associative overdominance through linkage disequilibrium in finite populations

Tomoko OHTA and Motoo KIMURA

Associative overdominance arises at an intrinsically neutral locus through its non-random association with overdominant loci. In finite populations, even if fitness is additive between loci, non-random association will be created by random genetic drift.

The magnitude of such associative overdominance is roughly proportional to the sum of σ_d^2's between the neutral and the surrounding overdominant loci, where σ_d^2 is the squared standard linkage deviation, defined between any two loci by the relation

$$\sigma_d^2 = E(D^2)/E\{p(1-p)q(1-q)\},$$

in which p and $1-p$ are frequencies of alleles A_1 and A_2 in the first locus, q and $1-q$ are frequencies of alleles B_1 and B_2 in the second locus, and D is the coefficient of linkage disequilibrium. A theory was developed based on diffusion models which enables us to obtain formulae for σ_d^2 under various conditions, and Monte Carlo experiments were performed to check the validity of those formulae.

It was shown that if A_1 and A_2 are strongly overdominant while B_1 and B_2 are selectively neutral, we have approximately

$$\sigma_d^2 = 1/(4N_e c),$$

provided that $4N_e c \gg 1$, where N_e is the effective population size and c is the recombination fraction between the two loci. This approximation formula is also valid between two strongly overdominant as well as weakly overdominant loci, if $4N_e c \gg 1$.

The significance of associative overdominance for the maintenance of genetic variability in natural populations was discussed, and it was shown that $N_e s'$, that is, the product between effective population size and the coefficient of associative overdominance, remains constant with varying N_e, if the total segregational (overdominant) load is kept constant.

The amount of linkage disequilibrium expected due to random drift in experimental populations was also discussed, and it was shown that $\sigma_d^2 = 1/(n-1)$ in the first generation, if it is produced by extracting n chromosomes from a large parental population in which $D=0$.

For details, see Ohta and Kimura (1970), Genet. Res. Camb. 16: 165–177.

The rate of decay of genetic variability in a two-dimensionally structured finite population

Takeo MARUYAMA

The rate at which a population approaches homozygosity is an important number in population genetics. In particular, the asymptotic rate is related

to the amount of genetic variability maintained in a population and to the speed of gene substitution. Previously, I have studied the rate for a population linearly subdivided into colonies.

Populations occupying two-dimensional habitats however are biologically more interesting than linear cases. In this note I want to investigate such cases of torus-like lattice structure. Considering the direct product of two circular lattices with n_1 points and with n_2 points, assume that a population consists of n_1n_2 colonies and that each colony occupies a grid point of the lattice. Assume furthermore that there are N diploid individuals in each colony.

Let m be the rate at which a colony receives immigrants from its four neighboring colonies in one generation. It receives immigrants from each at a rate of $m/4$ per generation. Let $f_{ij}^{(t)}$ be the probability that two randomly chosen homologous genes, one each from colonies which are i-steps apart in one dimension and j-steps apart in the other, are identical by descent. Let $f_{00}^{(t)}$ be the probability of identity by descent when the two genes are sampled from a single colony without replacement. If we assume that the mutation rate is u and every mutant is new to the population, it can be shown that the average value of $f_{00}^{(t)}$ over a long period time is given by

$$f_0 \equiv \lim_{T\to\infty} \frac{1}{T}\int_0^T f_{00}^{(t)}dt = \frac{S}{2N_T+S}$$

where

$$S = \sum_{k=1}^{n_1}\sum_{l=1}^{n_2} \frac{(1-u)^2\xi_{kl}^2}{1-(1-u)^2\xi_{kl}^2}$$

with

$$\xi_{kl} = \left[1-\frac{m}{2}\left(1-\cos\frac{2\pi k}{n_1}\right)\right]\left[1-\frac{m}{2}\left(1-\cos\frac{2\pi l}{n_2}\right)\right]$$

and $N_T = Nn_1n_2$. Now let

$$h_{ij}^{(t)} = 1-f_{ij}^{(t)} .$$

Assuming that reintroduction of lost alleles into the population by mutation or migration does not occur or occurs at a negligibly small rate, we are interested in determining the asymptotic rate at which the $h_{ij}^{(t)}$ approaches zero. If we let $H^{(t)}$ be the matrix $[h_{ij}^{(t)}]_{n_1\times n_2}$, we want to determine a con stant λ such that

$$H^{(t+1)}=\lambda H^{(t)}$$

for large t. According to Robertson (1964), we have

$$1-\lambda=\frac{1}{2N_T}\lim_{u\downarrow 0}\frac{1-f_0}{1-\bar{f}}=\frac{1}{2N_T}\lim_{u\downarrow 0}\frac{1-f_0}{1-\frac{1-f_0}{4N_T u}} \tag{1}$$

approximately. Several examples of the value of $1-\lambda$ calculated by (1) and the exact value obtained by an iteration method are compared in Table 1.

Table 1. Numberical comparison of the eigenvalues obtained by formula (1) and the exact value

$n_1\times n_2$	N	mN	$\lim_{u\downarrow 0} 1-\bar{f}_0/1-f$	Eigenvalue $(1-\lambda)$	
				from (1)	exact value
20×20	5	0.1	0.163	2.039×10^{-5}	2.040×10^{-5}
50×50	5	0.1	0.131	2.610×10^{-6}	2.616×10^{-6}
50×50	5	1.0	0.612	1.223×10^{-5}	1.222×10^{-5}
50×50	25	5.0	0.889	3.556×10^{-6}	3.554×10^{-6}
90×90	5	1.0	0.578	3.566×10^{-6}	3.570×10^{-6}
90×90	10	4.0	0.852	2.631×10^{-6}	2.632×10^{-6}
190×190	5	2.0	0.710	8.931×10^{-7}	8.920×10^{-7}

The values by the two different methods agree remarkably well.

Let $\bar{f}$ be the probability that randomly chosen two homologous genes from the entire population are identical by descent. Then the value of ratio $(1-f_0)/(1-\bar{f})$ is of considerable interest, because it tells us whether or not the population behaves like a panmictic one. I have calculated a large number of numerical examples and have found a fact that when the number of colonies is sufficiently large, the value of ratio depends only on the value of mN (number of migrating individuals), and it is almost independent of number of colonies. Furthermore when $mN\gg 1$, the value of $(1-f_0)/(1-\bar{f})$ is approximately equal to unity. Therefore if a population is of two-dimensional and $mN\gg 1$, it behaves very much like a random mating population, even if the population consists of large number of colonies. It is interesting that in a one-dimensional population, the ratio depends on both mN (local property) and the number of colonies (global property), but in a two-dimensional case it depends only on mN (local pro-

perty). Several examples of one-dimensional and of two-dimensional cases are listed in Table 2.

Table 2. Examples of numerical values of $\lim_{u \downarrow 0} 1-f_0/1-\bar{f}$

$n_1 \times n_2$	N	mN	$\lim_{u \downarrow 0} 1-f_0/1-\bar{f}$
100×100	10	0.01	0.0127
200×200	50	0.01	0.0114
500×500	10	0.01	0.0111
200×200	50	0.03	0.0329
500×500	5	0.05	0.0496
100×100	5	0.1	0.1145
300×300	5	0.1	0.0955
1000×1000	5	0.1	0.0811
100×100	10	0.2	0.2054
300×300	5	0.2	0.1755
1000×1000	5	0.2	0.1588
100×100	50	1.0	0.5638
500×500	50	1.0	0.5036
200×200	20	5.0	0.8603
500×500	50	5.0	0.8330
1000×1000	20	5.0	0.8242
500×500	100	10.0	0.9090
1000×1000	100	10.0	0.9008
100×100	200	50.0	0.9863
1000×1000	100	50.0	0.9809
100×1	5	0.5	0.1011
200×1	5	0.5	0.0501
500×1	5	0.5	0.0208
100×1	50	0.5	0.1007
100×1	500	50.0	0.9245
100×1	5000	50.0	0.9237

In the case of one-dimensional circular population studied, it was shown that if the number of colonies is large, the dominant eigenvalue can be approximated as the smaller of $m\pi^2/n^2$ or $1/2Nn$, where n=number of colonies. An analogous feature seems to exist in the two-dimensional case studied here. Here we assume that the number of colonies along both axes are the same, *i.e.*, $n_1=n_2\equiv n$. If $mN<1$ the value of $1-\lambda$ is approximately equal to m/n^2 and if $mN>1$ it is approximately equal to $1/2Nn^2$, *i.e.*,

$$1-\lambda \approx \frac{m}{n^2} \qquad \left(\text{more precisely } 1-\lambda < \frac{2m}{n^2}\right)$$

$$\text{if } mN<1$$

$$1-\lambda \approx \frac{1}{2Nn^2} \qquad \left(\text{more precisely } 1-\lambda < \frac{1}{2Nn^2}\right)$$

$$\text{if } mN>1.$$

This relationship is illustrated in Figure 1.

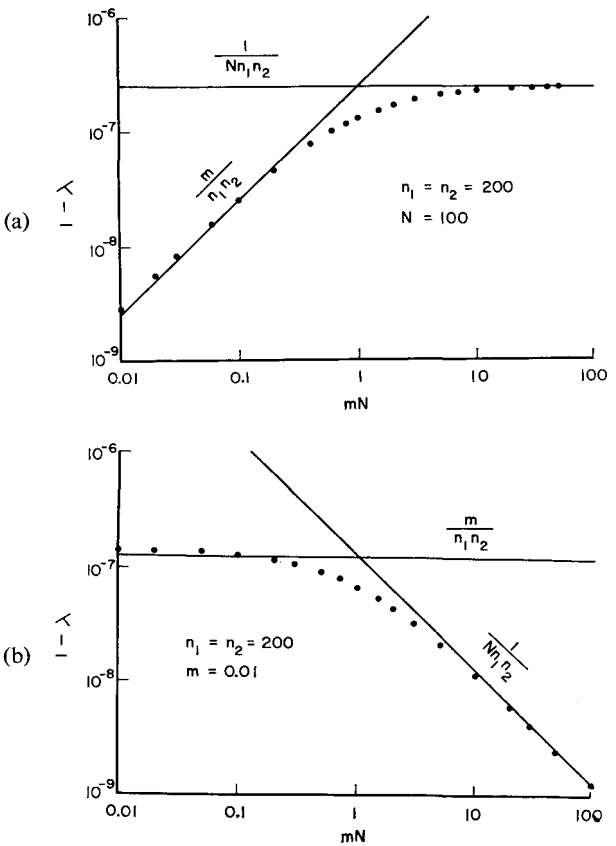


Fig. 1. The relationship between $1-\lambda$ and mN. The lines indicate the values of the approximations discussed in the text. In (a) the value of N is fixed and the value of m is varied, while in (b) m is fixed but N is varied.

