

NATIONAL INSTITUTE OF GENETICS
JAPAN

ANNUAL REPORT

No. 13

1962

Published by
THE NATIONAL INSTITUTE OF GENETICS

Misima, Sizuoka-ken, Japan

1963

Annual Report
of the
National Institute of Genetics
No. 13, 1962



Published by
The National Institute of Genetics, Japan
1963

CONTENTS

General statement	1
Abstracts of diary for 1962	2
Staff	2
Council	5
Projects of research for 1962	6
Foreign visitors in 1962.....	9
Researches carried out in 1962	11
A. <i>Genetics, cytology and biochemistry of animals</i>	
1. Pre-adult viability of heterozygous flies for lethal chromosomes (1) (OSHIMA).....	11
2. Pre-adult viability of heterozygous flies for lethal chromosomes (2) (OSHIMA and MINAMORI).....	12
3. Persistence of some recessive lethal genes in a natural population of <i>Drosophila melanogaster</i> (OSHIMA)	13
4. Pre-adult viability of heterozygous flies for semi-lethal chromosomes (OSHIMA).....	13
5. Studies on spontaneous polygenic viability mutations in <i>Drosophila melanogaster</i> 1. Method and representation of condensed data (MUKAI, CHIGUSA and YOSHIKAWA).....	15
6. Studies on spontaneous polygenic viability mutations in <i>Drosophila melanogaster</i> 2. Variance increasing rate and mean decreasing rate (MUKAI).....	16
7. Studies on spontaneous polygenic viability mutations in <i>Drosophila melanogaster</i> 3. Estimation of polygenic viability mutation rate (MUKAI).....	17
8. Studies on spontaneous polygenic mutations in <i>Drosophila melanogaster</i> 4. Dominance in polygenic viability mutations (MUKAI, CHIGUSA and YOSHIKAWA)	19
9. Studies on spontaneous polygenic viability mutations in <i>Drosophila melanogaster</i> 5. Polygenic mutations and genetic structure of natural populations (MUKAI)	20
10. Persistence of two recessive genes in experimental populations of <i>Drosophila melanogaster</i> (CHIGUSA and MUKAI).....	21
11. Syntheses of <i>Drosophila</i> eye pigments (NAWA and FORREST)..	23
12. Melamine action in phenocopic pigment elimination from hypodermal cells of the silkworm (TSUJITA and SAKURAI) ..	25

13. Specific protein combining with yellow pigments (dihydrop- terin) in the silkworm (TSUJITA and SAKURAI)	28
14. Conversion from guanine to pteridine in the silkworm (SAKA- GUCHI)	29
15. Analysis of marker genes in inbred strains of albino mice (KURITA and SAKAKIBARA)	30
16. Genetical studies of post-axial polydactyly in the house mouse (NAKAMURA, SAKAMOTO and MORIWAKI)	31
17. Cytological study of a transplantable ascites plasma-cell neoplasm in mice (KURITA and YOSIDA)	32
18. A characteristic chromosome found in the karyotype analysis of the mouse (NAKAMURA and TONOMURA)	32
19. Cytological studies of lymphogenic and hematogenic metas- tases in MH-134 mouse hepatoma (INUI).....	33
20. Chromatographic analysis of coat coloring substance in mice (NAKAMURA)	34
21. Biochemical changes in the skin of rhino mouse preceding the hereditary hair loss (MORIWAKI)	35
22. Viscosity changes in the deoxyribonucleoprotein of L-strain cells following treatment with a base analogue (MORIWAKI)	36
23. Endurance of one day old chicks under starvation (KAWAHARA)	37
24. Genetic studies on body weight at different stages of growth in the domestic fowl (KAWAHARA)	38
25. Use of rabbit anti-serum for muscle proteins of fowls for serological determination of homologous tissue proteins of <i>Triturus</i> (OGAWA and KAWAHARA)	39
26. Effects of p-hydroxy-benzyl-methyl-ether, a component of <i>Citrullus colocynthis</i> , on growth and differentiation of <i>Tri- turus</i> embryo (OGAWA and WATANABE)	40
27. Comparison of free amino acids among eye-color mutants of <i>Drosophila melanogaster</i> (SHINODA)	42
28. Spectrophotometric determination of trinitrophenyl deriva- tives of amino acids and peptides resolved by paper electro- phoresis and chromatography (SHINODA)	43
29. Paper electrophoretic heterogeneity of serum albumin (SHI- NODA)	44
 B. <i>Genetics, cytology and biochemistry of plants</i>	
30. Genome manifestation of wheat in <i>Aegilops</i> cytoplasm (KIHARA)	45
31. Increased occurrence of haploids and twin seedlings due to an alien cytoplasm (KIHARA and TSUNEWAKI).....	45

32. Comparative gene analysis of common wheat and its ancestral species I. Progressive necrosis (TSUNEWAKI and KIHARA) ..	47
33. Transmission of the monosomic condition through female gametes in a common wheat, Chinese Spring (TSUNEWAKI).	47
34. Chromosome number of Gramineae species collected in Pakistan, Afghanistan and Iran (SAKAMOTO and MURAMATSU) ...	48
35. Reciprocal translocations in Emmer wheat (NISHIKAWA).....	50
36. Production of polyploid wheat by nitrous oxide, II. N ₂ O-treatment during fertilization with reference to culture media (TSUNEWAKI)	51
37. Photoperiodic responses of <i>Oryza</i> species V (KATAYAMA).....	51
38. Developmental instability in tobacco-plants (SAKAI and SHIMAMOTO)	52
39. A method of estimation of genetic parameters in forest trees without raising progeny (SAKAI and HATAKEYAMA)	53
40. Cytoplasmic effect on the inheritance of seed weight in <i>Pharbitis Nil</i> (SAKAI and SUZUKI)	54
41. Effect of genotypes and cultural conditions on quantitative characters of tobacco varieties (SAKAI and SHIMAMOTO).....	55
42. Studies on developmental instability of quantitative characters in X-rayed progenies of rice (SAKAI and SUZUKI).....	56
43. Intra-clonal variation of pathogenicity in <i>Piricularia oryzae</i> (GOTO and SAKAI).....	57
44. Studies on blast-disease susceptibility of wild and cultivated groups of rice (GOTO and SAKAI)	58
45. Natural selection experiment in populations of wild rice, <i>Oryza perennis</i> (OKA and CHANG)	59
46. Some considerations on the evolutionary dynamics of cultivated rice (HINATA and OKA)	60
47. F ₂ segregation ratios in a hybrid between tetraploid <i>Oryza sativa</i> and <i>glaberrima</i> (MORISHIMA and OKA).....	60
48. Comparison of evolution modes of cultivated forms derived from two wild rice species, <i>Oryza breviligulata</i> and <i>O. perennis</i> (MORISHIMA, HINATA and OKA).....	61
49. Cytogenetic studies in the genus <i>Nicotiana</i> XV. Reduction divisions in three interspecific hybrids and one amphidiploid (TAKENAKA).....	61
50. Tumorous hybrids in <i>Nicotiana</i> (TAKENAKA and YONEDA)	63
51. Effects of extracts from <i>Aralia elata</i> Seem. and <i>Artemisia vulgaris</i> L. var. <i>indica</i> Maxim. on cell division in living plant tissue and growth of rat tumor (TAKENAKA and OGAWA)	64

52.	Experiments with carnations (LILIENFELD and SUZUKI)	65
53.	Comparison of flower color inheritance in pansy and morning glory (ENDO)	68
54.	Mutations of dominant green to recessive chlorina in heterozygous plants (FUJII)	69
55.	Effect of nitrous oxide on germinating seeds of einkorn wheat (FUJII)	70
56.	Cytological observations on chemically induced male sterility in sugar beets (OHTA and MATSUMURA)	71
C. <i>Mathematical genetics</i>		
57.	On the probability of joint fixation of mutant genes (KIMURA)	71
D. <i>Genetics and biochemistry of microorganisms</i>		
58.	Phase specific regulator of flagellin genes (H_1 and H_2) in <i>Salmonella</i> (INO)	72
59.	Further studies on non-flagellated mutants of <i>Salmonella</i> (INO and ENOMOTO)	73
60.	Effect of phenol on flagellation of <i>Salmonella</i> cells (INO and MITANI)	74
61.	Grouping of paralyzed mutants in <i>Salmonella</i> (ENOMOTO)	75
62.	Chromatography and electrophoresis of flagellin of <i>Salmonella abortus-equi</i> (ENOMOTO and IINO)	76
63.	Further studies on the nuclei of yeasts (YONEDA)	77
64.	Temperature dependence of nitrous acid effects on phage T4 (ISHIWA, YAN and KONDO)	78
E. <i>Radiation genetics in animals</i>		
65.	Effect of radiation-induced mutations on fitness of <i>Drosophila melanogaster</i> (HIRAIZUMI)	78
66.	Heterozygous effects of radiation-induced mutations on viability in homozygous and heterozygous genetic backgrounds in <i>Drosophila melanogaster</i> (MUKAI, CHIGUSA and YOSHIKAWA)	79
67.	Radiation-induced mutation rates of polygenes controlling the sternopleural bristle number in <i>Drosophila melanogaster</i> (MUKAI, YOSHIKAWA and CHIGUSA)	81
68.	Radiation sensitivity of infectious particles responsible for "sex-ratio" condition in <i>Drosophila willistoni</i> (SAKAGUCHI and KONDO)	82
69.	Revised interpretation of the non-recovery type dose-rate dependence of radiation induced mutation rates of silkworm gonidia (TAZIMA and KONDO)	85

70. Differential radiation-sensitivity of germ cells as a possible interpretation of sex difference in dose-rate dependence of induced mutation rates in the silkworm (TAZIMA and KONDO)	86
71. The increase in induced mutation frequency after fractionated irradiation of gonial cells of the silkworm (TAZIMA and MURAKAMI).....	87
72. Modification of X-ray-induced mutation rate in the silkworm by pre- or post-irradiation treatment with halogenated base analogues (MURAKAMI and TAZIMA).....	89
73. Cytological estimation of LD ₅₀ X-ray dose for secondary spermatogonia of the silkworm (SADO).....	91
74. Inactivation of <i>Salmonella</i> by ³² P disintegration combined with UV (ISHIWA, YAN and KONDO).....	92
75. Protective effect of sodium glucuronate on the differentiation of muscle tissue inhibited by X-irradiation (OGAWA).....	93
F. <i>Radiation genetics in plants</i>	
76. Radiation-induced variegations in einkorn wheat and their inheritance (MATSUMURA).....	96
77. Effects of chronic irradiation on einkorn wheat (MATSUMURA and FUJII).....	96
78. Relation of chlorophyll mutations to dose rates in γ -irradiated einkorn wheat (MATSUMURA and MABUCHI).....	97
79. Relation of radiation effects to dose rates of γ -radiation in rice (MATSUMURA and MABUCHI).....	99
80. A red-stem mutant in einkorn wheat induced by X-rays (FUJII).....	100
81. Multi-subunit target model for RBE versus LET relationship for radiation damage to higher forms (KONDO).....	101
G. <i>Human genetics</i>	
82. Embryonal sex ratio in the Japanese determined by the sex-chromatin test (MATSUNAGA, TONOMURA, INUI and HONDA) ..	102
83. Studies on selection in ABO blood groups (MATSUNAGA, HIRAZUMI, FURUSHO and IZUMIYAMA).....	103
84. Spontaneous and induced chromatid aberrations in human cells cultivated <i>in vitro</i> (TONOMURA).....	106
85. Sex-chromosome conditions in four cases of true hermaphroditism (TONOMURA and HONDA).....	107
86. Character of modified human hemoglobin (SHINODA).....	108
Books and papers published in 1962 by staff members.....	110

GENERAL STATEMENT

As shown in the photograph given above, two thirds of our main building were finished in 1962. The rest will be completed in the fall of 1963. Still we will need to add a few buildings, namely, an auditorium, an exhibit room, seed storage rooms and a dormitory for visiting scientists.

To erect the new concrete building has taken four years. It was high time to build it since the old one was partly destroyed by storms and was liable to catch fire.

The recent advances of genetics in many fields make it necessary for our Institute to accommodate to the diverse developments. We are planning to establish four new departments; namely, population genetics, molecular genetics, biophysics and a research center for fine structure investigations. However, we have to admit that the Institute should keep its moderate size and not become too large. Therefore it is necessary to reorganize old departments hitherto established to fit our needs.

To keep a continuous flow of young people, it is desirable to have post-graduate students who will be trained in our Institute. For this purpose, the Institute should be converted into a research center of genetic sciences affiliated with all national universities.

It was customary to list on this page the past achievements. However as our building is near completion the future prospects have to be mentioned.

A handwritten signature in black ink, appearing to read "H. V. Uehara". The signature is fluid and cursive, with a long horizontal stroke extending to the right.

ABSTRACT OF DIARY FOR 1962

- Jan. 13. 100th meeting of Misima Geneticists' Club.
 Feb. 14. 101st meeting of Misima Geneticists' Club.
 Feb. 24. 102nd meeting of Misima Geneticists' Club.
 Mar. 23. 103rd meeting of Misima Geneticists' Club.
 Apr. 10. 43rd Biological Symposia.
 Apr. 27. 104th meeting of Misima Geneticists' Club.
 May 11. 44th Biological Symposia.
 May 29. 105th meeting of Misima Geneticists' Club.
 June 16. 106th meeting of Misima Geneticists' Club.
 June 22. 45th Biological Symposia.
 June 29. 107th meeting of Misima Geneticists' Club.
 July 19-21. The 5th Summer Seminar on Genetics for High School
 Teachers of Biology.
 July 26. 46th Biological Symposia.
 Aug. 6. 47th Biological Symposia.
 Sept. 25. 108th meeting of Misima Geneticists' Club.
 Oct. 17-19. The 34th Annual Meeting of the Genetics Society of Japan.
 Nov. 9. 48th Biological Symposia.
 Nov. 20. 109th meeting of Misima Geneticists' Club.
 Dec. 21. 110th meeting of Misima Geneticists' Club.

STAFF

Director

Hitoshi KIHARA, D. Sc., M.J.A., Emeritus Professor of Kyoto University

Members

Department of Morphological Genetics

Yataro TAZIMA, D. Ag., Head of Department

1st Lab.	Yataro TAZIMA, D. Ag., Head of Lab. Kimiharu ONIMARU, Akio MURAKAMI*
2nd Lab.	Bungo SAKAGUCHI, D. Ag., Head of Lab. Toshihiko SADO, D. Ag. (in U.S.A.)

* Research member under grant from the Toyo Rayon Foundation

Department of Cytogenetics	
Yô TAKENAKA, D. Sc., Head of Department	
1st Lab.	Toshihide H. YOSIDA, D. Sc., Head of Lab. (in U.S.A.) Kazuo MORIWAKI, D. Sc., Akira NAKAMURA*
2nd Lab.	Yô TAKENAKA, D. Sc., Head of Lab. Yoshiaki YONEDA, D. Sc.
* Visiting researcher	
Department of Physiological Genetics	
Chozo OSHIMA, D. Sc., Head of Department	
1st Lab.	Chozo OSHIMA, D. Sc., Head of Lab. Toshifumi TAIRA, D. Sc. (in U.S.A.) Sumio MINAMORI, D. Sc.**
2nd Lab.	Hitoshi KIHARA, D. Sc., Head of Lab. Koichiro TSUNEWAKI, Ph. D., Sadao SAKAMOTO, Tadao C. KATAYAMA*, Kôzo NISHIKAWA**
* Research member under grant from the Rockefeller Foundation	
** Visiting researcher	
Department of Biochemical Genetics	
Mitsuo TSUJITA, D. Ag., Head of Department	
1st Lab.	Saburo NAWA, D. Sc., Head of Lab. (in U.S.A.)
2nd Lab.	Yoshito OGAWA, M. D., Head of Lab. Tôru ENDÔ, D. Ag.
3rd Lab.	Mitsuo TSUJITA, D. Ag., Head of Lab. Susumu SAKURAI
Department of Applied Genetics	
Kan-Ichi SAKAI, D. Ag., Head of Department	
1st Lab.	Yukio YAMADA, D. Ag., Head of Lab. (in U.S.A.) Takatada KAWAHARA, D. Ag.
2nd Lab.	Kan-Ichi SAKAI, D. Ag., Head of Lab. Shinya IYAMA, D. Ag. (in U.S.A.), Akira MIYAZAWA, Akio SUSUKI, Iwasaburo Goto*, Suekichi HATAKEYAMA*, Yoshiya SHIMAMOTO*
3rd Lab.	Hiko-Ichi OKA, D. Ag., Head of Lab. Hiroko MORISHIMA
* Visiting researcher	

 Department of Induced Mutation

 Seiji MATSUMURA, D. Ag., Head of Department

1st Lab.	Seiji MATSUMURA, D. Ag., Head of Lab. Kiyosi TUTIKAWA, Terumi MUKAI, Ph. D., Sadao CHIGUSA*, Isao YOSHIKAWA*
2nd Lab.	Seiji MATSUMURA, D. Ag., Head of Lab. Taro FUJII, D. Ag., Tomoo MABUCHI**
3rd Lab.	Sohei KONDO, D. Sc., Head of Lab. Hiromi ISHIWA, Yonhoi YAN***

* Research member under grants from the N.I.H. (U.S.A.)

** Research member under grant from the Rockefeller Foundation

 *** Research member under grant from the Toyo Rayon Foundation

 Department of Human Genetics

 Ei MATSUNAGA, M. D., D. Sc., Head of Department

1st Lab.	Ei MATSUNAGA, M. D., D. Sc., Head of Lab. Akira TONOMURA, D. Sc., Tomotaka SHINODA
2nd Lab.	Motoo KIMURA, Ph. D., D. Sc., Head of Lab. (in U.S.A.) Yuichiro HIRAIZUMI, D. Sc.

 Department of Microbial Genetics

 Hitoshi KIHARA, D. Sc., Head of Department

1st Lab.	Tetsuo INO, Ph. D., D. Sc., Head of Lab. Masatoshi ENOMOTO
-------------	---

Part-time Staff and Research Associates

Yoshinari KUWADA, D. Sc., M.J.A., Emeritus Professor of Kyoto University

Kan OGUMA, D. Ag., Ex-Director, Emeritus Professor of Hokkaido University

Yoshimaro TANAKA, D. Ag., D., Sc., M.J.A., Emeritus Professor of Kyushu University

Taku KOMAI, D. Sc., M.J.A., Emeritus Professor of Kyoto University

Flora A. LILIENFELD, Ph. D.

Tsuguo TATEOKA, D. Sc.

Shiro SHIRATO, M. D., Izu Teishin Hospital

Department of Administration

Tanryû OTOMO, Head of Department

Toyotake MINAMIGUCHI, Chief of General Affairs Section

Seiichi KOIZUMI, Chief of Finance Section

Hiroko NAKANO, Ryôji OYAMA, Kazuo ENOMOTO and Keiichi KAWASHIMA

Clerks, Librarian, Typists, Chauffeur, Field Laborers and Janitors...9

Misima Branch of Hatano Tobacco Experiment Station

Masao TANAKA, D. Ag., Head

Assistants3

Association for Propagation of the Knowledge of Genetics

Hitoshi KIHARA, President

Yô TAKENAKA, Managing Director

Yatarô TAZIMA, Managing Director

Seiji MATSUMURA, Manager

Bungo WADA, Manager

Yosito SINOTO, Manager

COUNCIL

Yô K. OKADA, Director of National Science Museum, Chairman

Daigorô MORIWAKI, Professor of Tokyo Metropolitan University, Vice
Chairman

Kan OGUMA, Ex-Director, Emeritus Professor of Hokkaido University

Masataka OMASA, Professor of Tokyo University

Seizô KATSUNUMA, Ex-President of Nagoya University

Minoru TACHI, Director of Institute of Population Problems

Yakichi NOGUCHI, Professor of Tokyo University

Akira KAWADA, Director of National Institute of Agricultural Science

Toshio SAITO, Governor of Sizuoka Prefecture

Taiji SAKATA, President of Japan Monopoly Corporation

Takeo SAKATA, President of T. Sakata Company

Hiroshi TAMIYA, Director of Institute of Applied Microbiology, Univ. of
Tokyo

Tanemoto FURUHATA, Director of Scientific Research Institute of Police

Bungo WADA, Professor of Sizuoka University

Kempo TSUKAMOTO, Director of National Institute of Radiological Sciences

PROJECTS OF RESEARCH FOR 1962

Department of Morphological Genetics

- Genetics of the silkworm (TAZIMA)
- Studies on food preference of the silkworm (TAZIMA)
- Chemical mutagenesis in the silkworm (TAZIMA, SADO and MURAKAMI)
- Cytological study of silkworm germ cells (SADO)
- Studies on dose-rate dependence of radiation-induced mutation rates (TAZIMA and ONIMARU)
- RBE of radiations for induced mutation frequency in the silkworm (MURAKAMI)
- Hereditary infections in *Drosophila* (SAKAGUCHI)

Department of Cytogenetics

- Cytology and genetics of tumors (YOSIDA, MORIWAKI and KURITA)
- Experimental breeding and genetics of mice and rats (YOSIDA, MORIWAKI, KURITA and NAKAMURA)
- Biochemical study on genetical abnormalities of mice (MORIWAKI and NAKAMURA)
- Determination and differentiation of sex in higher plants (TAKENAKA and YONEDA)
- Induction of abnormal mitosis and inhibition of growth by substances extracted from certain plants (TAKENAKA and OGAWA)
- Interspecific hybridization in *Nicotiana* (TAKENAKA and LILIENFELD)
- Genetics of *Pharbitis nil* (TAKENAKA)
- Origin of *Prunus yedoensis* (TAKENAKA)
- Cytogenetics of *Oryza* species (TAKENAKA and YONEDA)
- Comparative observations of chromosomes in haploid plants of *Oryza* (C. H. Hu and YONEDA)
- Cytological studies on the yeast cell (YONEDA)

Department of Physiological Genetics

- Genetical studies on insecticide-resistance in *Drosophila* (OSHIMA)
- Physiological studies on eye-pigment formation in *Drosophila* (TAIRA and OSHIMA)
- Population genetics of deleterious genes in natural populations of *Drosophila* (OSHIMA and MINAMORI)

- The persistence of some natural lethal genes in experimental populations (OSHIMA and FUWA)
Comparative gene analysis with reference to the origin of wheat (KIHARA, TSUNEWAKI and NISHIKAWA)
Nucleus substitution in wheat and related species (KIHARA)
Production of polyploids by N₂O treatment (KIHARA and TSUNEWAKI)
Genetic studies of wheat aneuploids (TSUNEWAKI)
Genetic basis of ecological differentiation in *Agropyron* (SAKAMOTO)
Collection and preservation of *Oryza* species (KIHARA)
Morphological study of *Oryza* (KIHARA and KATAYAMA)
Investigation of photoperiodic responses of *Oryza* species (KATAYAMA)

Department of Biochemical Genetics

- Biochemical genetics of insects and microorganisms (TSUJITA and NAWA)
Embryological and biochemical studies in the silkworm (TSUJITA)
Genetical and biochemical studies of chromoprotein (SAKURAI)
Biochemical studies on the differentiation of muscle proteins in animals (OGAWA)
Biochemical studies on the mechanism of cell division in animals (OGAWA)
Chemical research in anti-tumor substances (OGAWA)
Biochemistry of the mechanism underlying variations in flower color (ENDÔ)
Genetics of virus (TSUJITA)

Department of Applied Genetics

- Studies on breeding and genetics in poultry (YAMADA and KAWAHARA)
Theoretical studies on plant breeding techniques (SAKAI)
Studies on competition and migration in plants and animals (SAKAI, IYAMA and NARISE)
Genetic studies of alkaloid content in tobacco plants (SAKAI and SHIMAMOTO)
Biometrical study of cytoplasmic inheritance (SAKAI and SUZUKI)
Genetic studies on developmental stability in plants (SAKAI, SUZUKI and SHIMAMOTO)
A statistic-genetical study in forest-trees (SAKAI and HATAKEYAMA)
Statistic-genetical studies in cultivated and wild rice (SAKAI, NARISE and SUZUKI)
Genetic studies on the blast-disease resistance in wild and cultivated rice (SAKAI and GOTOH)
Comparison of evolutionary mode between series *Sativa* and series *Glaber-*

rima (MORISHIMA and OKA)

Survey of geographical variation in *O. perennis* (MORISHIMA, CHANG and OKA)

Crossing-experiments and sterility of hybrids between wild and cultivated rice strains (HINATA and OKA)

Studies of intermediate wild-cultivated forms in rice (OKA and CHANG)

Responses to growing conditions of wild and cultivated rice forms (OKA and CHANG)

Evolutionary-genetic studies in *Oryza* (OKA and MORISHIMA)

Department of Induced Mutation

Radiation genetics of mice (TUTIKAWA)

Population genetics of *Drosophila* (MUKAI and CHIGUSA)

Studies on the effects of irradiation on populations (MUKAI and YOSHIKAWA)

Estimation of polygenic mutation rates in *Drosophila* (MUKAI, CHIGUSA and YOSHIKAWA)

Relation between the quality of radiations and mutations (MATSUMURA and KONDO)

Radiation genetics of cereals (MATSUMURA, FUJII and MABUCHI)

Radiation genetics and its practical application (FUJII and MABUCHI)

Biophysical studies of radiation genetics (KONDO, ISHIWA and YAN)

Radiation dosimetry (KONDO and ISHIWA)

Male-sterility in sugar beets (MATSUMURA)

Genome analysis of *Oryza* species (MATSUMURA and MABUCHI)

Department of Human Genetics

Selection in ABO blood groups (MATSUNAGA and HIRAIZUMI)

Genetic studies on sporadic retinoblastoma in Japan (MATSUNAGA)

Studies on dimorphism in human normal cerumen (MATSUNAGA)

Cytogenetics in man (TONOMURA)

Sexual dimorphism in resting nuclei (TONOMURA (TOYOFUKU) and TONOMURA)

Biochemical studies on plasma proteins and haemoglobins (SHINODA)

Studies on individual difference in metabolism (SHINODA)

Theoretical studies in population genetics (KIMURA)

Effects of radiation-induced mutations on fitness (HIRAIZUMI)

Populational implications of meiotic drive with special reference to the *SD* locus in *D. melanogaster* (HIRAIZUMI)

Effect of radiation induced mutations on correlated characters of *D. melanogaster* (HIRAIZUMI)

Department of Microbial Genetics

Immunogenetics of *Salmonella* (INO and ENOMOTO)
 Genetics of multi-drug resistance in bacteria (INO)
 Genetics of motility in bacteria (ENOMOTO)
 Genetics of bacteriophages (INO and SASAKI)

FOREIGN VISITORS IN 1962

- Feb. 16. S. EL. MOUKHTAR (Gawhary Embassy of the United Arab Republic)
 17. A. H. MOSEMEN and Jean MOSEMEN (The Pockefeller Foundation, New York, U.S.A.)
- March 5. J. DRILON and Mercedes L. DRILON (The International Rice Research Institute, Manila, The Philippines)
 10. Edward KUCHLEWSKI (Elizabeth, N. J., U.S.A.)
- April 10. Ady Raul da SILVA (Institute Agronomico do Sul Pelota, Brazil)
- May 11. Joshua LEDERBERG and E. M. LEDERBERG (Dept. of Genetics, Medical School, Stanford University, California, U.S.A.)
- June 8. L. H. BAKER (Pioneer Hi-Bred Corn Co., Des Moines, Iowa, U.S.A.)
 21. E. R. DEMPSTER (Professor of Genetics, Genetics Department, Univ. of California, Berkeley, California, U.S.A.)
- July 13. H. M. BEACHELL (Rice Breeder, Rice Pasture Experiment Station, Beaumont, Texas, U.S.A.)
 13. Mrs. E. E. BEACHELL (Iowa Technology, Beaumont, Texas, U.S.A.)
 13. B. R. GALGALI (Plant Breeder, Mysore State, India)
 20. Te-Tzu CHANG (International Rice Research Institute, Los Baños, Laguna, The Philippines)
 26. Edward A. ADELBERG (Microbiology Department, Yale University, New Haven, Conn., U.S.A.)
 27. Engracio BASIO (Principal Librarian, College of Agriculture and Central Experiment Station, University of the Philippines, College, Laguna, The Philippines)
- Aug. 6. Allen S. Fox (Department of Biochemistry, Michigan State University, East Lansing, Michigan, U.S.A.)
- Sept. 7. Selim NAZIF (Director General, Field Crops Research Div., Ministry of Agriculture, Cairo, U.A.R.)

- 7. A. ABOUL-SBOUD (Director of Nutrition Section, Animal Production Dept., Ministry of Agriculture, Cairo, U.A.R.)
- 7. I. M. IBRAHIM (Fruit Research Section, Horticultural Dept., Ministry of Agriculture, Cairo, U.A.R.)
- 7. G. E. DICKERSON (Kimber Farms Inst., Fremont, California, U.S.A.)
- 15. S. H. OU (International Rice Research Institute, Los Baños., Laguna, The Philippines)
- 15. Jack A. SOULES (Office of Naval Research, Physics Branch, Code 421, Washington 25, D.C., U.S.A.)
- Oct. 3. S. S. SAINI (Rice Breeder, Regional Rice Research Station, Kapurthola Punjab, India)
- 3. Fouad NAGUIB (Rice Breeder, Rice Research Station, Department of Agriculture, Giza-Orman, U.A.R.)
- 3. Mohammad MOOFIZAD (Rice Research Station, Department of Agriculture, Rasht, Iran)
- 3. U-Thai WONGVISES (Pimai Rice Experiment Station, Rice Department, Ministry of Agriculture, Bangkok, Thailand)
- 3. Abdel-Wahab MUSTAFA (Rice Breeder, Rice Research Station, Department of Agriculture, Sakha Experiment Station, Kafre EL Shiekh, Egypt, U.A.R.)
- 9. Mihhail VASILJEVICH ORLOV, peti Efimovich BOZKKO, Genrikh Vyacheslanovich MILJINIKOV and Valentin Ivanovich KONOVALOY (Visiting Group of Specialists in Poultry Breeding, U.S.S.R.)
- 25. Otto LAPORTE (Professor of Physics, University of Michigan, Ann Arbor, Michigan, U.S.A. Currently, Science Attaché, American Embassy, Tokyo)
- Nov. 9. Arnold H. SPARROW (Brookhaven National Laboratory, Upton, N.Y., U.S.A.)

RESEARCHES CARRIED OUT IN 1962

A. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF ANIMALS

1. *Pre-adult viability of heterozygous flies for lethal chromosomes (1)*¹⁾

(By Chozo OSHIMA)

It was shown in the previous annual report (No. 3) that the mean relative viability of natural lethal heterozygotes was significantly lower than that of normal heterozygotes and that of double lethal heterozygotes within the same population was rather better than that of single lethal heterozygotes. To confirm this complementary effect, similar experiments were carried out with ten normal and ten lethal chromosomes isolated in 1961 from the Hiroshima and the Suyama-Juriki populations respectively. Both populations are about 700 kilometers apart, and are

Table 1. Relative viabilities of normal, single and double lethal trans-heterozygotes between the Hiroshima and the Suyama-Juriki populations, in comparison with those of normal heterozygotes within the populations.

Chromosome combination	Pooled basis		Line basis	
	No. of counted flies	Relative viability	No. of lines	Relative viability
$+h/+h$	15,858	1.119 ± 0.0186	45	1.139 ± 0.0239
$+s-j/+s-j$	13,950		45	
$+h/+s-j$	34,713	1.086 ± 0.0166	100	1.105 ± 0.0202
$+h/l_{s-j}$	70,388	1.083 ± 0.0115	200	1.100 ± 0.0139
$+s-j/l_h$				
l_h/l_{s-j}	37,678	1.117 ± 0.0165	100	1.121 ± 0.0179

The viability of *Cy/Pm* fly=1.0000 The same for the other experiment.

$+h, l_h$: normal and lethal chromosomes isolated from the Hiroshima populations respectively.

$+s-j, l_{s-j}$: normal and lethal chromosomes isolated from the Suyama and Juriki populations, respectively.

* significance at the 5% level.

1) This work was supported by the United States Public Health Service Grant, RG 7836.

assumed to be genetically unrelated. The results are given in Table 1.

The mean viability of single lethal heterozygotes was lower than that of normal heterozygotes irrespective of whether any coadaptation system existed or not. On the other hand, double lethal trans-heterozygotes were significantly superior in their mean viability to single lethal heterozygotes and similar to normal heterozygotes.

During the last period of the breeding season, the population size is gradually reduced, but this might be not followed by a decrease in relative frequency of lethals. If so, there may be chances that surviving lethal heterozygotes breed together and double lethal trans-heterozygotes are produced. Heterozygous flies for many lethal genes except the heterotic ones would be eliminated, but those double lethal trans-heterozygotes of nearly normal viability would remain alive through the dormant season.

2. *Pre-adult viability of heterozygous flies for lethal chromosomes (2)*¹⁾

(By Chozo OSHIMA and Sumio MINAMORI)

Several chromosomes on which two lethal genes are located, had been isolated from the Hiroshima populations in early summer of 1961. These lethal genes were separated by recombination and found again in the autumn lot of the same population. Sixteen normal, six single lethal and three double lethal chromosomes were used and the viability of heterozygotes for those chromosomes was estimated. The results are given in Table 1.

Table 1. Relative viabilities of double lethal cis-heterozygotes in comparison with those of normal and single lethal heterozygotes in the Hiroshima populations.

Chromosome combination	Pooled basis		Line basis	
	No. of counted flies	Relative viability	No. of lines	Relative viability
+/+	28,816	1.096 ± 0.0185	60	1.123 ± 0.0284
+/ <i>l</i>	25,970	1.072 ± 0.0201	60	1.093 ± 0.0293
<i>ll</i> /++	23,071	1.148 ± 0.0217	60	1.171 ± 0.0304

** significance at the 1% level, * significance at the 5% level.

1) This work was supported by the United States Public Health Service Grant RG 7836.

The mean viability of single lethal heterozygotes was a little less than that of normal heterozygotes, but double lethal cis-heterozygotes were significantly superior in their mean viability to single lethal heterozygotes and even normal heterozygotes.

In the early period of the breeding season, double lethal cis-heterozygotes which might have been originated from double lethal trans-heterozygotes by recombination were actually found by Minamori in a natural population in Hiroshima and by Karlik and Sperlich (1962) in a natural population on Lipari Island.

The complementary effects on viability of double lethal heterozygotes might be understood as an interaction between two loci carrying lethal genes in the genetic background which was subjected to natural selection.

3. *Persistence of some recessive lethal genes in a natural population of *Drosophila melanogaster*.*¹⁾

(By Chozo OSHIMA)

Lethal second chromosomes have been isolated every year from 1959 from the Suyama-Juriki natural populations in Shizuoka prefecture. Those chromosomes have been maintained by *Cy* balanced system in the following generations. Allelism test was performed between old and new lethal genes isolated during two successive years. The results showed that the two lethal genes have been maintained at least for two years and other two lethal genes have been retained at least for one year in the same population. The former two lethal genes supposed to have high fitness in heterozygous state in the natural population were found to be maintained with much higher frequencies for about forty generations in experimental populations (see previous annual report No. 1), as compared with the theoretical frequencies. The obtained results show that the heterozygous flies for those lethal genes were heterotic in both natural and experimental populations.

4. *Pre-adult viability of heterozygous flies for semi-lethal chromosomes*²⁾

(By Chozo OSHIMA)

Fifteen semi-lethal and sixteen normal chromosomes isolated from the

1) This work was supported by the United States Public Health Service Grant, RG 7836.

2) This work was supported by the United States Public Health Service Grant, RG 7836.

Table 1. Relative viabilities of normal, single and double semi-lethal trans-heterozygotes within the Suyama-Juriki populations.

Chromosome combination	Pooled basis		Line basis	
	No. of counted flies	Relative viability	No. of lines	Relative viability
+/+	53,851	1.160±0.0144	120	1.177±0.0184
<i>sl</i> /+	102,336	1.166±0.0105	240	1.186±0.0137
<i>sl</i> / <i>sl</i>	41,069	1.128±0.0160 _*	102	1.147±0.0214
+/+	55,302	0.999±0.0121	110	1.006±0.0136
<i>sl</i> /+	60,991	0.983±0.0114	121	0.982±0.0134
<i>sl</i> / <i>sl</i>	48,625	0.934±0.0122 _{**}	106	0.942±0.0124 _{**}

sl: semi-lethal chromosome

* significance at the 5% level, ** significance at the 1% level.

Table 2. Relative viability of single semi-lethal heterozygotes.

	First exp.	Second exp.
No. of semi-lethal chr.	15	11
No. of normal chr.	16	11
Relative viability	+/+ 1.160±0.0144 <i>sl</i> /+ 1.166±0.0105	0.999±0.0121 0.983±0.0114
	+/+ 1±0.0124 <i>sl</i> /+ 1.005±0.0091	1 ±0.0121 0.984±0.0114
Pooled	+/+ 1 ±0.0088 <i>sl</i> /+ 0.996±0.0071	

Suyama-Juriki populations in 1961 were used in the experiment and the viability of single and double semi-lethal heterozygotes was estimated and compared with that of normal heterozygotes. The results are given in Table 1.

The results obtained from the experiments of Table 1 were pooled together and the mean viability of semi-lethal heterozygotes was standardized by that of normal heterozygotes. The results are shown in Table 2.

The mean viability of single semi-lethal heterozygotes was not much inferior to that of normal heterozygotes and detrimental effects on the viability of two non-allelic semi-lethal trans-heterozygotes were increased.

The frequency of semi-lethal chromosomes causing 20-30 per cent

viability was lowest in the distribution curve manifested by individual homozygous second chromosomes isolated from a natural population. The causes for a similar finding have been discussed by Greenberg and Crow (1960) and Hadorn (1961). The semi-lethal chromosomes seem to have accumulated numerous mildly detrimental genes and the intensified deleterious effects on viability of double trans-heterozygotes may be due to duplication of some common mild mutant genes.