Let us now assume that a population is divided into partially isolated n colonies of equal size N. Then the inequality $1-\lambda \leqq 2m/n$ holds for a more general situation. The equality is atained if $h_{ii}^{(t)} \ll h_{ij}^{(t)}$ and $h_{ij}^{(t)} \approx h_{i'j'}^{(t)}$ for every $i \neq j$ and $i' \neq j'$. This seems to be the case in a two-dimensional population with very small amount of migration, *i.e.*, $mN \ll 1$.

IX. POPULATION GENETICS (EXPERIMENTAL)

Deleterious genes in natural summer and autumn populations of *Drosophila melanogaster*[1]

Chozo OSHIMA, Takao K. WATANABE and Jong-Kil CHOO[2]

Many flies were collected at three sites in Katsunuma locality on July 31st and October 12th of 1970. The summer population was not very large, but the number of flies increased remarkably since September and then the autumn population became very large.

Many second chromosomes, extracted from each male fly, were analyzed by Cy-Pm method and the results are given in Table 1.

Table 1. Frequencies of deleterious and normal second chromosomes isolated from summer and autumn populations of *D. melanogaster*

Season	Site	Frequency (%)				No. of chromosomes tested
		lethal	semilethal	subvital	normal	
Summer	Marufuji	14 (32.6)	4 (9.3)	4 (9.3)	21 (48.8)	43
	Koshūen	21 (42.9)	3 (6.1)	2 (4.1)	23 (46.9)	49
	Minaminoro	35 (32.7)	10 (9.3)	7 (6.5)	55 (51.4)	107
	Total	70 (35.2)	17 (8.5)	13 (6.5)	99 (49.7)	199
Autumn	Marufuji	21 (21.0)	12 (12.0)	10 (10.0)	57 (57.0)	100
	Koshūen	15 (25.9)	10 (17.2)	5 (8.6)	28 (48.3)	58
	Maruki	19 (34.5)	8 (14.5)	2 (3.6)	26 (47.3)	55
	Total	55 (25.8)	30 (14.1)	17 (8.0)	111 (52.1)	213

The frequency of lethal chromosomes in the summer population was much higher and also that in the autumn population was higher than the mean frequency in the autumn populations in the previous years.

The frequencies of allelism were determined by half diallel crosses between 65 lethal strains isolated from male flies of the summer population and 89 lethal strains isolated from male and female flies of the autumn population. The results are given in Table 2.

[1] This work was supported by a grant (0801) from the Ministry of Education.

[2] Visting researcher, Department of biology, Chung-Ang University, Seoul, Korea.

Table 2. Results of allelism between lethal strains

Population	Summer population	Autumn population
No. of lethal strains	65	89
No. of crosses	2,080	4,005
No. of allelic crosses	171	133
Frequency of allelism (%)	8.22	3.32

Summer population			Autumn population	
Allelic group	No. of lethal genes		Allelic group	No. of lethal genes
			I	8
I	14 (2*)	———	II	7
			III	5
II	11 (8*)	———	IV	7 (7*)
			V	4
			VI	7
III	11 (3*)	———	VII	10
			VIII	5
IV	7	———	IX	4
			X	2
V	3		XI	2
VI	2		XII	2
			XIII	2
Single letahl gene	18 (27.7%)		Single lethal gene	24 (27.0%)

* : visible mutant *rbl* gene ———: allelic relationship

The high frequency of allelism decreased with the frequency of lethals in the course of about two months. The frequency of single lethal genes, which had no allelic genes, was about 27 per cent in both populations. Other clustering lethal genes could be divided into 6 and 13 groups in the summer and autumn populations, respectively.

The representative 10 lethal strains of the summer populations were crossed diallelly with all 89 lethal strains of the autumn population and the frequency of allelism was 6.62 per cent. It was recognized that many lehtal genes of allelic groups I, II, III and IV of the summer population were allelic respectively with those of allelic groups II, IV, VII and X, XI of the autumn population. These lethal genes have persisted for about two months and were distributed over about 10^6 square meters in Katsunuma locality.

Most chromosomes of allelic group II in the summer population and all chromosomes of allelic group IV in the autumn population had not only an allelic lethal gene, but also a visible mutant gene for reduced bristle (*rbl*).

A few chromosomes of group II in the summer population were proved to have at least three deleterious genes.

Sterility genes in natural summer and autumn populations of *D. melanogaster*[1)]

Chozo OSHIMA, Takao K. WATANABE and Masaoki KAWANISHI

Frequencies of sterile male and female flies among several hundred flies collected simultaneously from the summer and autumn populations of 1970 in Katsunuma locality were found to be 4.3, 4.8 per cent and 3.4, 7.8 per cent respectively.

Among 342 second chromosomes extracted from each male fly in summer and autumn populations of 1970, 69 chromosomes were found to be sterility chromosomes. The results are shown in Table 3.

Table 3. Frequency of sterility chromosomes and frequencies of male, female and both sexual sterility chromosomes

Collection time	1970 (July, October)			
Class of viability	Semilethal	Subvital	Normal	Total
No. of tested chromosomes	46	41	255	342
No. of sterility chromosomes	13	12	44	69
Frequency (%)	(28.3)	(29.3)	(17.3)	(20.2)
No. of male sterility chromosomes (%)			32 (46.4)	
No. of female sterility chromosomes (%)			27 (39.1)	
No. of both sexual sterility chromosomes (%)			10 (14.5)	

The frequency of sterility chromosomes was higher than 12.6% in 1968.

By half diallel crosses between sterility lines, which have been maintained by the Cy-sterility balanced system, the frequency of allelism was determined in the summer and autumn populations as Table 4 shows.

The sterility strains, which were extracted from a natural population in Katsunuma locality in 1968 and have been maintained in our laboratory, were crossed diallelly with new sterility strains extracted from the summer populations of 1970. On the other hand, diallel crosses between the sterility strains of the summer and autumn populations were performed.

1) This work was supported by a grant (84097) from the Ministry of Education.

Table 4. Results of allelism between the strility genes

Collection time	July 31		October 12	
Sex	♀	♂	♀	♂
No. of sterility chromosomes	11	19	24	23
No. of crosses	55	171	276	253
No. of allelic crosses	10	1	14	26
Frequency of allelism (%)	(18.2)	(0.6)	(5.1)	(10.3)
Frequency of finding	No. of sterility genes			
1	6	17	14	10
2	—	1	1	—
3	—	—	1	2**
5	1	—	1*	—
7	—	—	—	1

*, **: persistent sterility gene.

One male sterility gene (*ms 801*) and one female sterility gene (*fs 801*) have persisted for two years. These male sterility genes (*ms 202, B, C*) in the autumn populations of 1970 were linked with different female sterility genes (*fs 206, fs 217*). Three different male sterility genes in the summer population of 1970 were found to be combined in double sterility chromosomes (*mss 203 A–C*). On the other hand, a female sterility gene (*fs 102*) has increased in the autumn population. The results are shown in Table 5.

The breeding pattern of *D. melanogaster* in Katsunuma locality has been scarcely known, but the places of hibernation and the dispersion of flies were probably localized in the district.

Table 5. Persistent and allelic sterility genes during two years in Katsunuma locality

Collection date	Oct. 2, 1968	July 31, 1970	Oct. 12, 1970
Female sterility gene	*fs801 A–F* (6 chromosomes)	*fs 102* (1 chromosome)	*fs 201 A–E* * (5 chromosomes)
Male sterility gene	*ms 801 A–M* (13 chromosomes)	*ms 113* (1 chromosome)	*ms 202 A* **
			ms 202 B·fs 206
			ms 202 C·fs 217 (3 chromosomes)
		ms 114	*mss 203 A*
		ms 103	*mss 203 B*
		ms 102	*mss 203 C*

——: allelic relationship *, **: persistent sterility genes.

Correlation between productivity and viability of *D. melanogaster*

Takao K. WATANABE

A total of 224 second chromosomes were extracted from a cage population, which has been maintained at constant 25°C for about 30 generations. Viabilities and productivities of homozygotes and heterozygotes for these chromosomes were estimated.

The mean viability of homozygotes for female sterility chromosomes was remarkably lower than that of other homozygotes and they reduced significantly the productivity of male flies, but on the contrary the homozygotes for male sterility chromosomes did not show such an effect on the viability and the productivity of female flies.

The correlations between productivities of both sexes, being homozygous and heterozygous for all chromosomes, were positive, and the correlation in the homozygotes was significant at 5 per cent level.

The correlations between productivity and viability of male and female heterozygotes and female homozygotes for all chromosomes or fertility chromosomes were not significant, but the correlations between productivity and viability of male homozygotes for all chromosomes or fertility chromosomes were positive and significant at 5 per cent level and 1 per cent level respectively.

Sterility genes of both sexes might be slightly deleterious in heterozygous condition for productivity of the same sex, but they should be neutral on viability.

Fecundity and longevity of *D. melanogaster* under constant and fluctuating temperature environments

Chozo OSHIMA and Masaoki KAWANISHI

Two cage populations were set up with about 1,000 F_2 flies from 192 female flies collected in October of 1966 from natural populations in Kofu and Katsunuma localities.

One cage population (F) has been maintained in an incubator, whose temperature fluctuated two times a day between 20 and 30°C by a program controller. Another cage population (C) has been maintained in the culture room, whose temperature was kept constant at 25°C.

After about two years, flies were sampled and used in the present experiment. Flies sampled from C population were divided into two groups and one group was exposed to constant temperature (C-c group) while another group was exposed to fluctuated temperature (C-f group). Flies sampled from the F population were similarly exposed to fluctuated temperature (F-f group) and to constant temperature (F-c group).

Individual longevity and fecundity of many female flies and hatchability of eggs laid in each vial were estimated under these constant and fluctuating temperature environments. The results for the four groups were compared.

The mean longevity of female flies in the F-f group was 31.3 days and that in the C-c group was 29.0 days. The longevity in constant temperature was generally shorter than in fluctuating temperature, but the mean longevity of female flies in the F-c group was remarkably reduced being 25.7 days.

The fecundity and the longevity of female flies may be closely related quantitative characters. Young female flies in the C-c group laid significantly more eggs until they were 10 days old than in the C-f group. However, the total number of eggs laid for 40 days in the F-c group having the shortest mean longevity was smaller than in other groups.

The hatchability of eggs laid by young female flies (1–10 days old) in all groups was higher than 90 per cent and no significant difference was found. However, the hatchability of the C-c group decreased faster with the age of female flies and that of old female flies (20 days old) was 67 per cent. On the other hand, that of old female flies (20 days old) of the F-f group was 81 per cent.