5. *Studies on spontaneous polygenic viability mutations in Drosophila melanogaster* 1. *Method and representation of condensed data*

(By Terumi MUKAI, Sadao CHIGUSA and Isao YOSHIKAWA)

Studies on spontaneous polygenic mutations which slightly reduce the viability in homozygous condition are scanty. From the study of this kind of mutations, a considerable amount of information could be drawn with respect to the genetic structure of natural populations. Accordingly,

Table 1. Basic statistic and genetic parameters obtained from data in Generations 10, 15, 20 and 25

Generation(temperature)	10(25°C)	15(21°C)	20(25°C)	25	
				(21°C)	(25°C)
No. of lethal lines	2	5	13	15	15
No. of homozygously detrimental lines	3	2	3	2	2
No. of quasi-normal lines tested	98	97	89	84	84
No. of missing lines	1	0	2	3	3
Estimated genetic variance (homozygote basis) (δ_G^2)	0.698	2.092	1.848	3.514	3.810
Average of the viability indices of quasi-normal lines (\bar{v})	31.60	27.89	30.93	28.36*	
Estimated control viability index (\bar{v})	32.94	29.69	32.36	32.45**	
Total no. of flies counted in quasi-normal lines	140,328	240,473	383,543	453,999	472,720

* Mean value of 21°C and 25°C experiments

** Pooled estimate of 21°C and 25°C experiments

a series of experiments were started in May, 1961. The experiments are still in progress, but results at hand are here reported.

A single male $Pm/+$ from a cross of $Cy/Pm \times +/+$ (an isogenic stock of Burdick: W160) was sampled and multiplied by the cross Cy/Pm ($\varnothing \varnothing$) $\times Pm/(1\delta)$ and 104 lines of $Cy/Pm \times Pm/+_i$ ($i=1, 2, \dots, 104$) were established. In each line, the second chromosome has been maintained through a single male by the cross Cy/Pm ($5\varnothing \varnothing$) $\times Pm/+_i$ (1δ) for the purpose of accumulating spontaneous mutations affecting viability.

In Generations 10, 15, 20 and 25, homozygous viability of each second chromosome was estimated by Wallace's Cy -method according to which the expected ratio of wild-type flies to the total is 33.3 per cent and 0 per cent in the absence of mutation and for recessive lethal mutations. Accordingly, the percentages of wild-type flies were employed as viability indices.

The basic statistic and genetic parameters were estimated by the aid of analysis of variance and other techniques. The results are presented in Table 1. In this table, the control viability index implies the mean of the distribution of viability indices of quasi-normal lines (having a larger than 20 viability index in homozygous condition) in Generation 0 in the same environment as that of a certain generation in which the test was conducted. Analysis was carried out with respect to quasi-normal lines.

6. *Studies on spontaneous polygenic viability mutations in Drosophila melanogaster 2. Variance increasing rate and mean decreasing rate*

(By Terumi MUKAI)

If the effects of polygenic viability mutations are additive or multiplicative, although in the latter case the effect of each mutation should be small as compared with the viability of standard normal homozygotes, the genetic variance among the lines increases approximately linearly in the course of generations and the mean of quasi-normal homozygote viabilities decreases also linearly. Although we do not have sufficient data for testing the linearity of variances and means, it might be tested graphically.

The control viability indices of Table 1 were estimated by the following procedure: for example, in the case of Generation 10, the line showing the greatest viability and four lines following in the order of degree were sampled in each of Generations 15, 20 and 25. In Generation 10,

phenotypically *Cy*-flies and wild-type flies in those selected lines were pooled, respectively, and the viability index was estimated as the control viability in this generation in terms of per cent wild-type flies on the pooled basis. Using this method, we assumed that the five lines selected above in each of Generations 15, 20 and 25 did not contain any mutations and the effects of errors in those generations were not correlated with those in Generation 10. The control viabilities for Generations 15 and 20 were estimated by the same procedure as that in Generation 10. That for Generation 25 was estimated on the basis of comparison between the estimates of viabilities at 21°C and 25°C.

The relationships between reduction of means and generation numbers and between variances and generation numbers are represented in Table 2. From Table 2, it might be said that the variances of viability increase and the means decrease approximately linearly. Accordingly, the following conclusions might be drawn: first, the average number of mutations per individual increases linearly with generations, and secondly, effects of interaction of new mutant genes with each other, and with already existing genes are not of appreciably large magnitude in homozygous genetic background.

Table 2. Genetic variances and decrements of the means of viability indices

Generation	0	10	15	20	25*	Regression coefficient on generation
Genetic variance	0	0.6981	2.0918	1.8481	3.6622	0.1120 ^{**} ±0.0185
Decrement of mean	0	1.34	1.80	1.43	4.09	0.1207 ^{**} ±0.0207

* Average of the estimates at 21°C and 25°C

** Significantly different from 0 at the one per cent level.

7. *Studies on spontaneous polygenic viability mutations in Drosophila melanogaster* 3. *Estimation of polygenic viability mutation rate*

(By Terumi MUKAI)

According to Bateman's (1959) calculation, the following formula (1) can be obtained concerning the reduction of mean viability and the

increase of genetic variance caused by mutations under the assumption that spontaneous mutations are distributed on homogeneous chromosomes according to Poisson.

$$\left. \begin{aligned} \bar{a}p &= \bar{v} - \bar{v} \\ (\bar{a}^2 + \sigma_a^2)p &= \hat{\sigma}_v^2 \end{aligned} \right\} \quad (1)$$

where \bar{v} is the mean of control viability indices, \bar{v} is the mean viability index of wild-type flies in quasi-normal lines, a is the effect of a single mutation whose mean and variance are \bar{a} and σ_a^2 , respectively, p indicates average number of mutations in the second chromosome, and $\hat{\sigma}_v^2$ is the estimated genetic variance of viability indices among quasi-normal homozygotic lines. For the sake of simplicity, $\bar{v} - \bar{v}$ is equated to A and $\hat{\sigma}_v^2$ to B . Consequently, A and B are the estimated values. From formula (1), the maximum σ_a^2 can be estimated. σ_a^2 should be 0 or positive. Thus, the range of σ_a^2 becomes as in formula (2).

$$0 \leq \sigma_a^2 \leq \frac{B^2}{4A^2} \quad (2)$$

The ranges of p and \bar{a} can be correspondingly calculated from formulae (1) and (2) under reasonable conditions; the results are presented in formulae (3) and (4).

$$\frac{B}{2A} \leq \bar{a} \leq \frac{B}{A} \quad (3)$$

$$\frac{A^2}{B} \leq p \leq \frac{2A^2}{B} \quad (4)$$

If we substitute the variance increasing rate and mean decreasing rate for A and B in formulae (3) and (4), we can estimate the average number of mutations per second chromosome per generation and the average effect of mutations. The results are tabulated in Table 3.

From Table 3 it can be concluded that the mutation rate of polygenes is of extremely large magnitude, when it is compared with that of

Table 3. The rate of polygenic mutations controlling viability, their average effect and variance

Item	Mutation rate (per 2nd chrom. per gen.)	Average effect	Range of variance of effect
Estimate	$0.130 \leq p \leq 0.260$	$0.464 \leq \bar{a} \leq 0.928$	$0 \leq \sigma_a^2 \leq 0.215$

recessive lethal genes (ca. 0.0063) per second chromosome per generation.

8. *Studies on spontaneous polygenic mutations in Drosophila melanogaster* 4. *Dominance in polygenic viability mutations*

(By Terumi MUKAI, Sadao CHIGUSA and Isao YOSHIKAWA)

When crosses in Generation 25 were conducted for estimating homozygous viabilities of the chromosomes in question, the viabilities of flies carrying random combinations of those chromosomes were estimated in the following combinations, 1×2 , 2×3 , 3×4 , \dots , $(n-1) \times n$, $n \times 1$, where numbers indicate the line designations. The lines in which lethal or semi-lethal mutations had taken place were not employed.

With the aid of analysis of variance, it was proven that there was a difference in heterozygous viability among random heterozygotes. In addition, the genetic variance in heterozygous combination of those chromosomes ($\sigma_{G'}^2$) was estimated. Phenotypic correlation ($r_{PP'}$), genotypic correlation ($r_{GG'}$) and covariance between heterozygote viabilities and the sums of the corresponding homozygote viabilities were estimated both in 25°C and 21°C experiments. These genetic statistic parameters are presented in Table 4 together with the mean viabilities.

Suppose that the average reduction of viability per second chromosome in homozygous condition is \bar{d} and their average degree of dominance is \bar{h} , then \bar{d} and \bar{h} can be estimated on the basis of the mean viabilities of control, homozygotes and heterozygotes; the results are $\bar{d}=4.09$, $\bar{h}=0.37$ on the average of the estimates at 21°C and 25°C in Generation 25. The estimated \bar{h} value, 0.37, is extremely large as compared with those for recessive lethals estimated for natural-origin chromosomes ($\bar{h}=0.02 \sim 0.04$).

On the other hand, if h and d are independent, then the average degree of dominance (\bar{h}) can be estimated by the following formula:

$$\bar{h} = \frac{\text{Cov}(\text{Hetero and Sums of Homo})}{2\hat{\sigma}_d^2} \quad (5)$$

If we substitute the estimated parameters in this formula, then \bar{h} 's at 21°C and 25°C become 0.72 and 0.80, respectively. Those values are much larger than the previous estimate ($\bar{h}=0.37$), and might confirm the large value of \bar{h} . In addition, a large amount of the difference between the two estimates might indicate that there is a positive correlation between the detrimental degree of viability mutations in

homozygous condition and the degree of dominance within the range of quasi-normal viability.

Table 4. Various genetic parameters in Generation 25

Temperature	21°C	25°C
Control viability	32.45	
Mean viability of homozygotes	28.37	28.36
Mean viability of heterozygotes	29.58	29.27
Phenotypic correlation (r_{PP})*	0.510	0.594
Genotypic correlation (r_{GG})	0.692	0.807
Covariance*	5.028	6.092
Genetic variance of heterozygotes	7.508	7.480
Genetic variance of homozygotes	3.514	3.811

* Relationship between heterozygote viabilities and the sums of the corresponding homozygote viabilities.

9. *Studies on spontaneous polygenic viability mutations in Drosophila melanogaster* 5. *Polygenic mutations and genetic structure of natural populations*

(By Terumi MUKAI)

If the classical hypothesis is valid, the genetic load in the greater fraction of loci is mutational, and its magnitude is 2μ per locus (μ =rate of mutation from wild-type gene to mutant gene) in autosomes in equilibrium of a random mating population (Muller 1950), disregarding completely recessive genes with respect to viability. For the sex chromosome, the magnitude is approximately 1.5μ (3μ in males and of a negligible amount in females). It should be stressed in these cases that the contribution of selection coefficients to the mutational load is too small to be considered. On the other hand, if the balance hypothesis is true, the major part of the genetic load is segregational load, and its magnitude is independent of the mutation rate and is determined by selection coefficients.

Following Kimura (1960), a test can be conducted to see whether the classical hypothesis is valid or not. Assuming this hypothesis, survival rate of eggs (S) can be expressed by formula (6).

$$S = e^{-2\sum\mu_i - 1.5\sum\mu_j} \quad (6)$$

where $2\sum\mu_i$ and $1.5\sum\mu_j$ stand for the mutational loads in autosomes and

sex chromosomes, respectively.

In the present experiment, the mutation rate of polygenes in the second chromosome p is $0.130 \leq p \leq 0.260$. We used the average of those two extreme values and calculated the total mutational load to be 0.9760 on the basis of proportionality between chromosome length and mutation frequency. The survival rate becomes $e^{-0.9760} = 0.3768$. This rate is inconsistent with the actual situation in natural or artificial random mating populations, where, for instance, the hatchability of eggs in *Sy* and *OR* populations was found to be as high as 95 per cent (King 1961) and consequently, this finding is unfavorable to the classical hypothesis, if there is no epistasis favoring the coexistence of deleterious genes.

In the present experiment, we have detected that the average degree of dominance of mutations which have not experienced natural selection (\bar{h}) is large. This finding is inconsistent with that of Wallace and Dobzhansky (1962). In their case, samples from artificial populations were used. The difference might have been caused by natural selection in the artificial populations. If this is the case, in natural populations, mutant genes having large h values should be eliminated rapidly by natural selection and/or natural selection operates so as to produce a genic system in which h is small or rather negative (overdominance).

10. *Persistence of two recessive genes in experimental populations of Drosophila melanogaster*

(By Sadao CHIGUSA and Terumi MUKAI)

Two cases of single gene heterosis have been tested associated with the recessive genes *sepia* and *expanded wing* (formerly x , discovered in Mukai and Burdick's (1959) Population 1, of *Dichaete* phenotype, the gene located at about 3-49±).

Four artificial populations (Pop. A-1 and Pop. A-2 for *se*, and Pop. B-1 and Pop. B-2 for *ew*) were established, and the gene frequencies of Pop. (A or B)-1 and Pop. (A or B)-2 in the starting generation were 0.5 and 0.1, respectively. The homozygosities of the genetic backgrounds in A-populations are extremely low, while those of B-populations are relatively high. Each population consisting of 8 subpopulations has been maintained by so-called Pearl's method, and the frequencies of recessive homozygotes were estimated in most of the generations. Four populations seem to have reached equilibria since Generation 20~25, which indicates the superiority of the heterozygote over either homozygote with respect to fitness; the equilibrium frequencies are given in

Table 1. Characteristics of artificial populations

	Initial gene frequency	Generation	Population count				Progeny test			Number of subpopulations	χ^2 (†)
			Population size	[+]	[se] or [ew]	Recessive homozygote frequency	+/+	+/se or +/ew	q(†)		
Pop. A-1 (se)	0.5	39	5052	4659	393	7.78 %	108	85	28.0%	8	207.576***
		40	4591	4188	403	8.78					
		41	3868	3549	319	8.25	84	81	30.8		
-2	0.1	31	3778	3244	534	14.13	81	85	36.0	7	334.332***
		33	2445	2082	363	14.85	68	71	36.5		
		34	3302	2861	441	13.36	73	93	37.8		
Pop. B-1 (ew)	0.5	37	2590	2533	57	2.20	131	53	16.2	8	11.506
		38	3799	3746	53	1.34	135	49	14.7		
		39	3335	3276	68	2.04	114	42	15.1		
-2	0.1	32	3982	3820	162	4.07	71	60	26.3	8	35.2957**
		33	3003	2931	72	2.34	96	80	24.4		
		35	3371	3234	137	4.06	98	83	26.2		

†): Recessive gene frequency. q is estimated by a simple method.

††): Test for heterogeneity among subpopulations.

*** Significant at a very low level.

** Significant at the 1% level.

Table 1 in addition to the characteristics of the four populations. From the progeny test, the goodness of fit of the observed data to those which are expected can be calculated on the assumption that the three classes of flies are distributed in accordance with the Hardy-Weinberg law. The results of the population counts show that heterozygote frequencies are larger than the expected ones in three counts out of five in A-populations and in all counts in B-populations.

The relative values of the fitness components of the three genotypes were estimated in other experiments and the results show that the heterozygote has been superior in each population to either homozygote in female fecundity, male mating ability and zygotic viability.

It can also be seen from Table 1 that the recessive gene frequencies have reached equilibria, but that the equilibrium frequencies of two populations both in A-populations and B-populations are remarkably different from each other. However, a more quantitative determination of the trend is desired. This is now under way.

The x^2 values of heterogeneity test among subpopulations are very large in A-populations but those of B-populations do not indicate a significant difference. The large x^2 values imply that subpopulations have reached equilibria at various levels. As mentioned above, A-populations have higher genetic variability in their genetic backgrounds than B-populations. Therefore, the above mentioned phenomena might have been caused by the effects of random genetic drifts of the genetic backgrounds.

We are still maintaining these populations, and details of the work will be published elsewhere.

11. *Syntheses of Drosophila eye pigments*¹⁾

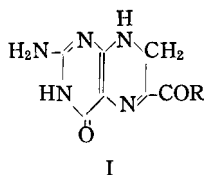
(By S. NAWA and H. S. FORREST)

Drosophila melanogaster has two kinds of yellow pigments and three kinds of red pigments. The structures of yellow compounds, isosepiapterin and sepiapterin, have been determined to be 2-amino-4-oxo-6-acyltetrahydropteridines, the acyl group in isosepiapterin being propionyl and in sepiapterin, lactyl (hydroxypropionyl). The red compounds, drosopterins (drosopterin, isodrosopterin and neodrosopterin), are believed to have structures related closely with sepiapterin, although their structures have not been confirmed.

Based on the findings that pyruvic acid gives a relatively stable

1) This work was done in The University of Texas, U.S.A.

carbanion (CH_3CO^-) in its decarboxylation by action of thiamine and that a reduced form of 2-amino-4 hydroxypteridine adds anionic reagents exclusively at the 6-position, formation of a pteridine having an acyl group in its 6-position would be expected on reaction with reduced pteridine and anion from α -keto acid. Indeed, incubation of reduced 2-amino-4-



hydroxypteridine, pyruvic acid and thiamine at pH 9 yielded a yellow compound. It is sure that the compound has a substituent in the 6-position, because permanganate oxidation of the compound gave 2-amino-4-hydroxy-6-carboxypteridine. Its structure is believed to be I ($\text{R}=\text{CH}_3$). Isosepiapterin (I, $\text{R}=\text{CH}_2\text{CH}_3$) was formed in the same

reaction at a reasonable yield, when α -ketobutyric acid was used instead of pyruvic acid.

Considering the structure of sepiapterin (I, $\text{R}=\text{CH}(\text{OH})\text{CH}_3$), its synthesis should be possible using the same procedure with α -keto- β -hydroxybutyric acid as a source of the side chain. No sepiapterin was, however, obtained under conditions developed for maximal isosepiapterin synthesis and even conditions modified variously. Nevertheless, the mixture of α -keto- β -hydroxybutyric acid and reduced pteridine became gradually red on standing at room temperature (even in the absence of thiamine). The reaction mixture contained three kinds of red compounds which were found to be identical, spectrophotometrically and chromatographically, with drosopterin, isodrosopterin and neodrosopterin respectively. On the contrary, if hydroxypyruvic acid was used instead of α -keto- β -hydroxybutyric acid, no red pigment was formed but a yellow compound was produced. The product was purified as a crystalline form. The properties of the compound were very similar to those of sepiapterin, and then its structure was identified to be I ($\text{R}=\text{CH}_2(\text{OH})$).

It is expected that in the reaction between reduced pteridine and a carbanion from a ketoacid an intermediate is a 6-acyl-5, 6, 7, 8-tetrahydropteridine. The intermediate will be then oxidized to a 7,8-dihydro compound. This seems to be true if its acyl group is such one as CH_3CO^- , $\text{CH}_3\text{CH}_2\text{CO}^-$ or $\text{CH}_2(\text{OH})\text{CO}^-$. However, the intermediate having $\text{CH}_3\text{CH}(\text{OH})\text{CO}^-$ as its acyl group was not oxidized to a corresponding 7,8-dihydro compound (sepiapterin) under conditions used. An unknown different type of oxidation gave drosopterins. The mechanism is obscure since structures of drosopterins have not been clarified.

The facts that the ratio of quantities of three kinds of drosopterins produced in the reaction is approximately the same to that found in *Drosophila* eye and that the reaction is carried out under mild conditions

(at neutral pH and room temperature) suggest strongly a possibility of their biosyntheses in this manner, although no accumulation of keto-hydroxybutyric acid was detected in *Drosophila*. On this assumption, it can be proposed that the mutant *sepia* which produces sepiapterin and not the drosoproteins, oxidises the intermediate exclusively to the 7,8-dihydro compound whereas in the wild type, a divergent pathway with some of the intermediate being converted to drosopterin and some to sepiapterin, is present.

12. *Melamine action in phenocopic pigment elimination from hypodermal cells of the silkworm*

(By Mitsuo TSUJITA and Susumu SAKURAI)

When mulberry leaves painted with 1% melamine solution were continuously given to lemon larvae (yellow colored larvae) from the beginning of 3rd, 4th or 5th instars, the hypodermis became translucent 2 days after the treatment and at the same time the yellow larval color disappeared, because the yellow pigments were lost from the hypodermal

Table. 1. Loss of uric acid from the hypodermal cells by eating mulberry leaves painted with melamine solution.

A. Before the treatment with melamine

No. of Experiment Strains	I	II	III	Mean
C124	mg/1 g dry matter 86.00	mg/1 g dry matter 84.00	mg/1 g dry matter 85.00	mg/1 g dry matter 85.00
<i>1 em/1 em</i>	87.50	79.00	84.30	83.60

B. After the change to translucent hypodermis

No. of Experiment Strains	I	II	III	Mean
C124	mg/1 g dry matter 5.00	mg/1 g dry matter 3.00	mg/1 g dry matter 8.00	mg/1 g dry matter 5.30
<i>1 em/1 em</i>	trace	trace	trace	trace

cells.

Each larva with translucent hypodermis was cut open from the dorsal side and almost all of the inner organs were removed and the remaining hypodermal tissue was dried. Then the amount of uric acid and pteridine compounds, such as yellow pigment or isoxanthopterin, was measured. Experimental results are shown in the following tables.

As shown in Table 1, a large part of uric acid is lost from the hypodermal cells of larvae eating mulberry leaves painted with melamine solution.

Table 2. Loss of pteridine compounds from the hypodermal cells by the effect of melamine

Strains	Pteridine compounds	Before treatment $\mu\text{g}/\text{individual}$	After the change to transparent hypodermis $\mu\text{g}/\text{individual}$
C124	Isoxanthopterin	13.2	1.2
KH19	Isoxanthopterin	15.0	6.0
<i>1 em/1 em</i>	yellow pigment	12.0	trace
	Isoxanthopterin	3.0	trace

As shown in Table 2, under the influence of melamine the amount of isoxanthopterin decreases markedly and the remainder differs with the three strains. On the contrary, yellow pigments are completely lost from the hypodermal cells of the treated larvae.

Further, the hypodermal cells of larvae which lost uric acid and pteridine compounds were microscopically examined. It was found that almost all chromogranules in their cytoplasm broke down and disappeared. To purify the chromogranules in the cytoplasmic layer of the hypodermis a procedure used in another experiment was applied. However we could not obtain the granular fraction. It may be said from these experimental results that pteridine compounds become released from the chromogranules as the result of their collapse.

Effects of melamine treatment on the substances showing fluorescence in the cytoplasm of hypodermal cells and in the body fluid of larvae were examined by paper electrophoresis and the following results were obtained.

As shown in Table 3, there is a substance showing sky-blue fluorescence in addition to yellow pigment and isoxanthopterin in the cytoplasm of hypodermal cells of lemon larvae and traces of a similar

Table 3. Substances showing fluorescence in the hypodermal cells and in the body fluid examined by paper electrophoresis.

Treatment	Strain	Tissues	Pteridine compounds		
			Yellow pigment	Isoxanthopterin	Substance showing sky-blue fluorescence
Before the treatment	lemon	hypodermal cells	++++	+	+
		body fluid	±	-	±
One day after the treatment	lemon	hypodermal cells	++	±	+++
		body fluid	-	±	++
	dilute lemon	hypodermal cells	+	+	+
		body fluid	±	-	++

+: Indicates relative amount.

-: Indicates the absence of the compound.

±: Indicates trace.

substance can be detected in their body fluid. One day after treatment with melamine the amount of yellow pigments decreases and that of the substance showing sky-blue fluorescence increases in the hypodermal cells. According to Table 3 the amount of the latter compound shows the tendency to increase in the body fluid of the treated larvae. The same increase in the amount of substance showing sky-blue fluorescence could be observed in the body fluid of dilute lemon larvae as compared with lemon larvae. It is inferred from the experimental results that yellow pigments released from the chromogranules are converted into other pteridine compounds, such as the substance showing sky-blue colored fluorescence, which are excreted from the hypodermal cells into the body cavity.

We mentioned in a previous report (Tsujiya 1963) that a large part of yellow pigments produced in the hypodermal cells of dilute lemon larvae is lost from the cells by the action of d-lem gene. The finding that owing to melamin treatment yellow pigments are lost from the hypodermal cells of lemon larvae can be considered as an example of phenocopy. However, it should be noticed that there is

remarkable difference between the action of d-lem gene and that of melamine; d-lem gene controls a small specific change, namely "the production of an altered specific protein" within the chromogranules, while melamine induces a drastic change, namely "disintegration of chromogranules."

13. *Specific protein combining with yellow pigments
(dihydropterin) in the silkworm*

(By Mitsuo TSUJITA and Susumu SAKURAI)

A large amount of spherical granules that contain yellow pigments and isoxanthopterin occur in fresh hypodermal cells of larvae of the strains lemon and dilute lemon. The presence of these chromogranules in the hypodermal cells gives a yellow color to the larvae.

The yellow granules were purified by a new method devised by us. The chromogranules obtained from the lemon and the dilute lemon larvae show almost the same shape, an irregularly spherical one. The granules obtained from the lemon larvae are much darker yellow in color than those from the dilute lemon larvae. Similarly shaped granules containing isoxanthopterin but smaller in size than those of the lemon and the dilute lemon larvae are present in the hypodermal cells of normal larvae.

The protein composition of the three types of chromogranules obtained from normal, lemon and dilute lemon larvae has been examined by paper electrophoresis, and characteristic differences have been found in their electrophoretic mobility. It was at first thought that these electrophoretic differences might reflect small structural dissimilarities among the three types of chromoprotein, comparable to those that have been found in mutant human hemoglobin (Ingram 1958). However, the digestion of the chromoprotein with trypsin showed differences in the peptide composition; the chromoprotein of the lemon sample contained two supernumerary peptides in addition to those commonly observed in the three types of chromoprotein, while the chromoprotein of the dilute lemon sample had only one additional peptide that had a different R_f value when compared with the supernumerary one produced by the 2-P of the lemon sample. The two supernumerary peptides found in the lemon sample are believed to be components of the specific protein which has the ability to combine with yellow pigments. The single supernumerary peptide found in the dilute lemon sample is apparently a component of the specific but altered protein.

On the basis of the present study, the biochemical interactions between *lem* and $+^{d-lem}$ or between *lem* and *d-lem* genes may be explained as follows. As reported in a previous paper (Tsujita 1961), the yellow pigments are produced abundantly in hypodermal cells in which the step leading from dihydropterin to tetrahydropterin is blocked by the *lem* gene. A specific protein is produced under the control of $+^{d-lem}$ gene action in the presence of yellow pigments. As the chromogranules in hypodermal cells of larvae with the genotype *lem/lem*; $+^{d-lem}/+^{d-lem}$ contain this protein, they are able to absorb yellow pigments and keep them within the cells. On the other hand, the altered protein produced under the control of *d-lem* gene action is present in the chromogranules of the dilute lemon larvae. Consequently in the dilute lemon larvae, the ability of the granules to absorb and retain the yellow pigments is markedly decreased, and such larvae have a dilute yellow color.

On the basis of other experimental results, it is believed that a large part of the yellow pigments which are not absorbed in the chromogranules are converted into other compounds, such as substances which show sky-blue fluorescence, and are excreted from the hypodermal cells into the body fluid.

14. *Conversion from guanine to pteridine in the silkworm*

(Bt Bungo SAKAGUCHI)

It is generally considered that the pteridine ring is biosynthesized from a purine precursor. But a direct evidence of the biosynthesis of the compound has not been found yet for the silkworm.

Guanine-2-¹⁴C was injected 1 μ c per head into females of early pupae of a normal strain (C 108). Young larvae were collected in the following generation from the adult insects. Pteridine and purine compounds were extracted from the young larvae with boiling water and the protein was removed from the extract by acidification. Paper chromatography and column chromatography (Dowex 1-Formate) were used for the isolation of pteridine and purine compounds which were detected by use of an ultraviolet light source and a spectrophotometer. For paper chromatography radioactive compounds were determined by autoradiography and scanning gas flow counter.

Purification of isoxanthopterin extracted from young larvae was achieved by performing four times paper chromatography with different solvents. Radioactivity of purified isoxanthopterin was tested by the gas flow counter and it was found to be radioactive. It is suggested from the

result that ^{14}C atom in carbon 2 of guanine molecule is contributed to isoxanthopterin. The turnover rate from the same precursor of guanine ^{14}C to isoxanthopterin- ^{14}C and uric acid- ^{14}C was shown to be one to seventeen.

In order to find out whether guanine- ^{14}C injected into pupae was being incorporated into nucleic acid components in the young larvae derived from the following next generation of the pupae, acid soluble, RNA and DNA fractions were separated from the young larvae by the method of Schmit *et al* (1945). The result is given in the following Table.

Table 1. Distribution of ^{14}C in the nucleic acid and other fractions prepared from young larvae

Fraction	Lipid	Acid soluble	RNA	DNA	Protein
cpm*	1617	2468	7419	433	150
%	12.8	23.6	58.9	3.5	1.2

* Counting value of extraction from one gram of wet weight.

As the Table shows high activity of ^{14}C originated from labeled guanine was found in both RNA and acid soluble fractions. The labeled components of nucleic acid in the fractions were separated and analyzed by the above method. It was found from the results that guanosine, guanylic acid and a small amount of pteridine derivatives were holding ^{14}C atoms liberated from guanine-2- ^{14}C .

Weygand *et al* (1961) have considered guanosine-5'-phosphate to be the precursor of leucopterin in the butterfly *Pieris brassicae*. Present results also indicate that guanine and guanylic acid directly contribute to the biosynthesis of the pteridine ring in the silkworm.

15. Analysis of marker genes in inbred strains of albino mice

(By Yoshinori KURITA and Katsumi SAKAKIBARA)

The marker genes of the main inbred strains of albino mice which have been maintained in Misima were analyzed. All albino mice were mated with C57BR and NH mice. It was found that the A/HeMs sub-strain consisted of genotypically three different families. The results of these analyses are summarized as follows:

Albino strain	Genotype
A/HeMs	AABBcc AAbbcc aabbcc
AKR/JMs	aaBBccSSPPDD
BALB/cJMs	AAbbcc
CFW/Ms	AAbbcc
DM/Ms	AAbbccSSPPDD
D103/Ms	AABBcc
MA/JMs	AABBcc
RF/Ms	aaBBcc
SL/Ms	AABBcc
SMA/Ms	AABBcc

16. *Genetical studies of post-axial polydactyly in the house mouse*

(By Akira NAKAMURA, Hitoshi SAKAMOTO and Kazuo MORIWAKI)

Post-axial polydactyly characterized by the presence of supernumerary fingers on the forefeet was found in an albino mouse obtained from a fancier in 1958. Since then it has been maintained by sister-brother matings. Strong (1934) has already reported similar features in his brief notes. Later, Phillips (1949) described a somewhat comparable condition which seemed to be heritable, but a detailed analysis has not yet been made.

In our experiments in which outcrosses were carried out of the polydactylous strain to various inbred strains (CBA, DM, A and C57BR), the abnormality in the forefeet appeared in various numbers in F_1 . But in a cross between Swiss albino and the polydactylous strain, the condition could be observed in 95% of F_1 and a 3:1 segregation was demonstrated in F_2 . Moreover, the backcross of F_1 to Swiss albino showed a 1:1 ratio.

In view of the above results it is probable that this character depends on a major dominant gene and possibly some minor genes which may modify its manifestation. The major dominant gene was named post-axial polydactyly gene (symbol Po).

17. *Cytological study of a transplantable ascites plasma-cell neoplasm in mice*

(By Yoshinori KURITA and Toshihide H. YOSIDA)

A transplantable ascites plasma-cell neoplasm in C3H mice has been obtained from an experimental intraperitoneal injection of X5563 solid plasma-cell neoplasm, and has been maintained by means of serial intraperitoneal transplantations. The averaged life span of the mice inoculated with the ascites was 15.3 ± 1.7 days. The modal chromosome number of the ascites neoplastic cells was 83, and four marker chromosomes have been ascertained, namely two large mediocentric, one metacentric and a diminutive one. The characteristic feature of this neoplasm is that it has a stable tetraploid cell population, consisting of more than 99% tetraploid cells. Its origin in connection with its stable heritable globulin production is under investigation.

18. *A characteristic chromosome found in the karyotype analysis of the mouse*

(By Akira NAKAMURA and Akira TONOMURA)

For a long time it has been believed that, in mice, all chromosomes are telocentric and morphologically indistinguishable from one another. Recently, the improved techniques for mammalian chromosome cytology has revealed that certain mouse chromosomes are characterized by the presence of a small knob near the centromeric region. The evaluation of this finding led us to re-examination of the classical chromosomal studies of the mouse.

The cells of normal mouse (DM/Ms strain) embryos were cultivated in media containing 20% of calf serum. Chromosome preparations from the cultured cells were made by acetic-dahlia squash technique, after pretreatment with 0.0001% colchicine solution and distilled water.

Most of the chromosomes were telocentric; a second arm was entirely imperceptible (Fig. 1). A small knob was observed in some chromosomes (Fig. 2), but it could not be always distinguished when the chromosomes were somewhat condensed by colchicine treatment. A detailed observation, however, disclosed one characteristic chromosome shown in Figure 3. It had a well-defined round or oval head separated from the main body of the chromosome by a thin chromatin strand. The head was almost $1/4$ as long as the body, and its diameter was considerably smaller than

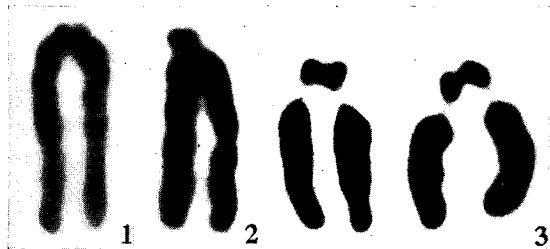


Fig. 1-3. Higher magnification of three structurally distinct chromosomes in the mouse. $7,000\times$.

Fig. 1; Ordinary telocentric chromosome. Fig. 2; Chromosome with small knobs. Fig. 3; A pair of chromosomes with well-defined oval heads. The heads are much larger than the knobs of Fig. 2.

that of the body. A detailed analysis of five cells revealed that this chromosome pair may be placed between the 14th to 16th pairs.

On morphological grounds, it is impossible to confuse this characteristic chromosome with the others, although whether its prominent head refers to the second arm of a submetacentric chromosome or is only a part of the chromosome due to a secondary constriction is still unknown.

19. *Cytological studies of lymphogenic and hematogenic metastases in MH-134 mouse hepatoma*

(By Naomichi INUI)

MH-134 mouse ascites tumor contains two different types of tumor cells having 40 ($2n$) and 80 ($4n$) chromosomes, their ratio being 1 to 0.97. In the present study, the proportion of diplo- to tetraploid tumor cells in metastases of the lymphnodes and parenchymal organs was examined and compared with that found in the original ascites tumor. Metastases to lymphnodes were induced by intracutaneous injection of ascites tumor cells into the tail of the mouse (Exp. A). Metastases to parenchymal organs were established by subcutaneous injection into the back.

Experiment A: Seven to ten days after transfer of the tumor cells, the tail was amputated at the base. About 20 days after the amputation, the mice were killed and caudal, lumbar and sciatic lymphnodes were removed. These lymphnodes showed a notable enlargement and contained a great number of tumor cells in 48 out of 50 animals. In seven mice remote metastases occurred in brachial, axillary and superfi-

cial lymphnodes. The ratios of diploid to tetraploid cells were almost similar to those found in the original ascites tumor.

Experiment B: About one month after the removal of the transplanted tumors, the animals were killed. In 24 out of 30 mice, metastases were observed in the parenchymal organs, such as lung, liver and kidney. The ratios of diplo- to tetraploid cells were found to be 1 : 1.06 in lung, 1 : 1.01 in liver and 1 : 0.86 in kidney. Detailed karyotype analysis revealed that 38 acrocentric chromosomes were in diploid cells and 76 acrocentric and 4 metacentric were in tetraploid cells. No chromosomal change could be found in the two types of tumor cells, as compared with those of the original ascites tumor cells.

The results here obtained suggest that, in MH-134 mouse hepatoma, the diplo- and tetraploid tumor cells invade both lymphnodes and parenchymal organs through the lymphatic and blood systems, and there is no preference for either diplo- or tetraploid cells in the specific organs.

20. *Chromatographic analysis of coat coloring substance in mice*

(By Akira NAKAMURA)

Rebell et al. (1957) have performed the extraction and chromatographic separation of L-kynurenine from the hairs of albino rats, and in the

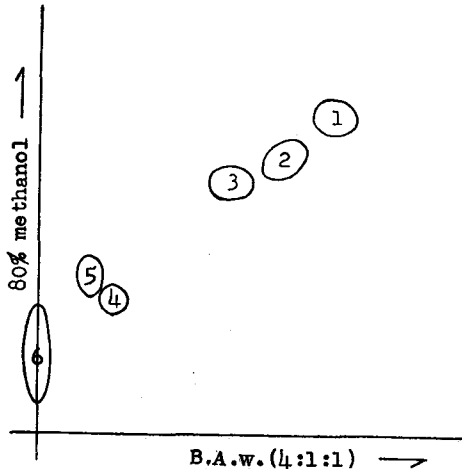


Fig. 1. Six typical spots on the two-dimensional paperchromatogram.

same year Hashimoto has observed kynurenine in mice skin. On the other hand Nachimas (1959) reported that kynurenine is not utilized for pigment formation in the skin of yellow mice (A^y).

In this study, the hair coat of 24 strains of mice was analysed by one-dimensional and two-dimensional paper chromatography. A summary of the results obtained is presented, and the positions of six typical spots (none for kynurenine) on the paperchromatogram are mapped in Figure 1.