Longevity, fecundity and hatchability may be polygenic characters. When their variances of all groups were calculated and compared with each other, homeostatic gene systems of flies in these F and C populations could be expected. The degree of the system in the former population was recognized to be higher than in the latter population, but the characters in the former population varied in manifestion under constant temperature environment. (Published in Environ. Cotrol in Biol. 7:21–29, 1969).

Effect of light on oviposition of *D. virilis*

Chozo OSHIMA and Koji INOUE[1)]

Many flies of *D. virilis* were collected from a warehouse of a brewery company in Tokyo in the spring of 1970. A cage population was set up with them and has been maintained in the culture room, whose temperature was kept at 25°C. After three months, 12 lines were established with the offspring of different female flies extracted from the population. Nine pairs, isolated from each line, were divided into three groups. When they were six days old, they were treated with three kinds of light conditions; 1. constant light (2500 Lux), 2. dark (15-minute light/day), and 3. periodical light and dark (9 hours 2500 Lux, 9 hours 0 Lux, 3 hours 2500$\rightleftarrows$0 Lux).

The first two groups were cultured under constant light and dark conditions, and the third group was cultured in an incubator, whose light condition was controlled periodically by a program controller. The experiment was performed at constant 25°C.

A pair of male and female flies were placed in a creamer and transferred every day to a new one. Eggs laid on a culture medium were scored. The experimental results for ten days are given in Tables 6 and 7.

The character of oviposition was remarkably affected by light conditions and it was observed that the mean number of eggs per fly increased linearly in the order of constant light, periodical light and dark, and dark (15-minute light/day) conditions. The phenotypic variances under those three conditions were significantly different and the variance under the periodical light and dark condition was the smallest while that underdark (15-minute light/day) condition was the largest.

Daily fluctuations of oviposition under these three conditions were com-

Table 6. Mean number of eggs per day per fly under three kinds of light conditions

Condition	Mean number of eggs	Range
Constant light	50.91	4—88
Periodical light and dark	54.16	29—77
Dark (15-minute light/day)	63.86	14—94

1) Temporary researcher, present address; Kurume Branch of Horticultural Research Station, M. A. F. Kurume, Fukuoka-ken.

Table 7. Result of analysis of variance for total number of eggs laid by three female flies of each line for 10 days

Source of variation	D.F.	S.S.	M.S.	F
Condition	2	326,977.02	163,488.51	3.408
C_L	1	301,994.01	301,994.01	6.295**
C_Q	1	24,983.01	24,983.01	
Line	11	1,574,402.77	143,127.52	6.706**
C×L	22	1,055,494.32	47,977.01	2.248**
Error	72	1,536,645.33	21,342.30	
	107			

pared by the run test method. The fluctuation was recognized only under the periodical light and dark condition. The total number of eggs laid in the first five days was higher than in the next five days under both constant light and dark (15-minute light/day) conditions.

Relationship between phototaxis and oviposition of *D. virilis*

Chozo OSHIMA and Koji INOUE

One cage population was set up with flies collected from a warehouse of a brewery company in Tokyo and has been maintained in the culture room. After three months, about three hundred flies were extracted, and male and female flies were separated. They were introduced into a maze apparatus having eleven choice points in order to select positive and negative flies with respect to phototaxis. About 30 pairs of photopositive flies and on the other hand, about 30 pairs of photonegative flies were crossed in vials and their offspring were subjective to selection. After several generations, the degrees of positive and negative phototaxis of flies seemed to reach the equilibrium state. The regression coefficient of selection curves was calculated to be 0.475.

Eleven lines were established with flies extracted from the 7th generation of photopositive population and twelve lines were established with flies extracted from the 9th generation of photonegative population. Nine pairs were isolated from each line and their oviposition under three kinds of light condition were examined as described in the previous experiment.

The mean number of eggs per day per fly increased in the order of constant light, periodical light and dark, and dark (15-minute light/day) conditions as shown in Table 8, but such a result could be observed more clearly in photopositive flies.

Table 8. Mean daily number of eggs laid by photopositive and photonegative flies under three kinds of light condition

Condition	Photopositive flies		Photonegative flies	
	First 10 days	Second 10 days	First 10 days	Second 10 days
Constant light	40.04	21.09	42.46	33.63
Periodical light and dark	44.90	30.47	40.86	36.76
Dark (15-minute light/day)	51.53	34.15	50.24	40.79

Flies laid more eggs in the first 10 days than in the second 10 days, and this pattern of oviposition was recognized clearly in photopositive flies. The difference between numbers of eggs laid for both first and second ten day periods by photonegative flies under the periodical light and dark condition was the smallest. The rhythm of oviposion may be most efficiently maintained by the same flies.

The running speed of photopositive flies through the apparatus was faster than that of photonegative flies.

X. EVOLUTION GENETICS

An intergeneric hybrid between *Agropyron tsukushiense* and *Elymus mollis*

Sadao SAKAMOTO

El. mollis Trin. is distributed widely from Korea to Japan, Kamchatka and Alaska, northeastern U.S.S.R., and Northern and Eastern Canada, Greenland and Iceland. This species is tetraploid ($2n=28$) and includes three subspecies. A strain collected at the seashore of Abashiri, Hokkaido, has been maintained in Misima as a clone. It usually does not head in Misima but two spikes were produced in 1969; their pollen grains were dusted on emasculated florets of *Agropyron tsukushiense* (Honda) Ohwi var. *transiens* Ohwi ($2n=42$) collected in Misima.

From this cross 22 F_1 plants were produced in 1970. Crossability was 30.6% calculating from the number of hybrid plants produced and the number of florets pollinated. Individual variation in growth behavior of F_1 plants was observed. Out of 16 F_1 plants observed only seven were heading. The shape of the F_1 spikes was of *Agropyron* type but the empty glumes were hairy as those of the *Elymus* parent. One of characteristics distinguishing *Elymus* from *Agropyron* is the production of two spikelets at almost all nodes of the rachis. This character was not expressed in F_1. Thus, this behavior was quite different from that observed in F_1 hybrids between *El. dahuricus* or *El. sibiricus* and *Ag. tsukushiense* where 1/3–1/4 of all nodes of a rachis had two spikelets (Sakamoto 1965). All pollen grains of F_1 plants were completely abortive.

Average chromosome pairing at MI of PMCs of the F_1 amounted to 2.0 bivalents and 30.9 univalents. Almost all bivalents ranging from one to seven were elongated and connected loosely by terminal chiasma. These results indicate a lack of genomic homology between the three genomes of *Ag. tsukushiense* and the two genomes of *El. mollis*.

Collection of wheats and various other species of the tribe Triticeae in Iraq, Turkey and Iran

Kosuke YAMASHITA[1], Masatake TANAKA[1], Sadao SAKAMOTO and Hirotoyo ISHII[1]

Northern Iraq, Southeastern Turkey and Southwestern Iran, or briefly, the eastern part of the so-called Fertile Crescent, was regarded from older findings to be the most important area which could provide interesting materials in respect of origin, differentiation and domestication of tetraploid wheats. For instance, a number of reports were known of massive stands of *Triticum dicoccoides* together with its putative progenitors, *Tr. boeoticum* and *Aegilops speltoides*. But only a few studies became known of their cytogenetic relations. The finding alone of *Tr. dicoccoides* in these parts was a powerful enough incentive to organize a new expedition and to collect a wealth of material of those plants which would at last allow a thorough study of their cytogenetic affinities to Palestinian *dicoccoides Tr. timopheevi* and *Tr. araraticum*.

Also, a thorough exploration of archaeological excavation of such age as 7,000 years B. C. promised to be highly interesting in view of the domestication problems in these parts.

Therefore, a botanical expedition of Kyoto University to the Northern Highlands of Mesopotamia was sent to Iraq, Turkey and Iran from May

Table 1. Specimens of Triticeae collected in Iraq, Turkey and Iran during the expedition

Genus name	No. of species	No. of samples
Aegilops	15	486
Agropyron	7	18
Crithopsis	1	13
Eremopyrum	5	81
Henrardia	1	5
Heteranthelium	1	55
Hordeum	8	179
Psathyrostachys	1	3
Secale	3	20
Taeniatherum	2	70
Triticum	5	221

[1] Kyoto University.

10th to July 25th, 1970, for the collection of wild as well as cultivated wheats and other Triticeae.

Along the expedition routes many large samples of *Triticum*, *Aegilops* and other taxa belonging to the tribe Triticeae were collected. The collected items are briefly summarized in Table 1. At the same time wild Gramineae indigenous to the area were also collected. The whole collection made by the expedition contains about 450 herbarium specimens of *Triticum*, *Aegilops* and their relatives. The specimens were placed on file at the Royal Botanic Gardens, Kew, the Smithsonian Institution, Washington, D. C., the National Science Museum, Tokyo and the Department of Botany, Kyoto University, Kyoto.

Dynamics of Plant Domestication: Cultivation Experiments with *Oryza perennis* and Its Hybrid with *O. sativa*

Hiko-Ichi OKA and Hiroko MORISHIMA

To look into the dynamics of domestication in rice, three population samples of the Asian form of *Oryza perennis* (the wild progenitor of *O. sativa*) collected from natural habitats, and seven hybrid populations derived from *sativa*×*perennis* crosses were grown for several generations at Taichung, Taiwan. They were observed for characters distinguishing wild from cultivated types. To investigate the influence of propagating methods, the hybrid populations were grown both in bulk and in pedigrees.

The results clearly indicated that when populations containing *sativa* genes were grown in the field, the frequency of alleles characterizing the cultivated type increased in response to "cultivation pressure". This genotypic change toward the cultivated type was more rapid in bulk populations than in pedigrees. Studies of character correlation showed that the pedigree populations gradually restored parental character associations in response to natural selection, but the same trend early realized in bulk populations could be masked by the elimination of wild types. It was also found that plants showing cultivated characters were more homozygous than those with wild characters, suggesting that natural selection for cultivated characters resulted in an increase of selfing rate. Comparing naturally sown populaions with those seeded by hand, seeding was found to be an effective agent causing "cultivation pressure". The details will be published

in EVOLUTION 25: 356–364.

Partial Self-incompatibility in *Oryza perennis*

Hiroko MORISHIMA and Hiko-Ichi OKA

The African form of *Oryza perennis* Moench (subsp. *barthii*) is partly self-incompatible (Chu *et al.* 1969. Japan Jour. Genet. 44: 225–229). To examine whether or not other geographical races of this species also show a tendency to self-incompatibility, four Asian, two American, and two Oceanian strains each represented by several plants were grown, and self- and cross-pollinations were made by hand. In so far as the materials used are concerned, self- and cross-pollination brought about the same amount of seed set indicating that they were self-compatible, though seed fertility differed among strains.