- 1) Kynurenine was not observed in any strain.
- 2) Spot 1 was present in all strains, and exhibited a yellowish white-blue fluorescence.
- 3) Spot 2 was present in all strains and turned rose-red in the presence of Ehrlich's diazo reagent. The color appeared immediately after spraying with the reagent and then gradually faded.
- 4) Spot 3 was present in all strains, especially in A^y strain, A and CFW. Ehrlich's aldehyde reaction of the spot showed yellowish color.
- 5) Spot 4 was positive in A^y strain, CBA, C57BR, D103, C57BL, A, C3H, CFW and DM, but negative in C^{ch} strain and Swiss albino. Color of the spot in Ehrlich's aldehyde reaction was purple.
- 6) Spot 5 was present in C57BL and C^{ch} , and exhibited purple fluorescence.
- 7) Spot 6 was positive only in A^y and turned red in the presence of Ehrlich's diazo reagent.

21. *Biochemical changes in the skin of rhino mouse preceding the hereditary hair loss*

(By Kazuo MORIWAKI)

A mouse homozygous for rhino gene (hr^{rh}) begins to lose the hair coat about 15 days after birth and becomes completely naked within one month. At that time acid phosphatase (acid Pase) and inorganic pyrophosphatase (PPase) activities in the skin show a markedly higher level than in the heterozygotes, as recently reported by the author (1962). Whether these changes in enzyme activity precede the beginning of depilation or not is an interesting problem for studying the manifestation mechanism of rhino gene in terms of biochemical genetics.

From this view point, the activities of acid Pase and PPase in the skin have been compared between homo- and heterozygotes at several stages of development. Some other biochemical characters, i.e., lipid phosphorus content, cathepsin activity and cystine content, which seem

to have certain intimate relations to the regulation of hair formation, were also compared in similar fashion. Among them, acid Pase activity has shown an apparent difference at the earliest stage, namely, an appreciable increase in enzyme activity could be observed in homozygous skin 8 days after birth. Differences in the other characters became recognizable after 10 days. Lipid phosphorus content began to increase to some extent on the 10th day in the skin of homozygotes, and PPase and cathepsin activities were also increased around the 25th day. Cystine content in the skins of both homo- and heterozygotes remained constant throughout the development.

Considering the precedence of skin acid Pase in the sequential biochemical changes accompanying the manifestation of rhino gene, a qualitative analysis of the enzyme was carried out using agar electrophoresis technique. In both homo- and heterozygotes, similar patterns consisting of two isozymes were obtained. Furthermore, a comparison between homo- and heterozygotes was performed on zymograms of esterases which have probably to some extent a substrate range in common with acid Pase. Also in this case, there was no difference between them, though seven isozymes, at least, could be separated.

These results suggest that acid Pase is an intermediate step in the biochemical sequence leading to the manifestation of rhino gene, though the change in activity certainly precedes the appearance of the phenotype.

22. *Viscosity changes in the deoxyribonucleoprotein of L-strain cells following treatment with a base analogue*

(By Kazuo MORIWAKI)

A crude unpurified deoxyribonucleoprotein (DNP) extracted with 1 *M* sodium chloride from rat thymus exhibits some viscosity losses after low X-ray exposures, which are understood as breakdowns in the fundamental units of chromosome structure (Fisher *et al*, 1959). On the other hand, Hsu and Somers (1961) reported that a base analogue, 5-bromo-deoxyuridine (BUDR), can induce chromosome aberrations in mammalian cell cultures similarly to ionizing radiations.

The present study was an attempt to find out a relationship between chromosome breaks and viscosity changes in DNP of cultured mammalian cells treated with a base analogue. *L*-strain cells treated with BUDR (25 μ g/ml) for 3 days showed chromatid breaks and simultaneously viscosity loss in DNP extracted with 1 *M* sodium chloride from the cells. Viscosity of DNP was measured using Cannon-Fenske viscometer modi-

fied by Fisher. Cell homogenates for DNP extraction were adjusted at the concentration of 500 mg% in wet weight. Specific viscosity in DNP decreased from 0.052 to 0.017 following treatment with BUDR.

This result suggests the possibility that chromosome aberrations induced by BUDR may be accounted for the breakdowns in the fundamental DNP units of chromosome structure.

23. *Endurance of one day old chicks under starvation*

(By Takatada KAWAHARA)

The time of surviving in starvation of one day old chicks was comparatively investigated in two purebreds, White Leghorns (WL) and Barred Plymouth Rocks (BPR) and their reciprocal hybrids. The starvation treatment was given in an incubator under the condition of relative humidity of about 55 percent and temperature of 37.6°C. Data were collected from 108 purebred and 138 crossbred chicks. The hatched body weight and average endurance of tested chicks are shown in Table 1.

The results of this investigation are summarized as follows:

1). The coefficient of correlation between the surviving time and

Table 1. Endurance under starvation of two breeds and their hybrids.

Breed or cross	Sex	Number of chicks	Body weight (g)	Endurance	
				Surviving time (hours)	Surviving time (hours) Body weight
WL	♂	28	38.23±0.88	156.14±2.93	4.13±0.09
	♀	26	37.00±0.60	164.77±3.62	4.44±0.08
	Both	54	37.64±0.55	159.63±1.93	4.28±0.07
BPR	♂	28	35.86±0.53	172.14±2.88	4.82±0.09
	♀	26	35.37±0.51	183.04±5.86	5.21±0.18
	Both	54	35.62±0.37	180.69±3.26	5.10±0.10
WL ♀ × BPR ♂	♂	44	38.09±0.50	166.73±1.60	4.39±0.07
	♀	45	37.71±0.44	168.76±3.32	4.49±0.09
	Both	89	37.90±0.19	167.75±2.06	4.44±0.06
BPR ♀ × WL ♂	♂	23	35.48±0.93	169.26±4.12	4.79±0.12
	♀	26	35.13±0.66	170.85±5.33	4.88±0.14
	Both	49	35.30±0.45	170.10±3.34	4.83±0.09

hatched body weight was -0.063 in the purebreds, while in the hybrids it was $+0.284$.

2). The difference between strains was remarkable, BPR showing a very high endurance in comparison with WL. BPR lived 21.06 hours longer than WL.

3). In all strains, females survived longer than males.

These results suggest that the endurance of chicks under starvation may be controlled at least partly by genes.

24. *Genetic studies on body weight at different stages of growth in the domestic fowl*

(By Takatada KAWAHARA)

Heritability of body weights during development and genetic and phenotypic correlations between weights at various growth stages were investigated in a closed flock of White Leghorns. Measurements of body weight were taken 4, 8, 12 and 18 weeks after hatching, at first egg laying, and after 48 weeks in adults. The estimates of heritabilities from analyses of variance are shown in Table 1.

Table 1. Heritabilities of body weights in various stages of growth.

	h_s^2	h_d^2	h_{s+d}^2
Body weight at:			
4 weeks	0.51	0.73	0.62
8 weeks	0.99	0.29	0.64
12 weeks	0.77	0.34	0.55
18 weeks	0.76	0.55	0.66
Age at 1st egg	0.53	0.73	0.63
Adult stage	0.40	0.48	0.44

Table 2. Correlations between body weights at different growth stages.

	Genetic correlation	Phenotypic correlation
4 weeks-Adult	0.573	0.445*
8 weeks-Adult	0.727	0.515*
12 weeks-Adult	1.100	0.590*
18 weeks-Adult	0.803	0.589*
Age at first egg-Adult	0.984	0.705*

*: Significant at the 1% level.

The estimate of heritability of (h_{s+d}^2) averaged for body weights at various stages of growth is 0.59.

Table 2 presents the estimates of coefficients of genetic and phenotypic correlations between adult body weight and body weight at various younger stages of growth.

25. *Use of rabbit anti-serum for muscle proteins of fowls for serological determination of homologous tissue proteins of Triturus*¹⁾

(By Yoshito OGAWA and Takatada KAWAHARA)

For immuno-chemical researches^{2),3),4)} the embryo of *Triturus pyrrhogaster* is a suitable material, because the developmental process is relatively slow and very typical. But it is very difficult to obtain enough pure actin or myosin for the preparation of the anti-serum, since *Triturus* is a very small animal and has scanty muscle tissue. The possibility to determine muscle proteins of *Triturus* by using the anti-sera for muscle proteins of White Leghorn was examined. Pure actin⁵⁾ and myosin⁶⁾ were isolated from muscle tissue of *Triturus* or White Leghorn by the method of Szent-Gyorgyi and the anti-sera of both proteins were prepared by injecting them into the rabbit intravenously. The precipitin test with anti-sera for *Triturus* muscle proteins was made as control. The titre of both anti-sera was adjusted to 1:512.

Triturus embryos from 120 to 192 hrs. after fertilization were mashed in ice cold potassium chloride solution (0.6 M). The homogenate was allowed to stand for one hour at 0°C under constant stirring and then was centrifuged. Before carrying out the precipitin reaction with the anti-serum, the clear supernatant extract was placed in a collodium-membrane tube and treated with large amounts of distilled water for 48 hrs. at 0°C, to eliminate the potassium chloride. Constant volume of anti-sera and progressively decreasing amounts of antigen, ranging from 1:10 to 1:160 in weight, were used for preventing a possible failure due

1) This work was supported by a Grant-in-Aid for Fundamental Scientific Research (No. 710267) of the Ministry of Education in Japan

2) Ogawa, Y.: *Nature*, **181**: 621, 1958.

3) Ogawa, Y.: *Nature* **186**: 1312, 1958.

4) Ogawa, Y.: *Exptl. Cell Res.*, **26**: 269, 1962.

5) Szent-Gyorgyi, A. G.: *J. Biol. Chem.*, **192**: 361, 1951.

6) Szent-Gyorgyi, A. G.: *Chemistry of Muscular Contraction*, (1951) Acad. Press, New York.

Table 1. Determination of developing skeletal muscle proteins in *Triturus* embryo

Hours after fertilization	Lowest antigen concentration giving positive results			
	Results given by the anti-sera for <i>Triturus</i> muscle proteins (Control)		Results given by the anti-sera for fowls muscle proteins	
	Actin	Myosin	Actin	Myosin
108	—		—	
120	—*		—*	
132	1: 40		1: 80	
144	1: 80		1: 160	
156	<1: 160	—	<1: 160	—
168		—*		—*
176		1: 160		1: 160
180		<1: 160		<1: 160
192		<1: 160		<1: 160

* Reaction at high antigen concentration (1:2) was added to confirm the negative result.

to excess of antigen. The experimental results are given in Table 1.

The results obtained by the anti-serum for White Leghorn's muscle proteins showed no marked difference from those found by the anti-serum for *Triturus* muscle proteins. This finding may be useful in research work on muscle protein development in Amphibian embryos.

26. *Effects of p-hydroxy-benzyl-methyl-ether, a component of Citrullus colocynthis, on growth and differentiation of Triturus embryo*

(By Yoshito OGAWA and Hiroyasu WATANABE)

P-hydroxy-benzyl-methyl-ether is a new compound. It was first isolated in 1961¹⁾ from the glycosid fraction of the fruits of *Citrullus colocynthis* SCHRAD. The influence of this substance on the early embryos of *Triturus pyrrhogaster* was examined, because this glucosid fraction shows an inhibiting action on the growth of animal tumors²⁾.

The embryos were raised in solutions of this compound (0.5%, 0.05%, 0.005% and 0.0005%) at 20°C from the 6th day after fertilization (Stage 20), and their body weight and length were measured. Furthermore, the growth progress with respect to organ formation was histologically

- 1) Watanabe, H., A. Ide and Y. Ogawa, : Agr. Biol. Chem., **25**: 269, 1961.
- 2) Ogawa, Y.: Ann. Rep. Natl. Inst. Genet., **9**: 94, 1959.

investigated. Non-treated groups were prepared for control.

It was found that *p*-hydroxy-benzyl-methyl-ether showed a significant promoting effect on the growth of embryos while a normal balance in organ formation was maintained. Its most effective concentration was 0.5 mg per 1000 cc. This promoting effect was observed approximately on the 6th day after treatment (Stage 34), but it did not last longer than 5 days. The difference between treated and non-treated plots seemed to correspond to about two developmental stages, representing a period equivalent to 54 hours at 20°C. The solutions of higher concentrations (0.5% and 0.05%) suppressed the growth of embryos and lower concentration (0.005%) showed no influence.

In order to examine the influence of the new chemical on the differentiation of early embryos, those of *Triturus* were raised in 0.005% solution at 20°C (most effective concentration to promote the growth) and the formation of muscle proteins, actin and myosin, in the developing embryos was investigated. The identification of muscle proteins was carried out by serological technique as already reported³⁾.

In the non-treated control group actin and myosin first became detectable in early embryos 132 and 176 hrs. after fertilization, respectively. But in the treated embryos, actin and myosin were first detected after

Table 1. Muscle protein development in the *Triturus* embryo treated with *p*-hydroxy-benzyl-methyl-ether

Hr. after fertilization	Lowest antigen concentration giving positive results			
	Control		Treated	
	Actin	Myosin	Actin	Myosin
60	—			—
84	—			—
108	—*	—		—*
132	1: 20	—	—	1: 80
156	1: 80	—*	—	1: 160
180	<1: 160	1: 160	—*	<1: 160
204		<1: 160	1: 10	
220		<1: 160	1: 20	
252			1: 40	

* Reaction at high antigen concentration (1:2) was added to confirm the negative results.

3) Ogawa, Y.: Nature, **182**: 1312, 1958.

204 and 132 hrs., respectively. Thus, this chemical suppressed markedly the synthesis of actin and promoted that of myosin, concerning formation rate and amount as shown in Table 1.

The order of formation of both proteins in embryos treated with *p*-hydroxy-benzyl-methyl-ether is therefore, reversed to that found in non-treated material. This result shows a similar tendency to my findings concerning the effect of glucuronolacton⁴⁾, *x*-irradiation⁵⁾ and temperature⁶⁾ on the development of muscle proteins during early embryonal stages. Thus, another new evidence was found of the independence of the formation of actin and myosin.

4) Ogawa, Y.: Ann. Rep. Natl. Inst. Genet., **11**: 40, 1961.

5) Ogawa, Y.: Nature, **186**: 77, 1960.

6) Ogawa, Y.: Nature, **194**: 978, 1962.

27. Comparison of free amino acids among eye-color mutants of *Drosophila melanogaster*

(By Tomotaka SHINODA)

To obtain some information on the differences concerning utilization of amino acids among eye-color mutants of *Drosophila melanogaster*, free amino acids were compared.

Three mutant strains of *Drosophila melanogaster*, *cn*, *bw* and *cn bw*, were cultured in the standard medium at 25°C. By homogenizing whole bodies of five day old flies with cold 0.7 M TCA at 4°C, free amino acids were extracted. The resulting samples were analyzed by paper chromatography followed by paper electrophoresis at pH 6.6. Trinitrobenzene sulfonic acid was employed in the quantitative determination of amino acids. As a control, flies of a wild strain were treated in the same way.

The result shows that (a) in *cn* mutants alanine and glutamine are increased while serine and glycine decreased as compared with the wild strain, (b) in *bw* mutants no significant increase of amino acid was found while glutamic acid, glycine, histidine and proline were decreased, (c) in *cn bw* mutants the values of cystine, glutamine and threonine were increased while proline, glycine and histidine were decreased.

Thus, there might be some differences in the utilization of amino acids between wild and eye-color mutant strains of *Drosophila melanogaster*.

Table 1. Free amino acid content in several mutant strains of *Drosophila melanogaster* (μ mol/100 mg wet weight)

Amino acids	wd	Mutant Strains		
		cn	bw	cnbw
Asp	.44	.34	.51	.36
Glu	1.56	1.26	.95	1.94
Cys/2	.66	.84	.64	1.22
Glun	.74	1.33	.68	2.10
Gly	2.04	1.41	.96	1.51
Ser	1.35	.60	1.38	1.27
Thr	.66	.60	.46	1.20
Ala	2.02	2.85	2.03	2.18
β -Ala	1.38	1.07	.97	1.24
Pro	2.15	1.80	1.57	.68
γ -ABA	.38	.42	.45	.31
Tyr	.02	.08	trace	.04
Try	trace	.04	trace	.02
Phe	.31	.23	.28	.21
Met	.17	.21	.08	.13
Val	.24	.31	.19	.27
Leu/ILeu	.11	.16	.21	.08
His	1.35	1.05	.80	.93
Lys	.60	.69	.50	.64
Arg	1.18	1.03	1.08	.98
X ₁ *	.57	.51	.38	.60
X ₂ *	.64	.42	.30	.39

* Not identified yet

28. *Spectrophotometric determination of trinitrophenyl derivatives of amino acids and peptides resolved by paper electrophoresis and chromatography*

(By Tomotaka SHINODA)

As already reported by the present author, amino acids and peptide contents in solution (0.01 to 1 μ mol) can be determined after quantitative trinitrophenylation with 2, 4, 6-trinitrobenzene sulfonic acid (TNBS). The present paper deals with an application of the TNBS technique

to quantitative determination of minute amounts of amino acids and peptides resolved on paper by electrophoresis and chromatography.

Paper electrophoresis was performed at a constant potential gradient of 20 volt per centimeter, using a medium of pyridine-acetate system (pH 6.65), and *n*-butanol-acetic acid-water system was used as a solvent for paper chromatography. The paper so developed was treated with bufferized TNBS solution, and the optical density of the solution was determined at 340 m μ in N HCl. The molar extinction coefficients of TNP-amino acids were 1.2 to 1.5 $\times 10^4$, while in the case of peptides the corresponding value was 1.05 $\times 10^4$ for an amino group.

The amino acid composition of insulin and fractionated globins was assayed by TNBS method. The result was in good agreement with the theoretical expectation for the insulin with an error of ± 4 per cent.

The present method was also applicable for the quantitative determination of total free amino groups in a polypeptide chain, and for limited modification of proteins under physiological conditions. Further, this method was effective for modifying the bases in RNA and DNA.

29. *Paper electrophoretic heterogeneity of serum albumin*

(By Tomotaka SHINODA)

Homogeneity of serum albumin has been reported by many investigators. However, its heterogeneous behaviour in acidic medium has also been reported. In order to study the heterogeneity of serum albumin in the alkaline range, the author employed paper electrophoresis combined with mediums which contained various concentrations of urea. The pH range tested was from 7.4 to 11.6. The materials subjected were crystallized human serum albumin (HSA) and crystallized bovine serum albumin (BSA). Analytical paper electrophoresis of the materials was carried out at constant potential gradient of six volts per centimeter for three hours at 10°C. The total ionic strength of the media was 0.1 in most cases and the concentration of protein solution to be analyzed was 10-15 mg per milliliter in each case.

Under the conditions employed, both serum albumins were resolved into two components, *F* and *S*, where *F* represents the fast moving component toward the anode side, while *S* is the slow one.

The amounts of the two components varied according to urea concentration or to pH variation. Their "finger-printing" indicated that there are no apparent differences in the tryptic digests between *F* and *S*.

The above results indicate that there are two components in albumins

with different stability or reactivity to some chemical agents such as urea, guanidine etc., although these differences could not be detected even by "finger printing".

B. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF PLANTS

30. *Genome manifestation of wheat in Aegilops cytoplasm*

(By Hitoshi KIHARA)

In order to investigate the effect of alien cytoplasm on genome manifestation, genome complements of $4x$ and $6x$ wheat have been introduced by successive backcrosses into the cytoplasm of *Aegilops caudata* or *Ae. ovata*. The backcrosses have been carried out 14 times for the most advanced materials and only twice in the least advanced ones.

Influence of the alien plasma has been noticed in morphological and physiological wheat characters. It was most remarkable in the floral organs. When their genomes were introduced into *caudata* cytoplasm, two varieties of $6x$ and four of $4x$ wheat showed complete or incomplete pistillody and all became male sterile. Fourteen other varieties or strains became also male sterile but no pistillody occurred. Those were seven varieties of $6x$ wheat, three of $4x$ wheat, three strains of synthesized $6x$ wheat and one Triticale. A $6x$ wheat, *Triticum compactum* var. No. 44, differed from the others in being normally male fertile. In general, genome manifestation of $4x$ wheat in this respect was more influenced by *caudata* cytoplasm than that of $6x$ wheat. This was critically shown in the difference between $4x$ wheat (pistilloid) and the hexaploids (male sterile but not pistilloid) synthesized from the former as one of the parents.

Genomes of four $4x$ wheats were introduced into *ovata* cytoplasm, and all became male sterile. Pistillody did not occur in any strains.

Effect of the alien cytoplasm was also noticed in the increased occurrence of haploid and twin seedlings. The extent of the increase varied greatly with the genotype.

31. *Increased occurrence of haploids and twin seedlings due to an alien cytoplasm*

(By Hitoshi KIHARA and Koichiro TSUNEWAKI)

The authors have been placing by successive backcrosses nuclei of

various wheats, including Triticale, in the cytoplasm of *Aegilops caudata*. During these experiments it has been noticed that the alien cytoplasm increased the frequency of haploids and twin seedlings. For convenience's sake wheat strains with their own cytoplasm are termed "auto-plasmic" and those with *Aegilops* cytoplasm "alloplasmic".

Records on the occurrence of haploids are at present available for two varieties, *Triticum aestivum* ssp. *vulgare* var. *erythrospermum* (*T. vulgare erythr.*) and Taylor's Triticale, which are summarized in Table 1.

Table 1. Occurrence of haploids in auto- and alloplasmic lines of *T. vulgare* var. *erythr.* and Taylor's Triticale

Materials	No. of plants grown (<i>N</i>)	No. of plants checked (<i>n</i>)	No. of haploids (<i>M</i>)	Haploid freq.(%)	
				<i>M/N</i>	<i>M/n</i>
<i>T. vulgare erythr.</i> : autoplasmic	1,633	312	0	0.0	0.0
" : alloplasmic	1,649	402	11	0.7	2.7
Taylor's Triticale: autoplasmic	19	19	0	0.0	0.0
" : alloplasmic	17	17	9	52.9	52.9

The occurrence of twin seedlings in auto- and alloplasmic lines of wheat and Taylor's Triticale was studied in detail this year. The results are summarized in Table 2.

Table 2. Occurrence of twin seedlings in auto- and alloplasmic lines of wheat and Taylor's Triticale

Materials	No. of seedlings		Freq. of twin (%)
	Total	Twin	
Wheat: autoplasmic	13,644	10	0.07
" : alloplasmic	580	5	0.86
Triticale: autoplasmic	54	0	0.00
" : alloplasmic	98	7*	7.14

* One of the seedlings was a triplet.

The present results clearly indicate that the occurrence of both haploid and twin seedlings in wheat and Triticale was increased by the alien cytoplasm introduced from *Ae. caudata*. The extent of the increase varied greatly with the genotype.

32. *Comparative gene analysis of common wheat and its ancestral species, I. Progressive necrosis*

(By Koichiro TSUNEWAKI and Hitoshi KIHARA)

Progressive necrosis in common wheat is controlled by three genes, Ne_1 located in chromosome 5B, Ne_2 in 2A and Ne_3 in 3D. Distribution of those genes in common wheat and its ancestors, Emmer wheat and *Aegilops squarrosa*, was investigated using *Triticum aestivum* Prelude (haploid genotype: $Ne_1ne_2Ne_3$), *T. aestivum* Kharkov ($ne_1Ne_2Ne_3$) and *T. macha subletshchumicum* ($Ne_1Ne_2ne_3$) as test varieties.

In Emmer wheat a majority of varieties were found to have the genotype Ne_1ne_2 , while minor fractions were either ne_1ne_2 or Ne_1Ne_2 .

All strains of *Ae. squarrosa* so far tested had Ne_3 .

In common wheat, most varieties were either $ne_1ne_2Ne_3$ or $ne_1Ne_2Ne_3$, while a small fraction were $Ne_1ne_2Ne_3$. One variety only was found to be $Ne_1ne_3ne_3$ and another only one to be $Ne_1Ne_2ne_3$, both belonging to *T. macha*.

From these results, the genotypes of Emmer wheat, that supplied the AB genomes to common wheat, are assumed to be Ne_1ne_2 or ne_1ne_2 . *T. dicoccoides spontaneo-nigrum*, some forms of *T. dicoccum*, *T. turgidum*, *T. persicum* and *T. orientale*, and many varieties of *T. durum* have these genotypes.

The donor of the D genome to common wheat must have possessed Ne_3 . All strains of *Ae. squarrosa* so far tested had this allele.

The presumable hexaploid progenitor must have had either $Ne_1ne_2Ne_3$ or $ne_1ne_2Ne_3$. In common wheat, some forms of *T. spelta*, *T. sphaerococcum*, *T. compactum* and *T. aestivum* have these genotypes. *T. macha*, that is an exception in possessing the ne_3 allele, is considered to be an isolated species among the hexaploids and seems not to have contributed to the origin of common wheat.

33. *Transmission of the monosomic condition through female gametes in a common wheat, Chinese Spring*

(By Koichiro TSUNEWAKI)

Since 1955 the monosomics of the Chinese Spring series have been crossed as female parents to a number of hexaploid wheats. In each cross a limited number of F_1 plants were cytologically examined and monosomic plants were selected. Up to the present about 150 plants have been examined in each monosomic line.

In order to know the transmission rate of the monosomic condition

Table 1. Frequency of monosomics in the F₁ generation of crosses between 21 monosomic lines* of Chinese Spring (♀) and various 6x wheat varieties (♂)

Homoeologous group	Freq. of monosomics (%)				Within-group heterogeneity (χ^2)
	Genome				
	A	B	D	Average	
1	78.1	64.8	75.8	72.9	7.04**
2	63.4	68.4	66.4	66.1	0.94
3	75.2	77.2	79.7	77.4	0.90
4	66.7	72.7	68.8	69.4	1.33
5	79.7	69.4	70.7	73.3	4.75
6	75.5	55.5	77.9	69.6	23.37***
7	66.7	68.8	69.3	68.3	0.28

* Monosomic lines are indicated by the genome and the homoeologous group, to which the monosomic chromosomes belong. ** Significant at the 5% level. *** Significant at the 1% level.

through the female, accumulated data on the occurrence of monosomics have been analyzed. The results are summarized in Table 1.

The three monosomic lines belonging to homoeologous groups 2, 4, 7, 5 and 3 showed similar frequencies, namely low in the first three groups, intermediate in the fourth, and high in the last group.

Two homoeologues of groups 1 and 6 showed high frequencies of monosomics, while the third member of both, namely 1B and 6B showed very low frequencies. Nucleolus-organizers of common wheat are located on chromosomes 1B and 6B. This fact seems to suggest that gametes or zygotes lacking either of the nucleolar chromosomes are less viable than the other normal ones.

From the present results the following conclusions can be drawn: (1) Transmission rate of the monosomic condition through female gametes is determined mainly by homoeology of the monosomes. (2) Nucleolus-organizing chromosomes show very low transmission of monosomic condition as compared with their homoeologues.

34. *Chromosome number of Gramineae species collected in Pakistan, Afghanistan and Iran*

(By Sadao SAKAMOTO and Mikio MURAMATSU*)

During the Kyoto University Scientific Expedition to the Karakoram

* Curtis Hall, Univ. of Missouri, Columbia, Mo., U.S.A.

Species	Collected locality	No. of strains examined	Chromosome number (2n)
<i>Agropyron intermedium</i> (Host) P. Beauv.?	Iran	1	56
<i>A. trichophorum</i> (Link) Richt	Afghanistan and Iran	2	42
<i>A.</i> sp. probably <i>A. repens</i> (Linn.) P. Beauv. or <i>A. intermedium</i>	Iran	1	56
<i>Alopecurus mysuroides</i> Huds.	Iran	1	14
<i>Brachypodium</i> sp.	Iran	1	18
<i>Bromus brizaeformis</i> Fisch. et Meyer	Iran	1	14
<i>B. Commutatus</i> Schrader	Iran	3	14
<i>B. Danthoniae</i> (Desf.) Trin.	Pakistan, Afghanistan and Iran	7	14
<i>B. macrostachys</i> Desf.	Afghanistan	2	28
<i>B. madritensis</i> Linn.	Iran	1	14
<i>B. racemosus</i> Huds.	Iran	5	14
<i>Cynosurus echinatus</i> Linn.	Iran	1	16
<i>Dactylis glomerata</i> Linn.	Iran	1	28
<i>Elymus dahuricus</i> Turcz.	Afghanistan	1	42
<i>Eremopyrum buonapartis</i> (Spreng.) Nevski var. <i>buonapartis</i>	Pakistan, Afghanistan and Iran	6	14, 28
<i>E. buonapartis</i> (Spreng.) Nevski var. <i>sublanuginosum</i> (Drob.) Melderis	Pakistan and Afghanistan	2	28
<i>E. distans</i> (C. Koch) Nevski	Afghanistan	1	14
<i>E. orientale</i> (Linn.) Jaub. et Spach	Pakistan and Iran	2	28
<i>Festuca elatior</i> Linn.	Iran	2	14, 42
<i>Henrardia persica</i> (Boiss.) C. E. Hubbard var. <i>persica</i>	Iran	3	14
<i>H. persica</i> (Boiss.) C. E. Hubbard var. <i>glaberrima</i> (Hausskn.) C. E. Hubbard	Iran	2	14
<i>Heterantheium piliferum</i> (Banks et Soland.) Hochst.	Afghanistan and Iran	3	14
<i>Koeleria phleoides</i> (Vill.) Pers.	Afghanistan	1	26
<i>Phalaris minor</i> Retz.	Afghanistan	2	28
<i>Polypogon monospeliensis</i> (Linn.) Desf.	Afghanistan	1	28
<i>Taeniatherum asperum</i> Nevski	Afghanistan	1	14
<i>T. crinitum</i> (Schreb.) Nevski	Iran	1	14

and Hindukush in 1955, considerable numbers of wild and cultivated Gramineae species were collected. In this article, the chromosome numbers of those species, excluding *Aegilops*, *Echinochloa*, *Hordeum*, *Lolium*, *Secale* and *Triticum*, are reported.

35. Reciprocal translocations in Emmer wheat

(By Kôzo NISHIKAWA)

The meiotic chromosome pairing was analyzed in ten F_1 hybrids among five Emmer wheat varieties, *T. dicoccoides spontaneo-nigrum*, *T. dicoccum liguliforme*, *T. dicoccum arras* (Khapli), *T. dicoccum* (Vernal) and *T. durum reichenbachii*.

As shown in Table 1 in hybrids of *spontaneo-nigrum* and *liguliforme* with Khapli, Vernal and *reichenbachii*, a quadrivalent which was either a ring of four (55-72%) or a chain of four (45-28%) was found in more than 85% of PMC's. Two quadrivalents which were also either of ring shape (71.8%) or chain shape (28.2%) were observed in 94% of PMC's of the hybrid between *spontaneo-nigrum* and *liguliforme*. The percentages of quadrivalents presented here are much higher than those reported by previous authors for comparable hybrids. In the other F_1 's which were obtained among Khapli, Vernal and *reichenbachii* no quadrivalent was found.

Table 1. Chromosome pairing at first metaphase in ten Emmer wheat hybrids

	<i>spontaneo-nigrum</i>	<i>reichenbachii</i>	Khapli	Vernal	<i>liguliforme</i>
<i>spontaneo-nigrum</i>	—	1 _{IV} +12 _{II}	1 _{IV} +12 _{II}	1 _{IV} +12 _{II}	2 _{IV} +10 _{II}
<i>reichenbachii</i>	—	—	14 _{II}	14 _{II}	1 _{IV} +12 _{II}
Khapli	—	—	—	14 _{II}	1 _{IV} +12 _{II}
Vernal	—	—	—	—	1 _{IV} +12 _{II}
<i>liguliforme</i>	—	—	—	—	—

From these results, it is concluded that the quadrivalents are attributable to reciprocal translocations. Thus, four cultivated varieties of Emmer wheat used here have at least one reciprocal translocation in common which is not found in the variety of wild Emmer, *T. dicoccoides spontaneo-nigrum*, and in addition to this one *liguliforme* carries another reciprocal translocation.

36. *Production of polyploid wheat by nitrous oxide, II. N₂O-treatment during fertilization with reference to culture media*

(By Koichiro TSUNEWAKI)

For the improvement of the practical use of nitrous oxide, the following three points have been investigated in wheat: (1) Effect of N₂O applied in the course of fertilization, (2) production of amphidiploids directly from cross-pollinated florets, and (3) supplementation of culture medium for treated spikes by glucose and penicillin.

N₂O-treatment of pollinated florets did not disturb fertilization, which seemed to proceed rather normally in N₂O. Polysperm fertilization resulting in triploidy was not induced. N₂O appeared to inhibit specifically cell divisions after fertilization, producing polyploid plants. By suitable treatment, all resulting seedlings became polyploid.

Amphidiploids can be directly produced by treating cross-pollinated florets with N₂O. This was demonstrated in the cross, *Triticum aestivum* × *Secale cereale*.

Supplement of glucose as a carbon source to culture media of the N₂O-treated spikes had only a slight effect on seed setting but improved markedly the endosperm development and, consequently, germination. This, in turn, allowed polyploid embryos a better chance to survive. Supplement of penicillin as an antibiotic to the culture media improved neither seed setting, endosperm development nor germination, but an increase in polyploid occurrence was noticed. Its cause must be further investigated.

37. *Photoperiodic responses of Oryza species V¹⁾*

(By Tadao C. KATAYAMA)

Several factors are responsible for different photoperiodic sensitivities among various *Oryza* strains. Among them, the aging effect and the critical day length were considered in the previous reports (Ann. Rep. Nat. Inst. Genet. 11: 48-49, and *Ibid.* 12: 57-58). In this report, the difference of photoperiodic sensitivity was analyzed from the viewpoint of differential heading acceleration of various strains under short day condition. 68 strains belonging to eleven *Oryza* species were used in this investigation.

1) This work was supported by Grant RF 62027 from The Rockefeller Foundation.

Three different methods for expressing the acceleration rate of heading date were devised: (1) by the coefficient of linear regression of growing period on sowing time (denoted by LRC-1), (2) by the coefficient of linear regression of growing period on day length at the time of flower bud formation (LRC-2), and (3) by the angle of the slope of the regression line used in (2) (A-LRC-2).

The three methods were applied to the data obtained from 16 strains and they were all found to agree well with the previous classification of those strains into sensitive and insensitive. The border line between the two groups was found to be 0.5 for both LRC-1 and LRC-2 and 30° for A-LRC-2.

LRC-1 indicates acceleration rate of heading date (in number of days) due to delaying the sowing time by one day, while LRC-2 or A-LRC-2 represents acceleration rate of heading due to shortening the day length by one minute; the latter is biologically more significant than the former. It was found that LRC-2 is less suitable than A-LRC-2 for estimating differences in photoperiodic sensitivity. For these reasons, it was concluded that A-LRC-2 is the best index among the three. With the help of this method, the correct degree of photoperiodic sensitivity was determined for each of the 16 rice strains.

Using index A-LRC-2, the acceleration rate of heading was investigated in another experiment with 54 strains. It was found that 39 strains were sensitive and the rest were insensitive. This classification is in perfect agreement with the previous one based on a different method.

Regardless of the species, strains that have short critical day length showed in general greater sensitivity than those having long critical day length. This finding explains why in low latitudes sensitive strains with short critical day length predominate; their sensitive response to a small change in day length gives them a selective advantage.

38. *Developmental instability in tobacco-plants*

(By Kan-Ichi SAKAI and Yoshiya SHIMAMOTO)

Investigation of developmental instability was conducted on foliar and floral characters in *Nicotiana tabacum* L.

Using eleven varieties, three foliar and three floral characters were recorded as measures of instability in organ development. They are: (1) fluctuation in vein distance, i.e., within-leaf fluctuation in distance between two adjacent veins, (2) indentation index, i.e., degree of non-

parallelism of two adjoining veins measured by the difference between the widest part and the narrowest one divided by the average length of two veins, (3) asymmetry, i.e., the absolute value of difference between left and right half of leaf blade, (4) intra-plant variability of style length, i.e., flower to flower variation in length of styles, (5) intra-plant variability of filament length, i.e., flower to flower variation in average length of five filaments and (6) intra-flower variability of filament length. Measurements in the first character were expressed in terms of coefficient of variability while those in the last three in terms of standard deviations.

Three plants of each variety were taken at random. Measurement of foliar attributes was made on all leaves from the bottom to top of each plant, while for floral attributes, twenty flowers were used which were collected at random from each plant.

By the analysis of variance of data obtained, it was shown that variation among varieties was highly significant for all characters, except intra-plant variability of filament length. Developmental instabilities in the three foliar characters were found to be positively correlated with each other and the same was the case for the three floral characters. It was found, however, that instability in foliar characters and that in floral characters did not appear to be parallel, the repeatability being so low as zero.

In order to find out correlation, if any, between foliar and floral instabilities, instability-indices for foliar and for floral characters were computed by the principle of the discriminant function. The correlation coefficient between the two instability-indices was negative and low with $r = -0.3327$, suggesting that the degree of instability in foliar characters and that in floral characters may be independent from each other.

Of interest is that the examination of varieties in respect of developmental instability in foliar characters showed that high stability was found in two tobacco varieties used for cigar production while our commercial varieties widely grown and highly productive were rather unstable in foliar characters.

39. *A method of estimation of genetic parameters
in forest trees without raising progeny*

(By Kan-Ichi SAKAI and Suekichi HATAKEYAMA)

A method of estimating heritability and genetic correlations of quantitative characters in forest trees has been investigated. The underlying principle is that in hybrid forests, variation between cluster means

consists of one- x^{th} of the genetic variance and one- $x^{b\text{th}}$ of the environmental variance, x being number of trees included in each cluster and b being a function of the variation pattern of the environmental conditions in the forest. b is expected to lie between zero and unity.

$$V_{(\bar{x})} = \frac{G}{x} + \frac{E}{x^b}$$

$V_{(\bar{x})}$ denotes the variance of means of clusters, each including x trees, and G and E stand for genotypic and environmental variances, respectively. The formula can be rewritten as

$$xV_{(\bar{x})} = G + x^{1-b}E = G + x^bE$$

where $B=1-b$. By repeated grouping of adjoining trees into clusters of various sizes, we get a number of simultaneous equations involving three unknown quantities, G , E and B . The trial and error method is adopted to find B_0 value which gives the best fit between observed mean squares and expected ones which are computed from the values of G and E obtained by the least squares method with a given value of B . If G_0 and E_0 with B_0 are obtained, then we can estimate the value of heritability. Genetic and environmental components of covariance are obtained in the same manner by replacing variances with corresponding covariances, and genetic correlations are computed therefrom.