Plants belonging to several African strains (*barthii*) were also tested. Most of them consistently showed partial self-incomaptibility, but one strain, ML3–2, was found to be self-compatible. It has some different morphological characteristics from typical *barthii* plants. The F_1 hybrid between this strain and other *barthii* plants showed partial self-incompatibility. Different *barthii* strains appeared to differ in the degree of self-incompatibility.

XI. HUMAN GENETICS

Inheritance of Total Finger Ridge Count

Ei MATSUDA and Ei MATSUNAGA

The distribution of total ridge count has been examined using finger prints from unrelated Japanese, *i.e.* 313 males and 313 females. The mean total ridge counts were found to be 151.28 ± 46.09 for the males and 142.20 ± 44.81 for the females, the difference being significant. The values of g_1 (skewness) and g_2 (kurtosis) obtained for the male distribution are

$$g_1 = -0.150 \pm 0.138, \quad g_2 = 0.016 \pm 0.275.$$

For the female distribution the corresponding values are respectively:

$$g_1 = -0.286 \pm 0.138, \quad g_2 = -0.009 \pm 0.275.$$

Based on family data, correlation coefficients for total ridge count between various types of relatives have been estimated, and the results are shown in Table 1.

Table 1. Correlations for total ridge count between relatives

	No. of pairs	Correlation coefficient
Parent-child	1,048	0.436 ± 0.028
Midparent-child	470	0.598 ± 0.037
Mother-child	533	0.390 ± 0.040
Father-child	515	0.480 ± 0.039
Parent-parent	195	0.015 ± 0.072

Using data on 187 sibships, the sib correlation was calculated, after correction for sex difference, to be 0.463 ± 0.038. Finally, the regression coefficient of total ridge count of offspring on midparent value was estimated to be 0.819. Although these estimated values are slightly lower than those obtained by Holt for the British population, (Ann. Eugen. 20: 270–281, 1956), it appears that they are consistent with the hypothesis that total ridge count is controlled by a number of autosomal genes with almost additive effect.

Polymorphic Red Cell Enzymes among Japanese

Tomotaka SHINODA

Polymorphisms of several enzyme systems in red cells have been investigated in 2,423 unrelated individuals, which were taken in three different areas. Among them, acid phosphatase, adenosine deaminase, 6-phosphogluconate dehydrogenase and phosphoglucomutase were found to be polymorphic, with practically the same frequencies in different population groups except for a few variants. Estimated gene frequencies for respective enzyme systems were as follows; AP: $P^a=0.20$, $P^b=0.80$; ADA: $ADA^1=0.97$, $ADA^2=0.03$; PGD: $PGD^A=0.93$, $PGD^C=0.07$; PGM_1: $PGM_1{}^1=0.77$, $PGM_1{}^2=0.22$. As to rare PGM variants, there were four types; three of them were identified to be PGM_1 5–1, 7–1 and 8–1, but the remaining type was not yet identified with certainty. In phosphoglucose isomerase about 99% were of type PGI 1, while the remaining 1% were accounted for by four different variants, namely PGI 3–1, 4–1, 5–1 and 6–1 J, their incidences in percent being respectively 0.2, 0.8, 0.1 and 0.05. No electrophoretic heterogeneity was found in the samples with respect to adenylate kinase type, in agreement with the previous result.

Additional enzyme systems, such as citrate dehydrogenase, hexokinase, malate dehydrogenase, succinate dehydrogenase, nucleoside phosphorylase and fumarase were examined in some of the samples, but no electrophoretic polymorphism was found by the current technique employed. One rare variant in tetrazolium oxidase was found among the samples; the phenotype was tentatively designated as TOX 2–1, and its mode of inheritance was determined by family studies.

Details of the above results were published in Jap. J. Human Genet. 15: 133–143 and 159–165, 1970.

Structure of Human Macroglobulin

Tomotaka SHINODA

The Fc fragment prepared from whole Ig M by trypsin treatment at high temperature was subjected to a selective cleavage at methionyl residues by cyanogen bromide. By combination of several procedures, at least five fragments were isolated in a pure form, and they were further characterized. Of these, a peptide with 11 amino acids having a carbohydrate branch was

located at the NH_2-terminal and an octapeptide was located at the COOH-terminal of the heavy chain. The order of the remaining cyanogen bromide fragments was not yet fully established. In addition to isolation and characterization of cyanogen bromide fragments from Fc, an extensive work has been carried out to isolate and locate disulfide bridges, both inter and intra chains in the whole macroglobulin. At present, amino acid sequences around five disulfide bridges were established. Of these, one was proved to be located in VH region, while the remaining four were located in the Fc region. Exact positions of each bridge in the Fc fragment were not yet established. Further attempts to isolate disulfide bridges are now going on.

Birth Weight in Down's Syndrome: Comparison of interchange trisomics and G trisomics

Ei MATSUNAGA

From cytogenetical evidence it is assumed that the amount of excess chromatin mass in interchange trisomics with t(DqGq) must be slightly less than in regular G trisomics. As far as physical signs of Down's syndrome are concerned, however, no difference has been noted between patients with interchange trisomy and G trisomy types. The author examined birth weights of 58 pairs of interchange trisomics/G trisomics, matched for sex, maternal age and birth order. The mean difference in birth weight within a pair (interchange trisomic minus G trisomic) was calculated to be only −8.2g, with standard deviation of 86.3 g. This finding indicates that the two types of chromosome aberration have quantitatively a comparable effect upon intrauterine development of the affected fetus.

Parental Ages in Translocation Down's Syndrome

Ei MATSUNAGA

Parental ages were analyzed for 49 cases of D/G and 38 cases of G/G translocation Down s syndrome. These cases were collected from more than 1,700 patients with this syndrome karyotyped in various laboratories

in Japan. With respect to parental karyotypes, they were classified into inherited (19 D/G and 4 G/G), sporadic (18 D/G and 21 G/G) and unknown classes (12 D/G and 13 G/G). When the data were combined for sporadic and unknown classes, the mean paternal ages in years were 30.9 for D/G and 31.4 for G/G, both being close to the population average (30.9), while the mean maternal ages were 26.1 for D/G (1.6 years below the population average) and 29.1 for G/G (1.5 years above the population average), the difference between these two figures being significant. This finding suggests that maternal age may have some selective effect upon interchange of the acrocentric chromosomes.

Distribution of Fast Renaturing DNA in Human Chromosomes

Yasuo NAKAGOME

The distribution of fast renaturing (repetitive) DNA in human mitotic chromosomes was studied by *in situ* denaturation-renaturation technique (Arrighi, F. E. *et al.* 1970. Chromosoma 32: 224). After the denaturation with NaOH, chromosome preparations were allowed to renature *in situ* in either 2 or 6 X SSC solution at 66°C for 15 hours. The renatured segments of chromosomes were identified as darkly stained bands along the chromatids in Giemsa stained preparations. Every band observed was located at the centromeric region of a chromosome (centromeric heterochromatin).

The same procedure, however, was found to be not satisfactory in studying the nuclei of certain kinds of cells. Polymorphnuclear leukocytes and sperms were among the hard-to-analyse cells. To overcome this difficulty in identifying renatured regions, cells were stained with acridine orange instead of Giemsa solution. Experiences with viruses revealed that double-strand DNA showed yellow-green fluorescence but RNA and single-strand DNA showed flame-red fluorescence under certain conditions (Mayor, H. D. 1961. Proc. Soc. Exper. Biol. Med. 108: 103). Any segments of chromosomes or cells that show yellow-green fluorescence, presumably, contain renatured DNA. On the other hand, the flame-red regions(RNAase treatment required) should contain denatured DNA. Both the sensitivity and the specificity of this method in identifying renatured segments of chromosomes are expected to be much better than the usual Giemsa staining procedure. This particular experiment is still in progress.

A Cp+mat Chromosome Found in a Malformed Boy

Hidetsune OISHI, Ei MATSUDA and Yasumoto KIKUCHI

During our current survey of chromosomes in patients with some clinical disorders, a case with a Cp+mat chromosome was observed.

The patient was a 27 months old boy who was the first-liveborn child of a 31 years old mother and a 30 years old father. The gestation lasted normally for 40 weeks and the birth weight was 3,100 g. The parents were healthy and not consanguineous. The mother had previously experienced one stillbirth.

Main clinical features found in the patient were: epicanthal folds, high-arched palate, micrognathia, undescended testes, polydactyly of toes and protuberant heels. The results of dermatoglyphic analysis of the patient were as follows, a transverse palmar crease was noted on his left hand: *Right hand.*—Palmar formula, 11.9.7.1-tt″-L^u.O.O.L.O. *atd* angle=55°, 123.5°; digits=U,A,W,W,W. *Left hand.*—Palmar formula, 3.3.3.1-t-L^u. O.O.O.O, *atd* angle=54°; digits =U,U,W,W,W. Total ridge count=86. Right toes=A,A,A,A,A. Left toes=A^t,A,A,A,A.

Chromosome analyses of the patient revealed that a missing C-group chromosome was substituted by a metacentric chromosome resembling a no. 3. On the other hand, the karyotype of the mother was characterized by the presence of two extra chromosomes, one in no. 3 and the other in group G, in accordance with the missing two chromosomes one from group C and the other from group D. Since the mother was phenotypically normal, the apparently balanced karyotype was interpreted as the result of a reciprocal translocation between a C- and a D-group chromosome. Autoradiography disclosed the consistent presence of a normal, late replicating X chromosome in mother's cells and indicated that the extra large chromosome was not a no. 3. In addition, the D-group chromosome involved was identified with D_1 by means of autoradiography, though the exact numbering of the C-group chromosome involved was unknown. Therefore, the karyotype of the mother could be written as 46, XX, t(Cp+; Dq−). The Cp+ chromosome found in the patient was transmitted from the mother, and he was considered to represent trisomy for the distal half of the long arm of a D_1 chromosome. The father had a normal male karyotype.

Chromosome Studies in Congenitally Abnormal Children

Yasuo NAKAGOME and Hidetsugu MATSUMOTO

Among 20 cases studied, 2 showed rare chromosome disorders.

A fourteen year-old girl was referred to us because of short stature (128.8 cm) and sexual infantilism. On physical examinations a few additional minor anomalies such as shield-like chest, short and wide neck and short fourth metacarpal bones were observed. Dermatoglyphic studies revealed no unusual findings except for a very high total ridge count (226). Buccal smear studies revealed very small as well as scanty sex chromatin spots. No drumstick was observed in 2,000 polymorphnuclear leukocytes. The karyotype, based on the standard leukocyte-culture technique, showed two cell lines. One of them had a 45, X complement and the other showed a 46, XX, Cq- karyotype. The Cq- chromosome was smaller than No. 12 but larger than No. 16 and was median centric. Its chromatids were always attached to each other and looked fuzzy. Autoradiography revealed that the Cq- chromosome replicated itself at the end of the S-period, *i.e.* it represented, in fact, an X chromosome with the deletion of a part of the long arm. In other words, her karyotype was 45, X/46, XXq-. The size of the deletion was estimated to be 45% of the long arm. This case represents one of the mildest deletions of the long arm of the X chromosome so far reported.

The second case was a three month-old boy with the typical clinical features of Down's syndrome. Fourty-seven chromosomes were observed by the standard leukocyte-culture technique. However, there were only five G group chromosomes. None of them showed the characteristic appearance of the Y chromosome. There was an extra acrocentric chromosome in the D group. In most metaphases the extra chromosome was identified by its peculiar shape. The both chromatids of the long arm were usually close together and looked fuzzy. It was not satellited and was slightly smaller than the other members of the D group. Based on these findings, the patient's karyotype was interpreted as 47, XYq+, G+. The pattern of quinacrine mustard fluorescence in this unusually long Y chromosome is under study.

Clinical Conditions of Patients with Apparently Normal Chromosomes, VII

Hidetsune OISHI

Based on leucocyte cultures of peripheral blood, patients with various pathological conditions were found to have apparently normal chromosome assortments of 46 chromosomes. Cases of some interest are listed below.

Case no.	*Age*	*Legal sex*	*Clinical conditions*
a) Multiple deformations with metal retardation			
122 M.N.	10 days	M	Microcephaly; narrow temples; sloping forehead; hypertelorism; coloboma chorioideae; cleft palate; short neck; hemangioma
123 T.U.	3 years	M	Hypertelorism; antimongoloid slant eyes; a transverse palmar crease (left hand); hemangioma
124 Y.T.	5 years	M	Microcephaly; antimongoloid slant eyes; large ears; spasticity
125 U.M.	4 years	F	Dubois' sign; incurved fifth fingers; large ears; logopathy
126 M.A.	1 year	F	Microcephaly; micrognathia; hypotonia
127 S.H.	1 year	M	Microcephaly; high-arched palate; ptosis; malformed and low-set ears; micrognathia; polydactyly; syndactyly; cryptorchidismus; hypertonia
128 R.T.	2 months	F	Congenital heart disease; anophthalmia (right); coloboma chorioideae (left); hydrocephalus
129 Y.N.	3 years	M	Open anterior fontanelle; antimongoloid slant eyes; high-arched palate; a transverse palmar crease (left hand)
b) Sex anomalies			
130 H.O.	5 years	F	Webbed neck (Turner's syndrome?, 46, XX)
131 S.N.	14 years	F	Dwarfism; cubitus valgus (Turner's syndrome?, 46,XX)

132 Y.K. & M.K. (sibling) 22 & 26 years		F	Testicular feminization (46,XY)
133 C.Y.	15 years	F	Short stature; shield chest; cubitus valgus; small uterus (Turner's syndrome?, 46, XX)
c) Hereditary diseases			
134 K.K.	6 months	M	Familial ectodermal dysplasia
135 M.S.	3 years	F	Familial chronic nephritis (Alport's syndrome)

XII. APPLIED GENETICS

Geographic Variation on the Isoenzyme Level of *Abies sachalinensis* in Hokkaido

Kan-Ichi SAKAI and Takashi MATSUURA

Abies sachalinensis is one of the most important conifer species in Hokkaido, growing wild in hilly as well as in mountainous regions. For the purpose of a provenance test, seedlings from different localities have been grown in a hilly land at the outskirts of Sapporo. The present study deals with the electrophoretic study of needle-leaves collected from 25 trees from each of six strains. The locations from where the six strains had originated are designated from the east to the west by N (Nemuro), S (Saroma), U (Urakawa), P (Piuka), K (Kotanbetsu) and H (Higashi-setana). The trees were six years of age. Examined were the peroxidase isoenzymes in the leaf sap. It has been found from the study that their maximum number was 27. On the basis of those isoenzymes, the following comparisons were made among the six populations. The first comparison dealt with counting the average number of isoperoxidases per tree. It was found that the mean number as well as its standard deviation was variable among the populations. The second comparison was made from the frequency of each of the 27 isoenzymes in the six populations. It was found that some populations were slightly different from each other, whereas in others the difference was very significant. The third comparison was made by measuring the number of azygous isozyme bands or the "disagreement count" among trees within and between populations. It was found that the disagreement count among some populations was very small, whereas others showed high values. Combining the results of such comparisons, it was concluded that *Abies sachalinensis* may have been originally propagated in the eastern to the north-eastern district of Hokkaido. The trees may have first migrated to the central parts and finally to the western and south-western parts of the island. The details of this study will be reported in some other journal.

Genetic Differentiation within a Natural Forest of *Cryptomeria japonica* D. Don

Kan-Ichi SAKAI and Young-Goo PARK

The present study aimed at detecting intra-population genetic differentiation in a natural forest of *Cryptomeria japonica* growing in Kochi Prefecture on the Shikoku island. At first, three sites were allocated in the forest. They were located at the apices of an isosceles triangle, the straight line distances among the three subpopulations being 760 m, 1300 m and 1300 m, respectively. In each of these subpopulations approximately 50 trees were selected at random and their needle-leaves were collected on individual tree basis. An electrophoretic analysis was conducted with those leaves, in order to measure the variability of isozyme peroxidase patterns. The total number of isozyme bands of the peroxidase counted and identified in the present species was 36, though the average number of isozyme bands actually exhibited per tree in the three subpopulations was 11.68 ± 0.28, 13.73 ± 0.35 and 11.35 ± 0.29, respectively.

The three subpopulations were comparatively examined by twos for the incidence of each of the 36 bands. It was found that the number of statistically significant differences between subpopulations was 10, 12 and 15 out of 36 comparisons. It shows that they were far more numerous than expected if due only to chance. Thus, it is concluded that a natural forest of *Cryptomeria japonica* is not genetically homogeneous but very heterogeneous, most probably involving numberless subpopulations which are more or less genetically different from one another. It is suggested that a random genetic drift followed by effective isolation due to a higher occurrence of inbreeding than expected on the basis of wind-pollination may have occurred in a natural forest of *Cryptomeria japonica.*

Genetic Studies of Peroxidase Isozymes of the Japanese Red Pine, *Pinus densiflora* Sieb. & Zucc.

Young-Goo PARK and Kan-Ichi SAKAI

It has been proved recently that isoenzymes are of great use in the study of genetic variation of forest trees, especially in natural forests of *Cryptomeria japonica, Thujopsis dolabrata,* and *Abies sachalinensis.*

The present paper describes the results of a study enquiring into the genetic basis of peroxidase isozyme formation in Japanese red pine, *Pinus densiflora* Sieb. et Zucc. Materials for this study were selfed progenies and F_1 hybrids of a diallel cross among 15 parental trees collected from different regions of Tohoku district of Japan Proper. The laborious work of collection of mother-trees and the subsequent cross-hybridization have been performed by Dr. Y. Takayama and his collaborators in Kitakami Silviculture Station of Jujo Paper Manufacturing Co. Ltd., due to their kind help we have been able to make use of the valuable materials for the present study.

The electrophoretic analysis has shown that the total number of peroxidase isozymes in the present species was thirteen. Of those thirteen, four, *i.e.* H, K, M and N have been investigated. Inferences from comparison among the phenotypes of parents, selfed progenies and F_1 hybrids allowed the authors to assume that independent pairs of genes, Ph^+ vs. Ph^-, Pk^+ vs. Pk^- together with the repressor gene, Rk vs. rk, Pm^+ vs. Pm^-, and Pn^+ vs. Pn^-, were responsible for the formation of H, K, M and N bands, respectively. There was no indication of multiple allelism among those alleles. It was found that H behaved independently against either K, M or N, whereas K was negatively correlated with M and N, while M and N were positively correlated with each other though the correlation coefficients were rather low.

An Analysis of Sexual Dimorphism in Body Size in the Japanese Quail

Takatada KAWAHARA

In the Japanese quail, the sex difference in body weight is pronounced, the female being heavier than the male. The difference becomes apparent at the age of 2 or 3 weeks after hatching, and it continues throughout whole life. The main purpose of the present investigation was to elucidate the cause of the sex difference in the body weight. Data have been collected from three stages at the age of 2, 4 and 25 weeks. Three hundred and eighty-two birds were used in total. The number of birds examined was 42, 36 and 304 birds at 2, 4 and 25 weeks after hatching, respectively. They consisted of the same number of males and females collected from a number

of full-sib families. The total body weight was partitioned into the following components, *i.e.* bones, heart, lungs, liver, gizzard, intestines, pancreas, spleen, kidneys, gonads, oviduct, egg materials in oviduct and eviscerated body weight.

Results of this study are summarized as follows:

1) Whole body weight in the female was significantly heavier than that of the male at each of the three developmental stages. Almost all female organs showed a tendency to show heavier weight than the male organs at younger stages (2 and 4 weeks of age), though the difference was not statistically significant, except for a few organs such as digestive and the related organs and eviscerated body weights. Sexually matured birds (25 weeks of age), however, exhibited statistically significant difference between the sexes in all organ weights, except heart weight. Organs which showed the greatest difference between the sexes were sexual organs, liverin and testines.

2) Of interest is the eviscerated body weight in relation to the developmental stages of the bird. Total body weight in the three stages was always larger in females than in males. The eviscerated body weights were heavier in female than in males in the early two stages, but that at mature stage showed apparently reversion, that is, the male was heavier than the female. The difference between sexes or female minus male in the eviscerated body weight was +2.0 g. at 2 weeks, +4.1 g. at 4 weeks, but −11.6 g. at 25 weeks of age, respectively.

3) Comparison between male and female with regard to genetic and environmental variances of eviscerated body weight at 25 weeks of age showed that the environmental variance was approximately the same between the sexes, whereas the genetic variance in males was larger than in the female. Such a sexual difference in genetic variance was not found in other organs. Further investigation into the genetic mechamism of this sexual dimorphism in the quail is under progress.