Our investigations of three forests, one being a clone of *Populus euramericana*, the remaining two consisting of seed-propagated *Abies sachalinensis*, gave the following results: G values calculated from the *Populus* clone were so small as regarded as zero, suggesting the validity of the method. Heritability values calculated for five characters in the two *Abies* forests which were genetically not related at all, were reasonably comparable for the same characters though b values were rather different. Genetic correlations could also be computed in the two forests. Thus, this method is considered to be usable for genetic investigation of forest trees. The advantage of this method over others is that it requires neither clonal forests nor families grown from randomly selected mother trees. Accordingly, hardships involved in genetic studies with forest trees due to extremely long time periods required for growth can be surmounted by this method of analysis. The details will appear in *Silvae Genetica* before long.

40. *Cytoplasmic effect on the inheritance of seed weight in Pharbitis Nil*

(By Kan-Ichi SAKAI and Akio SUZUKI)

We have noticed that the weight per seed of morning glory, *Pharbitis*

Nil., is matroclinously inherited. This report deals with the result of a breeding experiment of reciprocal crosses between two varieties U and H , differing from each other with respect to seed size. The weights per seed in miligrams in different generations after the hybridization were as follows:

	$x(H \times U)$	$y(U \times H)$
P	$x_0=26.9$	$y_0=52.6$
F_1	$x_1=35.5$	$y_1=42.6$
F_2	$x_2=42.5$	$y_2=46.6$
F_3	$x_3=45.8$	$y_3=47.4$

Second degree statistics in different generations were calculated and estimates of the coefficient of maternal effect, m , were computed therefrom. The formulas used for this estimation were those given by Chandraratna and Sakai (1960: Heredity 14: 365-373). It was found that m for H was 0.51 while that for U was 0.38. The regression of F_3 line means upon F_2 measurements was also computed. The regression coefficient in $H \times U$ was 0.658 while that in $U \times H$ was 0.438, again suggesting stronger effect of H than of U cytoplasm.

Weight per seed in F_2 plants was found not to be correlated with the number of seeds per capsule, but negatively correlated with the total number of seeds per plant. The correlation coefficients were as follows:

Weight per seed and	$U \times H$	$H \times U$	Average
Number of seed per capsule	-0.17	0.20	0.014
Total number of seed	-0.31**	-0.37**	-0.34

It seems rather reasonable to conclude that seed weight may be affected by cytoplasm, which may bring about increase or decrease in the total number of seeds produced per plant.

41. *Effect of genotypes and cultural conditions on quantitative characters of tobacco varieties*

(By Kan-Ichi SAKAI and Yoshiya SHIMAMOTO)

Seven varieties of tobacco plants, *Nicotiana tabacum* L., were tested for their response to four growing conditions in two successive years, 1961 and 1962. The purpose of this experiment was primarily finding

out the relation between different characters in different genotypes exposed to different growing conditions.

The treatment variables were: (1) spacing between hills, (2) doses of fertilizer application, (3) topping effects and (4) sowing dates. Characters measured were the total yield in grams of fresh leaves, content of alkaloids in dry leaves, number of leaves per plant, length and width of largest fresh leaf, thickness of dry leaves and diameter of stem.

Analysis of variance of data revealed that varietal differences as well as treatment effects were statistically significant for every character. Interaction between treatment and variety was in some cases significant while not in others.

Computation of genotypic correlation showed that alkaloid contents and leaf thickness were positively highly correlated with $r=0.7022\sim 0.9048$ while alkaloid contents and yield were negatively correlated with $r=-0.8043\sim -0.8876$.

Environmental correlations among different characters in different genotypes under different growing conditions were investigated by partial correlations. It was concluded that alkaloid contents were in most cases positively and highly correlated with leaf thickness, suggesting that genetic as well as environmental factors producing thicker leaves may bring about a higher content of alkaloids, in other words, a common factor might be responsible genetically as well as environmentally for the two characters.

42. *Studies on developmental instability of quantitative characters in X-rayed progenies of rice*

(By Kan-Ichi SAKAI and Akio SUZUKI)

One hundred and sixty X₁ lines derived from a single rice variety were grown in comparison with the same number of control lines selected at random from the same variety in two replicated plots with twelve investigated plants in each plot. The present report describes the occurrence of increased developmental instability in various quantitative characters due to X-ray treatment. The characters investigated were length and width of seed, plant height, weight per plant, culm number per plant, number and length of panicles per plant and heading date. It was found that intra-plant variability of seed length and seed width measured in terms of within plant standard deviation increased significantly in the X-rayed lines. The same was found for intra-plot variability of other quantitative characters in X-rayed lines. The between-line

genetic variability in these was significantly higher than in control lines, suggesting polygenic mutations occurring in all characters. There was a general impression that with respect to polygenic mutations, X-ray treatment caused them to occur in minus direction, while developmental instability was mostly directed toward increase by X-ray treatment. In addition, mutations in developmental instability were not necessarily associated with quantitative ones of the given character.

From the result of this experiment, we conclude that there should be genes or genic systems responsible for developmental stability which can mutate by the effect of X-rays resulting in deficient regulation of organ development or "buffering against developmental noise," according to Waddington's terminology (1957).

43. *Intra-clonal variation of pathogenicity in Piricularia oryzae*

(By Iwasaburo GOTO and Kan-Ichi SAKAI)

Pathogenicity of rice blast fungus, *Piricularia oryzae*, has been known to be not a stable character, but variable according to the culture conditions of the fungus. It is the purpose of this report to describe results of our observations concerning seemingly intra-clonal pathogenicity segregation in the fungus. The method of investigation consisted of isolating a certain number of subclones or sub-subclones from clonal races and examining intra- racially their pathogenicity each on five or more plants of a rice variety. The number of initial clonal races was six, each started from single spore isolates. They were different from each other with respect to pathogenic reaction of the host plants. From each clone were thirteen subclones isolated in the first experiment by plate-culturing and they were tested for their pathogenicity under microscope by sheath inoculation. The seedlings to be inoculated were grown under as uniform conditions as possible in zinc cases kept in the greenhouse. Analysis of variance of the data obtained showed that in four of the six clonal races, variation among subclones within the same clone was statistically significant. One of the four clonal races, named K-373, which showed significant variation among subclones was selected and again tested for intra-clonal variability with 15 subclones by three replications. The result of experiment was quite in accord with the first test in that the between-subclone variation was statistically highly significant.

Three subclones of K-373 were again divided into 7 or 10 sub-subclones

each and inter-sub-subclone variation was tested. It was then found that the inter-sub-subclone variation was highly significant, the variation being so great that variation among parental subclones tested against it failed to reach the level of significance. The examination of correlation between pathogenicity of subclones and that of their sub-subclones is left to future investigation.

From the results of this experiment, we may state that a segregation seems to occur between vegetative isolates from a clone. For the present, we are unable to explain the phenomenon, but it is doubtless that it deserves further study not only from the standpoint of plant pathology but also genetics.

44. *Studies on blast-disease susceptibility of wild and cultivated groups of rice*

(By Iwasaburo GOTO and Ken-Ichi SAKAI)

Two cultivated species of rice, *Oryza sativa*, including *indica* and *japonica* groups, and *O. glaberrima*, are thought to have developed from *O. perennis*-*O. spontanea* and *O. breviligulata*, respectively. This paper deals with a comparative study of those species with respect to susceptibility to the blast-disease fungus, *Piricularia oryzae*. The strain of fungus used is 53-33, kindly supplied by the National Institute of Agricultural Sciences, Tokyo. This strain has been isolated from a population collected in Japan, and accordingly, it seems to be more adapted to Japanese varieties of rice than to other forms. In descriptions to follow, Japanese varieties will be dealt as a control, and comparison will be made among the other groups of species. The evaluation of susceptibility of rice plants was made by the method of leaf-sheath inoculation.

Comparison among 90 varieties of *O. sativa* and *O. glaberrima* showed that *O. sativa indica* was less susceptible than *O. sativa japonica* and *O. glaberrima*. Among different local groups of *O. sativa indica*, varieties from South America tended to be more susceptible than those collected from Asiatic countries.

Comparison between cultivated and wild forms revealed no substantial difference in general. A distinct difference, however, was observed between two species groups, *glaberrima-breviligulata* and *sativa-perennis*, the former being apparently more susceptible than the latter.

Genetic variability between varieties or strains within species or subspecies was not much different from that observed between species,

except for *O. sativa indica* which showed the least variability. This means that so far as susceptibility to the present fungus race is concerned, varieties belonging to *indica*-group were rather uniform though they were collected from different continents.

Three populations of wild rice of *perennis-spontanea* group were investigated for disease susceptibility. They differed from each other, but variability in each population was quite apparent. The effect of individual selection was investigated. It was then found that variation contained in each population was heritable, the heritability being so high as 0.7.

By testing individuals of a wild population of *O. perennis* for three races of *Piricularia oryzae*, it was found that a wild population involved different genotypes with respect to reaction against different races. The interaction between genotypes and pathogenic races was statistically significant.

Degree of inherent susceptibility, that of environment-respondent susceptibility and the degree of interaction between the two were investigated (For details see the preceding issue of this Report, No. 12, 1962). Degree of inherent susceptibility (D_c) was very low in *japonica*-group. Among other groups, *indica*-group was the highest, *breviligulata* and *glaberrima* being intermediate and *perennis-spontanea* group was the lowest. The same tendency was again found for the degree of environment-respondent susceptibility (D_b). Degree of interaction between the two was positive in *japonica* group, but negative in all other groups.

Conclusions drawn from this study are: (1) *O. glaberrima* and its wild ancestor species are more susceptible on the average than *O. sativa* and its ancestral wild species. (2) Wild populations of *O. perennis-spontanea* involved a good deal of genetic variability. (3) They also involved different genotypes with respect to reaction to different races of *Piricularia oryzae*. (4) Variation in the inherent susceptibility was small in Japanese varieties, while *indica* varieties involved the highest variability. (5) *Indica* varieties were again variable with respect to environment-respondent susceptibility.

45. *Natural selection experiment in populations of wild rice, Oryza perennis*

(By Hiko-Ichi OKA and Wen-Tsai CHANG)

This experiment was carried out at Taichung, Taiwan, with three populations of *Oryza perennis*, i. e., a *spontanea* type (India), a *perennis*

type (India), and a hybrid-swarm type (Taiwan) population. They were grown in bulk for three generations in an experimental field, and were compared with the populations raised from the original seeds, regarding the degree of grain shedding, grain weight, seed dormancy, and other characters. The results showed that the population genotype of *perennis* type, as well as that of the hybrid swarm, had changed toward cultivated rice in three generations, but that of *spontanea* type remained unchanged. This indicates that populations of wild rice readily respond to natural selection under cultivation, if they contain a large enough amount of genetic variation.

46. *Some considerations on the evolutionary dynamics of cultivated rice*

(By Kokichi HINATA and Hiko-Ichi OKA)

This study was attempted with the view to looking into the evolutionary dynamics of cultivated rice. A review of the previous theoretical studies on partly self-pollinated plant populations was given. Further, the writers' computations were reported dealing with the effects of seed dormancy and vegetative propagation on population structure, and the change in frequency of a gene controlling selfing probability. It was pointed out that the breeding system of the *perennis* type of wild rice, which is partly cross-pollinated, capable of propagating vegetatively and has long seed dormancy, would be quite suitable for accumulating genetic variation in its populations. It was also suggested that selfing genes can increase by themselves unless counteracted by another force favoring the heterozygotes. Therefore, if the habitat assumes conditions of cultivated fields and homozygous plants have relatively an advantage, the populations will tend toward selfing. (Publ. in Jap. Jour. Genet. 37: 329-342, 1962).

47. *F₂ segregation ratios in a hybrid between tetraploid Oryza sativa and glaberrima*

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

Observations of the F₁ hybrid between induced tetraploid strains of the two species were reported by Hinata and Oka (Ann. Rep. 12: 1962). This year, about 500 F₂ plants were observed with regard to endosperm character, phenol reaction, pollen fertility and other traits. Few recessive homozygotes were found, their frequency being lower than 1/36

expected from random chromosome pairing. The frequency of simplex heterozygotes for the glutinous gene, estimated from the frequency of glutinous pollen grains in each plant, was also lower than expected. There might be a slight tendency to preferential pairing of chromosomes derived from the same parent. Pollen fertility of most of the plants ranged between 35% and 70%.

48. *Comparison of evolution modes of cultivated forms derived from two wild rice species, Oryza breviligulata and O. perennis*

(By Hiroko MORISHIMA, Kokichi HINATA and Hiko-Ichi OKA)

Two cultivated rice species, *Oryza sativa* L. and *O. glaberrima* Steud., seem to have independently arisen from their respective wild progenitors, *O. perennis* Moench (Asian *perennis* type) and *O. breviligulata* Chev. and Roehr., for the following reasons: First, the two species series, called *Sativa* (*sativa* and *perennis*) and *Glaberrima* (*glaberrima* and *breviligulata*), are separated by a pronounced F₁ sterility barrier, and secondly, in certain particular regions continuous arrays of intergrades are found connecting *perennis* with *sativa* and *breviligulata* with *glaberrima*. The patterns of intra-specific variations and population structure of *O. breviligulata* were compared with those of the Asian forms of *O. perennis*. The results suggest that between the two species series certain similarities and differences may be pointed out regarding the mode of evolution of cultivated forms, which seem to be partly attributable to different breeding systems of the wild progenitors. (Publ. in *Evolution* 17: 170-181)

49. *Cytogenetic studies in the genus nicotiana XV.*

Reduction divisions in three interspecific hybrids and one amphidiploid

(By Yô TAKENAKA)

The reduction divisions in PMC's were studied in three hybrids, *N. paniculata* × *N. benavidesii*, *N. debneyi* × *N. glutinosa* and *N. tabacum* × *N. forgetiana*, and one amphidiploid of *N. rustica* × *N. tabacum* produced by colchicine treatment.

1) F₁ of *N. paniculata* ($n=12$) × *N. benavidesii* ($n=12$).

At MI of PMC's of this hybrid, twelve bivalents were usually counted whose partners were of same size and shape and very seldom eleven bivalents and two univalents were found. At 2nd division, a few ir-

regularities were very rarely observed such as a chromosome bridge and one to two chromosomes or a fragment strayed in the cytoplasm.

In the same hybrid, Goodspeed (1954) found that twelve bivalents were the norm and univalents, trivalents and quadrivalents occurred very rarely. Although there are some differences between Goodspeed's observations and mine, considering the findings mentioned above, the two species are closely related but it is thought that the genomes of *N. paniculata* and of *N. benavidesii* are somewhat differentiated by inversions, translocations and other irregularities.

2) F_1 of *N. debneyi* ($n=24$) \times *N. glutinosa* ($n=12$).

So far as I know, this hybrid has been previously reported. The nuclear plate in MI of PMC's of this hybrid consisted of bivalents and many univalents and it was somewhat difficult to count them clearly. But the number of univalents did not reach generally to one half of the total chromosome number of 36 and many bivalents were observed. Therefore it is assumed that *N. debneyi* is somewhat related to *N. glutinosa* on one hand but on the other hand intra-genomic bivalents may be formed in the former, since it is probably of amphidiploid origin.

3) F_1 of *N. tabacum* ($n=24$) \times *N. forgetiana* ($n=9$).

So far as I know, no report on this hybrid had been published. At MI of PMC's of this plant, the bivalent range was from 1 to 8, with the mode at 3. The frequency of PMC's with 4 and 5 bivalents followed that of PMC's with 3 bivalents. PMC's with 2, 6, and 7 bivalents were occasionally found but those with 1 and 8 bivalents were very rare. Some of them may be due to autosyndetic affinities between the two subgenomes of *N. tabacum* which is of amphidiploid origin and others may be caused by allosyndetic affinities between the genomes of the parents. Accordingly *N. tabacum* is assumed to be slightly related to *N. forgetiana*.

4) Amphidiploid hybrid of *N. rustica* ($n=24$) \times *N. tabacum* ($n=24$).

This amphidiploid was produced by colchicine treatment of F_1 of *N. rustica* \times *N. tabacum* with the purpose of breeding a new strain containing the genomes of *N. tabacum* and *N. rustica*. But this plant was highly sterile and the offspring showed segregation for various characters.

At MI of PMC's of this amphidiploid, the number of univalents ranged from 0 to 12, with the mode at 4. The frequency of PMC's with 2, 3, 5, 6, 7, 8 and 9 univalents followed that of PMC's with 4 univalents, and PMC's with 0, 1, 10, 11 and 12 univalents were rare.

F_1 of *N. tabacum* \times *N. rustica* showed the bivalent range from 0 to 10

with the mode at 4 (Takenaka, 1955). Accordingly, it is assumed that in the amphidiploid allosyndetic affinity between the genomes of the parents leads to conjugation.

50. *Tumorous hybrids in nicotiana*

(By Yô TAKENAKA and Yoshiaki YONEDA)

For researches on phyletic relationships of *Nicotiana* species and introduction of useful genes into tobacco breeding, we have produced many interspecific hybrids from 1950 to 1962. Among them the following ten tumorous hybrids were found, four of which are new (Asterisk), as far as we know.

- (1) F₁ *N. glauca* × *N. langsdorffii*
- (2) F₁ *N. glauca* × *N. longiflora*
- (3) F₁ *N. glauca* × *N. plumbaginifolia*
- (4) F₁ *N. paniculata* × *N. langsdorffii*
- (5) F₁ *N. rustica* × *N. cavanillesii*
- (6) F₁ *N. suaveolens* × *N. langsdorffii*
- (7) *F₁ *N. suaveolens* × *N. plumbaginifolia*
- (8) *F₁ *N. gossei* × *N. alata*
- (9) *F₁ *N. gossei* × *N. longiflora*
- (10) *F₁ 4x *N. tabacum* × *N. alata*

Kostoff (1930) first observed tumorous hybrids in *Nicotiana* and reported sixteen of them (1941-1943). Since his observations, some researchers found new non-parasitic tumors as well as those observed by him in seven interspecific *Nicotiana* hybrids (Whitaker 1930, Brieger and Roster 1942, Kehr 1950, Hilter and Izard 1951).

Näf (1958) also found four tumorous hybrids in *Nicotiana* and summarized all such hybrids in a table from the results mentioned above. He advocated the establishment of one plus- and one minus-group among *Nicotiana* species, indicating that specific hybrids between the two groups produce non-parasitic tumors, whereas the hybrids within each group do not.