Monte Carlo Studies on the Relationship between the System of Reproduction and Inbreeding

Shin-ya IYAMA

To maintain a population of wild organisms or breeding material under artificial control, it is required to find an appropriate structure of breeding

population to protect it from inbreeding depression. The author has undertaken two Monte Carlo experiments to investigate the effect of control of the reproductive system on the progress of inbreeding in a population. The following assumptions were made in these experiments: 1) non-overlapping generations, 2) constant population size, 3) no polygamy allowed; mating performed by twos between a male and female pair, 4) sex ratio 1:1, and 5) no relationship among members of the initial population.

Wright's coefficient of relationship for all possible combinations among the members was computed and recorded for each generation. Inbreeding coefficient of the progeny population derived from given matings was calculated on the basis of the above record up to 100 generations. Effective population number (N_e) was estimated by fitting the formula known for a dioecious population, $f_n=(1/2N_e)+(1-(1/N_e))f_{n-1}+(1/2N_e)f_{n-2}$, to the results, where f_n stands for the inbreeding coefficient at the n-th generation.

Experiment 1: In a random mating population, two types of propagation were considered. One was that the number of progeny from each mating to the next generation was variable. Since the population size was constant, the number of male or female progeny per mating was assumed to follow the Poisson distribution with mean of unity. The other was that each mating pair contributes always one male and one female to the next generation. Monte Carlo experiment was carried out for various population sizes (N) and the effective population number (N_e) was estimated (Table 1). In the case of variable progeny number, N_e was found consistent with N as expected. N_e for the case of constant progeny number agreed well with $2N-1$ which has been suggested by Wright (1938) theoretically. It is concluded that the control of the reproduction to produce an equal number of progeny from each mating pair makes the effective population size almost as large as double of the case with variable progeny number which is probable in uncontrolled population.

Experiment 2: When the population consists of more than two subpopulations, circular group mating was proposed by Kimura and Crow (1963) to keep inbreeding as low as possible. Practically it might be, however, troublesome since it is necessary to be done for every generation. Then, effectiveness of mixing the subpopulations with certain generation interval was examined as an alternative. In this experiment, equal number of progeny was assumed. Random mating was performed within subpopulations but they were mixed together and redistributed at random into

Table 1. Estimated effective population number for the matings producing variable and equal number of progeny

Actual population number (N)	Variable number of progeny	Equal number of progeny
10	10.0	17.9
20	19.7	38.0
30	28.3	57.8
40	37.2	77.5
50	48.9	96.9
60	55.8	117.0
100	104.2	195.7
150	150.8	294.4
200	197.9	392.9

Table 2. Estimated effective population number for the population consisting of more than two subpopulations

Total population number	No. of subpopulations	Size of subpopulation	Mixing type	Effective population number
60	2	30	none	57.8
			10	110.2
			5	113.8
			circular	118.1
60	3	20	none	38.0
			10	104.1
			5	110.9
			circular	118.6
60	5	12	none	22.0
			10	92.6
			5	104.5
			circular	115.9
60	6	10	none	18.0
			10	85.9
			5	99.5
			circular	114.5
60	10	6	none	10.0
			10	68.1
			5	87.5
			circular	98.5

subpopulations at every 10th or 5th generation. Results are shown in Table 2 in comparison with those of non-mixing and circular group mating.

N_e in circular group mating was nearly equal to the effective number of total population considered as an undivided one. Mixing effect improved the situation largely towards that of the circular group mating, frequent mixing making it still better. Furthermore, it was shown by the investigation of inbreeding coefficient at each generation that the more frequently mixing occurs, the smaller was the progress of inbreeding in the period during the mating was performed within each subpopulation.

Selection in Rice: An Experiment

Shin-ya IYAMA

The experiment was arranged in order to investigate what changes would occur when selection was made for low-yielding genotypes. The main material used in this study is represented by five populations, all derived from a cross between two or three *japonica* varieties and kept unselected until the fifth or sixth generation. They were:

CR141 F_6 Kinmaze × San-in No. 63
CR143 F_6 Hoyoku × 36–1
CR157 F_5 Sachikaze × Yamabiko
CR161 F_5 Shizukei Mochi No. 5 × Yamabiko
CR164 F_5 Norin No. 29 × (Hokushin Shuho × Chushin)

Throughout the experiment, the material was cultivated by single plant per hill planting with 25 cm × 15 cm spacing. The characters examined were panicle yield, plant weight, plant height and panicle number per plant. In 1968, selection for panicle yield was performed on an individual plant basis for each population. Each 50 plants were selected towards low and high yield from about 2,000 plants. In 1969, lines derived from the selected plants were cultivated with randomized block design with three replications, each plot consisting of 20 plants. Characters were measured on plot basis except panicle length which was obtained from ten samples chosen at random from each plot, and then converted into plant basis.

Analysis of variance of data showed that the difference between high and low selected groups in panicle yield was significant for all populations, indicating that the selection was effective. Heritability values obtained from the ratio of realized gain against selection differential were 0.07 to 0.17. Selection of panicle yield showed a statistically significant effect on

plant weight, plant height, panicle length and panicle weight, except plant weight and panicle length in CR141 and panicle length in CR157. It was suggested that the attained change in minus direction in panicle yeild by selection might be due rather to the change in weight per panicle than that panicle number per plant.

Growth Pattern and Its Plasticity in Wild and Cultivated Types of Rice

Hiroko MORISHIMA and Hiko-Ichi OKA

To compare the growth pattern and its plasticity between wild and cultivated types of rice, four cultivated, two wild and five intermediate (hybrid derivative) strains were grown in paddy and in gravel culture at two fertilizer levels from 1967 to 1970. Measurements for the length of various organs and dry-matter weight were taken several times during the growing period and at maturity. Yielding characters were also recorded at maturity. From the dry-weight data, logistic growth curves were computed to obtain various growth parameters.

The results may be summarized as follows: 1) In the cultivated type, the panicle and third internode elongate simultaneously and the following elongation of the first and second internode results heading. In some wild types, however, elongation of the third internode resulted in heading and the first and second internodes elongated after heading. 2) The wild type differs from the cultivated type in that the increase in dry weight after heading is partly due to vegetative growth and does not necessarily imply grain production. 3) Modern cultivars showed late vigor and sustained growth, while primitive ones showed early vigor and rapid decline. 4) Cultivated types responded to fertilizers mainly by increasing panicle number and grain yield, the size of organs remaining relatively constant, while wild types tended to be plastic as to organ size in response to fertilizers. 5) Annual fluctuation in characters was more pronounced in wild than in cultivated type. It may be concluded from the above experimental results that the wild type is highly responsive to natural environmental conditions, while the cultivated types are responsive to man-controlled conditions.

Inter-genotypic Interaction in Rice

Tsuguhiro HOSHINO, Hiroko MORISHIMA and Hiko-Ichi OKA

A mix-planting experiment with rice was conducted to investigate inter-genotypic competitive interaction. Ten F_{11} lines taken from pedigrees of a cross between two varieties, Taichung 65 (Japonica) × Pai-ku (Indica), were grown in pure stands and in mixture with each parental strain with three replications. They were observed for heading date and various yield characters at maturity. During the growing period, dry-matter weight was measured three times, and logistic growth curves were computed to obtain growth parameters. The effect of mix-planting on each genotype was estimated by subtracting the value of pure stand from that of mixed stand, and was designated as α for tested lines (resistance to aggression) and β for tester strains (aggressivity of a tested line). The values of $\alpha+\beta$ and $\alpha-\beta$ were considered to show synergism and competition between two component genotypes in mixed plot, respectively.

Main conclusions obtained may be summarized as follows: 1) Analysis of variance revealed that not only α and β but also the effects of synergism and competition significantly differed between the tested lines. 2) α and β were negatively correlated in most characters, though the correlation coefficients were low. 3) Principal component analysis of character correlations in α and β showed that, in addition to a general factor, a differential factor is involved, namely, variation between the lines which are plastic as to panicle size but stable as to panicle number and those having the opposite trends. 4) The larger is the difference in growth pattern between two component genotypes, the more synergistic they are in grain production.

Evaluation of an Electronic Instrument for Estimation of Plant Weight

Hiroko MORISHIMA, Hiko-Ichi OKA and Tsuguhiro HOSHINO

To estimate the weight of standing plants, an electronic instrument called "Herbage meter" was developed in collaboration with the Central Research Institute of Electric Power Industry. The instrument is essentially an electric capacitance meter. When the measuring head is placed over the standing plants, a change in the electrical capacitance of the system is caused which is closely related to the amount of herbage. Plant weight can be estimated from meter reading by calibration.

Using ten rice strains, reading of the herbage meter and actual dry-matter weight of sampled plants were compared every week during the growing period. Before heading, they were highly correlated with each other, though the regression coefficient in the prediction equation for cultivated strains significantly differed from that for a wild strain having a quite different plant type. Coefficients of determination (r^2) were 0.92 in cultivated strains and 0.96 in a wild strain. When the data for wild and cultivated strains were pooled, the coefficient of determination was 0.88, and was raised up to 0.89 by adding plant height to meter reading in the form of multiple regression. After heading, however, meter reading gradually decreased, while dry-matter weight increased with carbohydrate accumulation in the grains. This might be due to a change in electric resistance of plants after heading. Meter reading may indicate fresh weight rather than dry weight. This technique, therefore, seems to be applicable when the water content remains within a certain range. It was concluded that sequential record of vegetative growth can be taken by this technique more efficiently than by the ordinary sample-cutting method which reqires much labor and is often unreliable owing to a large inter-plant variation in dry weight.

BOOKS AND PAPERS PUBLISHED IN 1970 BY STAFF MEMBERS

(1) BLOOM, A. D., Y. NAKAGOME, A. AWA and S. NERIISHI 1970. Chromosome aberrations and malignant disease among A-bomb survivors. Amer. J. Public Health. **60**: 641–644.

(2) CHU, Y. E. and H. I. OKA 1970. The genetic basis of crossing barriers between *Oryza perennis* subsp. *barthii* and its related taxa. Evolution. **24**: 135–144.

(3) CHU, Y. E. and H. I. OKA 1970. Introgression across isolating barriers in wild and cultivated *Oryza* species. Evolution. **24**: 344–355.

(4) CROW, J. F. and M. KIMURA 1970. An Introduction to Population Genetics Theory. xiv+591. Harper and Row (New York).

(5) FUJII-KAWATA, I., K. MIURA and M. FUKE 1970. Segments of genome of viruses containing double-stranded ribonucleic acid. J. Mol. Biol. **51**(2): 247–253.

(6) FUKATAMI, A. and D. MORIWAKI 1970. Selection for sexual isolation in *Drosophila melanogaster* by a modification of Koopman's method. Japan. J. Genetics. **45**: 193–204.

(7) FURUICHI, Y., Y. WATAYA, H. HAYATSU and T. UKITA 1970. Chemical modification of yeast tyrosine tRNA with bisulfite. A new method to modify isopentenyladenosine residue. Biochem. Biophys. Res. Commun. **41**(5):1185–1191.

(8) HOTANI, H., T. OOI, H. KAGAWA, S. ASAKURA and S. YAMAGUCHI 1970. Biochemical evidence for identical structure of *p*-filament and flagellin. Biochim. Biophys. Acta. **214**: 207–215.

(9) KADA, T. 1970. Studies on the mutability of *Escherichia coli* K12. I. Suppression and high spontaneous mutation in a threonine auxotroph. Mutation Res. **10**: 91–102.

(10) KADA, T. 1970. Studies on the mutability of *Escherichia coli* K12. II. Modification of radiation sensitivity by reversions in a threonine auxotroph. Mutation Res. **10**: 103–109.

(11) KADA, T. 1970. Radio-sensitization with iodine compounds. II. Studies on mutant strains of *Escherichia coli* K12 resistant to radiation-induced toxic products from iodoacetic acid, potassium iodide or potassium iodate. Int. J. Radiat. Biol. **17**: 419–430.

(12) KADA, T., T. NOGUTI and M. NAMIKI 1970. Radio-sensitization with iodine compounds. I. Examination of damage in deoxyribonucleic acid with *Bacillus subtilis* transformation system by irradiation in the presence of potassium iodide. Int. J. Radiat. Biol. **17**: 407–418.

(13) KADA, T., Y. SADAIE and T. NOGUTI 1970. Radio-sensitization with extracellular halogenated purines and pyrimidines and related compounds. Int. J. Radiat. Biol. **18**: 281–285.

(14) KATO H. and T.H. YOSIDA 1970. Nondisjunction of chromosomes in a synchronized

cell population initiated by reversal of colcemid inhibition. Exptl. cell Res. **60**: 459–464.

(15) KIKUCHI, Y. and H. OISHI 1970. Internal asynchrony in late replication of human X chromosomes with structural abnormalities. Japan J. Human Genet. **15**: 114–123.

(16) KIMURA, M. 1970. Stochastic processes in population genetics, with special reference to distribution of gene frequencies and probability of gene fixation. Biomathematics Vol. 1, Mathematical topics in population genetics (ed. K. KOJIMA). 178–209 Springer-Verlag (Berlin).

(17) KIMURA, M. 1970. The length of time required for a selectively neutral mutant to reach fixation through random frequency drift in a finite population. Genet. Res. Camb. **15**: 131–133.

(18) KIMURA, M. and T. OHTA 1970. Probability of fixation of a mutant gene in a finite population when selective advantage decreases with time. Genetics **65**: 525–534.

(19) KIMURA, M. and T. OHTA 1970. Genetic loads at a polymorphic loci which is maintained by frequency-dependent selection. Genet. Res. Camb. **16**: 145–150.

(20) KURODA, Y. 1970. Differentiation of ommatidium-forming cells of *Drosophila melanogaster* in organ culture. Exptl. Cell Res. **53**: 429–439.

(21) KURODA, Y. 1970. Cytodifferentiation of *Drosophila melanogaster* in organ culture (in Japanese with English summary). Nucleus and Cytoplasm **14**: 49–55.

(22) KURODA, Y. 1970. Effects of X-irradiation on sorting-out property of animal cells in rotation culture (in Japanese). Japan. J. Develop. Biol. **24**: 69–70.

(23) KURODA, Y. 1970. Biology of cultured animal cells (in Japanese). The Heredity (Tokyo) **24**(5): 2–9.

(24) MARUYAMA, T. 1970. Rate of decrease of genetic variability in a subdivided population. Biometrika **57**: 299–311.

(25) MARUYAMA, T. 1970. Analysis of population structure. I. One-dimensional stepping-stone models of finite length. Annals of Human Genetics **34**: 201–219.

(26) MARUYAMA, T. 1970. On the rate of decrease of heterozygosity in circular stepping stone models of populations. Theoretical Population Biology **1**: 101–119.

(27) MARUYAMA, T. 1970. Effective number of alleles in a subdivided population. Theoretical Population Biology **1**: 273–306.

(28) MARUYAMA, T. 1970. Stepping stone models of finite length. Advances in Applied Probability **2**: 229–258.

(29) MARUYAMA, T. 1970. On the fixation probability of mutant genes in a subdivided population. Genet. Res. Camb. **15**: 221–225.

(30) MARUYAMA, T. and N. YASUDA 1970. Use of graph theory in computation of inbreeding and kinship coefficients. Biometrics **26**: 209–219.

(31) MATSUNAGA, E. 1970. Chromosomal aberrations and maternal age (in Japanese) Rinsho-Kagaku **6**: 648–655.

(32) MATSUNAGA, E. 1970. Report of the study group on medico-legal determination of disputed paternity (in Japanese). Japan J. Legal Med. **24**: 193–230.

(33) MATSUNAGA, E. 1970. Future of the world population (in Japanese). The Heredity **24**(11): 2–5.

(34) MATSUNAGA, E., K. OMOTO, T. SHINODA, H. OISHI and E. MATSUDA 1970. A further study on the family with anomalous inheritance of haptoglobin types. Japan J. Human Genet. **15**: 166–175.

(35) MIURA, K. 1970. Nucleic acid chemistry (in Japanese). The *Nucleic Acids*. (eds. Y. Takagi and Y. Miura) 1–35 Asakura-shoten (Tokyo).

(36) MIURA, K. 1970. Structure and function of the tRNA (in Japanese). Biochemical Genetics (eds. Y. Kajiro *et al.*) 123–141 Igakushoin (Tokyo).

(37) MIURA, K. and H. HAYASHI 1970. On the transfer RNA (in Japanese). Jyoho-kagaku-kōza (Information Sciences) B·7·2. Genetic Information II (ed. A. Tsugita). 27–62 Kyoritsu-shuppan (Tokyo).

(38) MORISHIMA, H. and H. I. OKA 1970. A survey of genetic variations in the populations of wild *Oryza* species and their cultivated relatives. Japan J. Genetics **45**: 371–385.

(39) MORIWAKI, D., Y. N. TOBARI and Y. OGUMA 1970. Spontaneous crossing-over in the male of *Drosophila ananassae*. Japan. J. Genetics **45**: 411–420.

(40) MORIWAKI, K. and H. T. IMAI 1970. The mechanism and significance of periodic ploidy alterations in the mouse myeloma, MSPC–1. Acta. Haem. Japon **33**: 67–78.

(41) MURAKAMI, A. 1970. A comparison of mutagenicity of 14 MeV fast neutrons on primordial germ cells among five different X-ray sensitive silkworm strains. Int. J. Radiat. Biol. **17**: 479–482.

(42) MURAKAMI, A. 1970. Strain sensitivity of silkworm eggs to killing and mutation by ultraviolet light. Radiat. Res. **44**: 146–153.

(43) NAKAGOME, Y. and A. D. BLOOM 1970. The satellite associations of the D group chromosomes in translocation carriers. J. Med. Genet. **7**: 371–376.

(44) OHTA, T. and M. KIMURA 1970. Statistical analysis of the base composition of genes using data on the amino acid composition of proteins. Genetics **64**: 387–395.

(45) OHTA, T. and M. KIMURA 1970. Development of associative overdominance through linkage disequilibrium in finite populations. Genet. Res. Camb. **16**: 165–177.

(46) OKA, H. I., H. MORISHIMA, T. T. CHANG and O. TAGUMPAY 1970. Analysis of genetic variations in plant type of rice. V. Early vs. sustained vigor types in growth and their bearing on yielding potential. Theoretical and Applied Genetics **40**: 50–55.

(47) SAKAI, K. I., S. HAYASHI and K. TOMITA 1970. Clone analysis and genetical study of quantitative characters in a natural forest of *Cryptomeria japonica*. Silvae Genetica **19**(4): 124–128.

(48) SHINODA, T. 1970. Polymorphism of red cell adenosine deaminase in the Japanese population. Japan J. Genetics **45**: 147–152.

(49) SHINODA, T. 1970. Inherited variation in tetrazolium oxidase in human red cells Japan J. Human Genetics **15**: 144–152.

(50) SHINODA, T. 1970. Inherited variations in red cell phosphoglucose isomerase among Japanese. Japan J. Human Genetics **15**: 159–165.

(51) SHINODA, T. and E. MATSUNAGA 1970. Polymorphism of red cell phosphoglucomutase among Japanese. Japan J. Human Genetics **14**: 316–323.

(52) SHINODA, T. and E. MATSUNAGA 1970. Studies on polymorphic types of several

red cell enzymes in a Japanese population. Japan J. Human Genetics **15**: 133–143.

(53) SHINODA, T., K. TITANI and F. W. PUTNAM 1970. Amino acid sequence of human λ chains. I. Tryptic peptides of protein Ha. J. Biol. Chem. **245**: 4463–4474.

(54) SHINODA, T., K. TITANI and F. W. PUTNAM 1970. Amino acid sequence of human λ chains. II. Chymotryptic peptides and sequence of protein Ha. J. Biol. Chem. **245**: 4475–4487.

(55) TAZIMA, Y. 1970. Studies on radiation genetics of the silkworm and their application to sericulture. Ann. Rept. of Nippon Agr. Res. Inst. 1969 pp. 36–51.

(56) TAZIMA, Y., and A. MURAKAMI 1970. Analysis of strain differences in radiosensitivity of the silkworm. Gamma-Field Symposium **8**: 53–66.

(57) TSAI, K. H., Y. C. LU and H. I. OKA 1970. Studies on soybean breeding in Taiwan. 4. Adaptability to fall cropping explored by disruptive seasonal selection of hybrid populations. SABRAO Newsletter **2**: 91–102.

(58) TSAI, K. H. and H. I. OKA 1970. Genetic studies of yielding capacity and adaptability in crop plants. 4. Effects of an earliness gene, *mb*, in the genetic background of a rice variety, Taichung 65. Botanical Bulletin of Academia Sinica **11**: 16–26.

(59) TSUJITA, M. and S. SAKURAI 1970. Lethal effects of a genetic abnormality in the pteridine metabolism of the silkworm larvae. In "Chemistry and Biology of Pteridines." Proc. 4th Intern Symp. Pteridines. The Chemistry and Biology. Intern. Print. Co. Ltd., Tokyo pp. 425–434.

(60) WATANABE, T. K. and C. OSHIMA 1970. Persistence of lethal genes in Japanese natural populations of *Drosophila melanogaster*. Genetics **64**: 93–106.

(61) YOSIDA, T. H. 1970. Chromosomes in cancer cells (in Japanese). Clinic All-Round **19**(5): 832–838.

(62) YOSIDA, T. H., H. T. IMAI and K. MORIWAKI 1970. Chromosomal alteration and development of tumors, XXI. Cytogenetic studies of primary plasma cell neoplasms induced in BALB/c mice. J. Nat. Cancer Inst. **45**: 411–418.

(63) YOSIDA, T. H., T. KUROKI, H. MASUJI and H. SATO 1970. Chromosomal alteration and the development of tumors, XX. Chromosome change in the course of malignant transformation *in vitro* of hamster embryonic cells by 4-nitroquinoline 1-oxide and its derivative, 4-hydroxyaminoquinoline 1-oxide. Gann **61**: 131–143.

ABSTRACTS OF DIARY FOR 1970

January	23	176th Meeting of Misima Geneticists' Club
February	27	177th Meeting of Misima Geneticists' Club
March	26	178th Meeting of Misima Geneticists' Club
April	23	179th Meeting of Misima Geneticists' Club
May	1	90th Biological Symposium
	4	91st Biological Symposium
	22	180th Meeting of Misima Geneticists' Club
June	25	181st Meeting of Misima Geneticists' Club
July	13–15	Summer Seminor of the Institute
	31	182nd Meeting of Misima Geneticists' Club
August	28	183rd Meeting of Misima Geneticists' Club
October	1	184th Meeting of Misima Geneticists' Club
	12	92nd Biological Symposium
November	20	185th Meeting of Misima Geneticists' Club
	24	93rd Biological Symposium

FOREIGN VISITORS IN 1970

March	1–4	STICH, H. F., University of British Columbia, Canada
April	4	WILSON, W. O., University of California, U.S.A.
	15	LEVY, S., National Institute of Health, U.S.A.
	17	SAMS, J. R., Indian River Poultry Farms, U.S.A.
	17	ARVIDSON, R. B., Hy-Line Poultry Farms, U.S.A.
May	1–2	KING, R. C., North Western University, U.S.A.
	4	GILLOIS, M., French Nat'l Organisation for Agricultural Research, France
June 15–September	1	CROW, J. F., University of Wisconsin, U.S.A.
October	7	GUTTMAN, R., Hebrew University of Jerusalem, Israel
November	3	KIMBER, G., University of Missouri, U.S.A.
	9	COLE, R. K., Cornell University, U.S.A.
	24	SCHAEFFER, P., Institute de Microbiologie, Université de Paris, France
	27	LAW, G. R. J., Hy-Line Poultry Farms, U.S.A.
December	8	MENGESHA, N. M., Agricultural College of the Haile Sellassie University, Ethiopia

ACKNOWLEDGEMENT

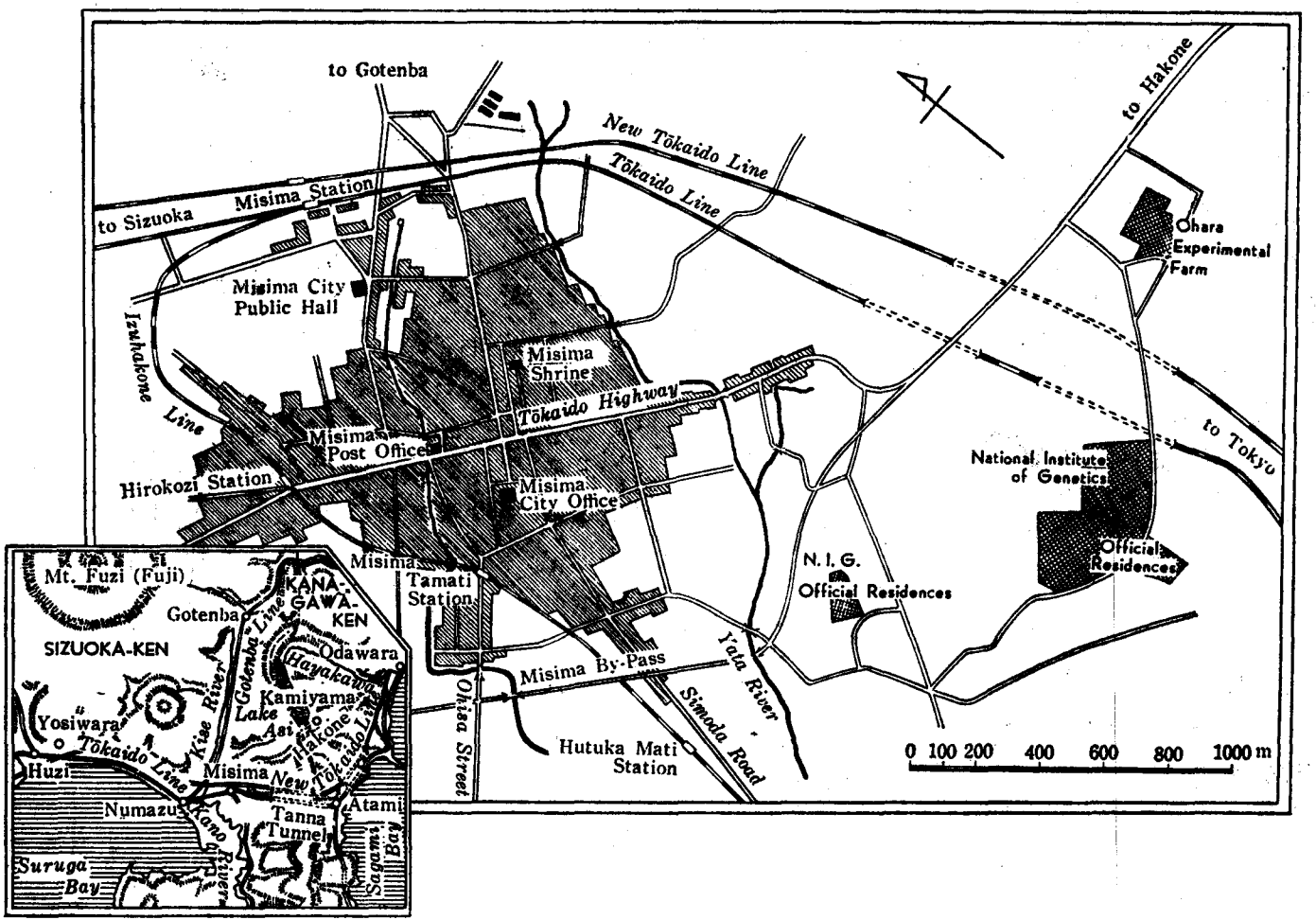
The editor wishes to express his sincere gratitude to Dr. F. A. LILIENFELD for her kindness in reading the original manuscripts. (T. MARUYAMA)

AUTHOR INDEX

Amano, E. 74, 75
Ando, T. 67
Choo, J. K. 88
Endo, T. 27
Enomoto, M. 22
Fujii, T. 76, 77
Fukase Y. 60, 61
Furuichi, Y. 11, 13
Hayashi, M. 52
Hoshino, T. 117
Iino, T. 14, 18, 21
Inoue, K. 94, 95
Ishidsu, J. 14, 16, 17
Ishii, H. 98
Ishiwa, H. 22
Iyama, S. 112, 115
Kada, T. 67, 69, 70, 72, 73
Kato, H. 49, 51, 63
Kawahara, T. 111
Kawanishi, M. 90, 92
Kikuchi, Y. 105
Kimura, M. 79, 80, 81
Kuroda, Y. 41, 42, 43
Maruyama, T. 82
Matsuda E. 101, 105
Matsumoto, H. 106
Matsunaga, E. 101, 103
Matsuura, T. 109
Mayeda, K. 37, 38
Minato, K. 44, 45
Miura, K. 11, 12, 13
Morishima, H. 99, 100, 116, 117
Moriwaki, K. 34, 36, 37, 38, 50, 56
Murakami, A. 57, 65, 65, 66
Nakagome, W. 104, 106
Nawa, S. 24, 26
Noguti, T. 69
Ohta, T. 79, 80, 81
Oishi, H. 105, 107
Oka, H. I. 99, 100, 116, 117
Onimaru, K. 63
Oshima, C. 88, 90, 92, 94, 95
Pai, C. 27
Park, Y. G. 110
Sadaie, Y. 70, 72
Sakai, K. I. 109, 110
Sakamoto, S. 97, 98
Sakurai, S. 28, 29, 31, 33, 67
Sato, T. 34, 36, 37, 56
Shibuya, A. 12
Shimotono, K. 12
Shinoda, T. 102
Suzuki, H. 18, 20
Tanaka, M. 98
Tazima, Y. 60, 61, 62, 63
Toshima, H. 14
Tsuchiya, K. 34, 37, 46, 47, 48, 49, 50, 54
Tsujita, M. 26, 28, 29, 30, 31
Tutikawa, K. 72, 73
Watanabe, T. K. 88, 90, 92
Wu, W. C. 21
Yamada, M. A. 25, 26
Yamaguchi, S. 14, 18
Yamashita, K. 98
Yosida, T.H. 46, 47, 48, 49, 51, 52, 54, 55

国立遺伝学研究所年報　第21号
昭和46年11月5日　印刷
昭和46年11月11日　発行

発行者　森脇大五郎
国立遺伝学研究所内
編集者　丸山毅夫
国立遺伝学研究所内
印刷者　笠井康頼
東京都新宿区山吹町184
印刷所　株式会社 国際文献印刷社
東京都新宿区山吹町184
発行所　国立遺伝学研究所
〒411　静岡県三島市谷田1111
電話・(三島0559)(75)0771,0772,4228
(夜間) 3492


to Gotenba
New Tōkaido Line
Tōkaido Line
to Sizuoka
Misima Station
to Hakone
Ohara Experimental Farm
Misima City Public Hall
Izuhakone Line
Misima Shrine
Tōkaido Highway
Misima Post Office
to Tokyo
Hirokozi Station
National Institutes of Genetics
Misima City Office
Misima Tamati Station
N. I. G. Official Residences
Official Residences
Misima By-Pass
Yata River
Ohara Street
Simoda Road
Hutuka Mati Station
0 100 200 400 600 800 1000 m
Mt. Fuzi (Fuji)
KANA-GAWA-KEN
Gotenba
Gotenba Line
SIZUOKA-KEN
Odawara
Hayakawa
Kamiyama
Lake Asi
Yosiwara
Kise River
Tōkaido Line
Hakone
Tōkaido Line
Huzi
Misima
New
Numazu
Kano River
Tanna Tunnel
Atami
Suruga Bay
Sagami Bay