Although his classification is not yet completely confirmed, it is very interesting. According to Näf, *N. alata* and *N. longiflora* belong to the plus-group and therefore *N. gossei* must be placed in the minus-group, since we newly obtained the following two tumorous hybrids, F₁ *N. gossei* × *N. alata* and F₁ *N. gossei* × *N. longiflora*. In fact, *N. gossei* is closely related to *N. suaveolens* which is a minus-species. Although Näf observed no tumor in a hybrid between *N. suaveolens* (minus-species)

and *N. plumbaginifolia* (plus-species), the same hybrid produced by ourselves showed marked tumors on stems and roots.

Kostoff (1930) found non-parasitic tumors in F_1 of *N. tabacum* \times *N. alata*, and we also found some tumors in F_1 of $4x$ *N. tabacum* \times *N. alata*.

We succeeded in obtaining *in vitro* cultures of tumorous tissue from stem of F_1 *N. glauca* \times *N. plumbaginifolia* and preliminary studies have been started with this material.

The tissues grew slowly and were rather compact in texture when they were cultured on White or White+NAA agar media, while they grew rapidly and had soft and crumbling texture on White+yeast extract, White+yeast extract+2,4-D and White+coconut milk+2,4-D agar media.

Differentiations of shoot-like structures were observed mainly on White or White+NAA agar media in the dark at 25°C after second to third transfers from isolation. On rare occasions, some roots were produced directly from the cultured mass on semi-solid medium containing NAA. This phenomenon is now under study. Concerning this problem Skoog (1944) reported that roots only arose secondarily on differentiated stems of the cultured tumor tissue of *N. glauca* \times *N. langsdorffii*. In light, the cultured tissues became green to pale green and showed no differentiation on all media tested.

51. *Effects of extracts from Aralia elata Seem.
and Artemisia vulgaris L. var. indica Maxim. on cell division
in living plant tissue and growth of rat tumor.*

(By Yō TAKENAKA and Yoshito OGAWA)

The extracts of *Aralia elata* Seem. and *Artemisia vulgaris* L. var. *indica* Maxim. were investigated regarding their effects on cell divisions in root tips of *Allium scorodoprasum* var. *viviparum* and on the growth of Yoshida sarcoma.

Leaves of *Aralia elata* were treated with methanol and stems and leaves of *Artemisia vulgaris* were extracted with ethy-ether at room temperature. The yields of methanol and ethy-ether fractions of both plants were 7.6% and 4.8% of dried original tissue weight, respectively.

Water solutions of various concentrations were prepared from the above two extracts and living roots of *A. scorodoprasum* var. *viviparum* were placed in the solutions for 4 to 24 hours. Living root-tips were treated according to the hydrochloric-acetic acid-orcein method and observed under the microscope. Water solutions of *Aralia elata* extract

caused faint radiomimetic phenomena after 4 hour treatment with 4% solution; they were remarkable after 4 hour treatment with 8% solution. Using the extract of *Artemisia vulgaris* var. *indica*, remarkable radiomimetic phenomena were found in the dividing cells after only 4 hour treatment with 0.5% water solution.

24 hrs. after transplantation of the ascites tumor, both fractions were injected subcutaneously at the axilla (0.5 g per 100 g body weight of rat) and their influence on the sarcoma was observed in respect to toxicity, systemic symptoms (lack of appetite, ascites, icterus and metastasis) and prolongation of the host's life.

The extract of *Aralia elata* produced some inflammation at the site of injection and some collapse of the regional tissue was found in the first 7 days after injection. But the group treated with this extract showed a remarkable recovery in systemic symptoms 5 days after injection and 50% of the treated animals remained alive for 14 days, though the non-treated control group died out within 10 days after transplantation.

The extract of *Artemisia vulgaris* showed no remarkable toxicity except for a slight inflammation at the injected site observed only in the first three days after injection, but no effect was found on the prolongation of the life of rats bearing the sarcoma.

52. *Experiments with carnations*

(By F.A. LILIENFELD and K. SUZUKI)

1. A case of albo-variegation

In 1958 a commercial seed sample of *Dianthus chinensis* L. was sown as No. 51. Fifteen plants raised had fairly uniform appearance and one was selected for further propagation. Among its offspring (No. 72) a considerable number of plants, namely 14 of 63, were in varying degree albo-variegated. Variegation started with the first leaf whorl (cotyledons were green) and continued upward through several whorls with increasing participation of green, until apparently uniform green pigmentation was reached. Only exceptionally a plant remained in variegated condition throughout the whole development. Later experience showed that such plants sometimes formed green, colorless and variegated branches.

Offspring of a green, a medium variegated and 2 highly variegated plants of No. 72 are given in the table. As the table shows, even an apparently green plant like 72.16 was throwing off variegated plants,

Table

Material	Offspring of No. 72, selfing and intra-cross				
No. 72 (commercial sample) 49 green, 14 var.	72.16 green, self	72.2 var., self	72.8 high var., self	76B high var., self	72.16×72.8 F ₁
	No. 77 18 green, 5 var.	No. 75 1 green, 20 var.	No. 76 0 green, 20 var.	No. 84 0 green, 15 high var.	No. 78 10 green, 7 var.
	Other crosses				
No. 80 (another commercial sample) green <i>D. superbus</i> green	76B(var.)×80.1 green No. 85 (F ₁) all green	80.1×76B No. 86 (F ₁) all green	76B×80.5 No. 81 (F ₁) 2 plts green No. 88 (F ₂) 31 green, 9 var.	80.5×76B No. 87 (F ₁) all green	72.1(var.)× <i>D. sup.</i> No. 79 (F ₁) all green No. 83 (F ₂) 12 green, 5 var., 3 albino

while the 2 throughout variegated gave only variegated offspring (72.8 and 76B). The green 72.16 was crossed by high variegated 72.8; the cross yielded green and variegated, more of the latter than were obtained from 72.16 selfed. When branches of a highly variegated plant like 76B showed different degrees in variegation, the differences were reflected in the progeny (colorless branches gave no seeds).

The above observations indicated the presence of a highly mutable gene in the whole original sample. This was confirmed by further crosses with *D. chinensis* from another source and *D. superbus* L. As may be seen from the table, the F_1 's obtained from both directions of cross 76B (high variegated) \times *chinensis* (80.1 and 80.5) were entirely green. In F_2 31 green and 9 variegated were obtained. Likewise, 72.1 (variegated) \times *D. superbus* gave a green F_1 and in F_2 variegated plants were segregated. The investigation will be continued.

2. Prickly leaf

In one of the further generations from crossing experiments with "ise-nadeshiko" (*D. chinensis* L. var. *laciniatus* Koern.), a plant was found with many scattered projections on the upper leaf side. They resembled of prickles or spines except for the blunt tips. These emergences were due to local hyperplasia of parenchyma and palisade tissues. A fully developed prickle consisted of a green base and a colorless blunt tip from which sometimes secondary small processes were arising. Inside, a well developed system of spiral tracheary elements was found. The whole plant had stunted habit and dark green leaves. Its seeds did not germinate. The plant was crossed by a typical *chinensis*, of very different growth habit, having long stems and light green leaves. Only two seeds germinated and the two F_1 plants showed predominance of the *chinensis* parent. They had no prickles but only once in one of them a very weak indication of the abnormality was recorded. This F_1 plant is still alive and all its leaves are normal.

In F_2 (No. 92) 24 plants were obtained; their growth habit was in general like that of F_1 . No plant approximating the prickly parent was found in the small F_2 . Since the first observations revealed in some plants only very weak indications of the abnormality, records were taken 10 times in succession. The plants which continuously, or almost so, showed sure, though weak, indications of prickles were classified as corresponding to prickly homozygotes. Six such plants were found and five more were alternately recorded as with or without indication of prickles. On this assumption the abnormality was simply segregating (18 normal+doubtful: 6 with indications). The fact that in F_2 the abnormality was only weakly expressed may be attributed to the entire-

ly different genetic background in respect to growth habit introduced by the cross.

53. *Comparison of flower color inheritance in pansy and morning glory*

(By Toru ENDO)

It has been well-known that pansy and morning glory exhibit an abundant variation in flower colors, the former varying in bright cyanic and acyanic colors and the latter in dull cyanic ones. The historical survey of Wittrock (1896) undoubtedly shows that most of flower color variations in pansy resulted from hybridization among some *Viola* species, mainly *V. lutea* and *V. tricolor*, in the early days of nineteenth century. On the other hand, the flower color variations in the Japanese morning glory, *Pharbitis nil*, have originated from a prototype with blue flowers through natural mutations and their combinations from seventeenth to the present century, while hybridization with other *Pharbitis* species was not tried at all until recently.

It was observed in twenty-one combinations among seven pansy varieties (Endo 1959 and 1961) that there were complicated F_1 and F_2 segregations in general, especially continuous quantitative variations in the amount of pigment in most of the combinations between cyanic and acyanic varieties. This observation suggests that each pansy variety examined has a unique genetic background, derived from original hybridization, contributing to flower color. It is noteworthy that there are among the varieties, in quality and/or quantity, mutually additive relations among three kinds of pigments, anthocyanins, flavonols and xanthophylls (Endo 1954).

In contrast to this, it has been established by Imai (1931), Hagiwara (1931) and others that inheritance of flower color in morning glory is always subjected to simple Mendelian ratio among numerous combinations. The results of gene-analysis obtained by them are summarized in Fig. 1. It seems that there is no detectable differentiation of the genetic background so far as flower color is concerned. However, there were found considerable effects of some mutant genes on glycosylation of anthocyanins. For example, the genotype, *i dy pr mg* (brownish red), produces at least seven peonidin-glycosides, most of which are glycosylated to a lower level than those produced by *i + pr mg* (purplish red), in which eight peonidin-glycosides are produced.

but *chlorina* stripes from somatic mutation appeared in some tillers of 201 among 1,809 plants. The mutation appeared on leaf and stem as longitudinal stripes. Only one plant had striped and pure *chlorina* tillers, together with green tillers within the same individual. In some cases, width of leaf and *chlorina* stripes was measured in the middle of the second, third or following leaves. Some tillers had only one stripe in each leaf, and two or more stripes were observed in other cases. The area of stripes shows large variation.

The *chlorina* stripes mostly appeared in one tiller of each individual; two or more tillers with *chlorina* stripes appeared in some cases in successive offshoots from striped primary tillers. One tiller only of one individual had *chlorina* stripes in 118 plants; two or more striped tillers within the same individual were found in 83 plants and the maximum number of striped tillers was 12.

From the results, the number of initial cells which contributed to tillering was estimated to be about 100 in the embryonic stage.

55. *Effect of nitrous oxide on germinating seeds of einkorn wheat*

(By Tarô FUJII)

60 seeds for each lot were soaked in water at room temperature. Germinating seeds were picked up 24 and 48 hours after the start of the experiment and were treated with nitrous oxide for 8, 16 and 24 hours, each treatment at the pressure of 3 and 6 atmospheres. Nitrous oxide treatment apparently does not affect either germination or survival rates and seedling length or the number of offshoots. Induction of polyploidy was examined by the observation of chromosomes at MI with smear technique. Each spike per most of the surviving plants was checked. All spike, except two, had seven bivalents like those of the control lots. Thus tetraploid or any kinds of chromosome irregularities were not obtained. One plant in each of the lots subjected to 6 atm. for 24 hr. and 3 atm. for 8 hr. was haploid but this was not due to the effect of nitrous oxide because sporadical appearance of haploid is known in *T. monococcum*. On the basis of cytological observation the upper confidence limit on the proportion of induced polyploids at the 95% level was calculated as 0.8%. Nitrous oxide did not produce polyploids when it was applied to germinating seeds for up to 24 hours at pressures of 3 and 6 atm.

56. *Cytological observations on chemically induced male sterility in sugar beets*

(By Tomoko OHTA¹⁾ and Seiji MATSUMURA)

According to F. WIT's method, 0.4% solution of Na-2,3-dichloroisobutyrate was sprayed on the leaves of sugar beet variety GW 359, when the beets began to head and had young inflorescences with small flower buds. Black spots were observed on the leaves and young tissues around the growing point began to etiolate 5 days after the treatment. Degeneration of pollen grains began after a week and completely sterile pollen was found after 2~4 weeks. Later pollen fertility was slightly recovered, while on the female side no degeneration occurred. Therefore, this method should be useful in sugar beet breeding.

The induced male sterility was investigated cytologically and histologically. In the treated inflorescence young anthers had a normal appearance before meiosis, but later the tapetal cells developed abnormally and showed hypertrophy, as observed in the case of genetical male sterility. They stained very deeply and thickened and remained unchanged. The pollen grains, not being able to absorb nutrients from the tapetum, finally degenerated. In this case the abnormal development of tapetal cells was most striking. Thus, the relation between pollen grains and the tapetal tissue is thought to be the main cause of male sterility.

1) Kihara Institute for Biological Research, Yokohama.

C. MATHEMATICAL GENETICS

57. *On the probability of joint fixation of mutant genes*

(By Motoo KIMURA)

Let us consider n independent loci each with a pair of alleles, conveniently called the normal and the mutant genes and denote by $p^{(i)}$ the initial frequency of the mutant gene in the i^{th} locus ($i=1, 2, \dots, n$). Let $u(p^{(1)}, p^{(2)}, \dots, p^{(n)}; t)$ be the probability that all the n mutant genes become fixed in the population by the t^{th} generation given that their initial frequencies at $t=0$ are $p^{(1)}, p^{(2)}, \dots, p^{(n)}$ respectively. As in the single locus case, we will be mainly concerned with the ultimate probability of joint fixation defined by

$$u(p^{(1)}, p^{(2)}, \dots, p^{(n)}) = \lim_{t \rightarrow \infty} u(p^{(1)}, p^{(2)}, \dots, p^{(n)}; t).$$

Under the assumption of random mating and constant (but not necessarily equal) fitness of individual genotypes, it is possible to show that

$$(1) \quad u(p^{(1)}, p^{(2)}, \dots, p^{(n)}) = \frac{\int_0^{p^{(1)}} \int_0^{p^{(2)}} \dots \int_0^{p^{(n)}} e^{-2N\bar{a}} dx^{(1)} dx^{(2)} \dots dx^{(n)}}{\int_0^1 \int_0^1 \dots \int_0^1 e^{-2N\bar{a}} dx^{(1)} dx^{(2)} \dots dx^{(n)}}$$

where $\bar{a} = \bar{a}(x^{(1)}, x^{(2)}, \dots, x^{(n)})$ is the average fitness of the population measured in Malthusian parameters and is a function of gene frequencies $(x^{(1)}, \dots, x^{(n)})$, in which $x^{(i)}$ ($i=1, \dots, n$) stands for the frequency of the mutant gene in the i^{th} locus in the population. If selective values are used to measure the fitness of individual genotypes (discrete model of generation time), $\log \bar{w}$ may be substituted for \bar{a} in the above formula, where w is the relative selective value in the sense used by Wright; w coincides with relative viability if no fertility differences are involved. The above formula (1) can be obtained as the steady state solution of the Kolmogorov backward equation for n variables by assuming random mating and constant fitness of individual genotypes. The formula enables us to study the effect of epistasis in fitness on the chance of joint fixation of mutant genes.

D. GENETICS AND BIOCHEMISTRY OF MICROORGANISMS

58. *Phase specific regulator of flagellin genes (H_1 and H_2) in Salmonella¹⁾*

(By Tetsuo IINO)

H_1 and H_2 are the structure genes of Salmonella flagellar protein (flagellin), in antigenic phase-1 and phase-2, respectively. The presence of an H_1 -specific regulator, designated ah_1 , was reported previously (Iino, Annual Report, No. 8, 1958). The functional relationships between ah_1 and H_1 were studied in detail. The heterogenotes occurring by transduction of an ah_1 - H_1 segment from a diphasic strain to an ah_1^- monophasic-2 strain produce trails on the semisolid plate containing the anti-

1) This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (E-2872), Public Health Service, U.S.A.

sera against phase-2 antigens of both donor and recipient. But they do not produce trails when the antiserum against phase-1 antigen of the donor together with the anti-phase-2 sera is added to the medium. These results indicate that ah_1^+ is dominant to ah_1^- , but the function of ah_1^+ is effective to H_1 only when it is in cis-position with it. It was further shown that ah_1^- mutants do not produce phase-1 flagellar protein at all even in phase-1. H_1 and ah_1 may behave as two component parts of a genetic transcription unit analogous to the operator-structure gene system in the *lac*-region of *Escherichia coli* (Jacob and Monod, 1961).

Transductional analysis of monophasic-1 mutants of *S. typhimurium* and *S. paratyphi B* disclosed a phase-2 specific regulator ah_2 . The function of ah_2 to H_2 is in parallel with that of ah_1 to H_1 . Diphasic strains carry an allele, ah_2^+ , which switches on the production of phase-2 flagellin. A mutant allele ah_2^- switches off the production. Consequently, the mutant carrying ah_2^- is stable in phase-1. Ah_2 is adjacent to H_2 on the chromosome.

59. Further studies on non-flagellated mutants of *Salmonella*¹⁾

(By Tetsuo IINO and Masatoshi ENOMOTO)

The *fla*-genes of *Salmonella* are antigenic phase non-specific regulators of flagella production. They are complementary with the structure genes, H_1 and H_2 , in trans-position. A *fla*⁻ mutation in any one of these genes causes the loss of the ability to produce flagella in both phase-1 and phase-2. The wild type allele, *fla*⁺, is dominant to all of the known *fla*⁻ alleles. The genetic complementation test reported in the Annual Report No. 8 (Iino, 1958) was extended to forty three *fla*⁻ mutants obtained from *S. typhimurium* TM2 and *S. abortus-equi* SL23. They are classified into twelve complementation units. At least three of them are closely linked to H_1 .

Production of 'flagellin-CRM', which immunologically cross-reacts with wild type flagellin, was examined on twenty five stable mutants among them. They included both deletion types and point mutants. The procedure of immunological tests was the same as that described in the Annual Report No. 12 (Iino and Haruna, 1961). Among the mutants examined, one strain, SJ28, of *S. abortus-equi* produced flagellin-CRM which is immunologically indistinguishable from wild strain

1) This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (E-2872), Public Health Service, U.S.A.

flagellin (Annual Report No. 12). The remaining twenty four strains did not produce flagellin-CRM at all.

From these results, it is inferred that the *fla*-gene mutated in SJ28 is responsible for the formation of flagella from their component protein molecules. The remaining *fla*-genes may regulate the synthesis of flagellin, presumably either producing an internal inducer of flagellin or controlling the production of ribosomes specific for flagellin synthesis.

60. *Effect of phenol on flagellation of Salmonella cells*¹⁾

(By Tetsuo INO and Michiko MITANI²⁾)

The cells of *Salmonella typhimurium* TM2 grown in the broth containing 0.13% phenol lose the ability to synthesize flagella. They can divide, and the number of flagella per bacterium decreases as the cells multiply, although the preformed flagella are not destroyed at that concentration. At the concentration of 0.2% not only the *de novo* synthesis of flagella is stopped but also the preformed flagella are paralyzed, and the cells become non-motile. The paralyzed cells recover motility immediately after they are transferred to phenol-free broth: the paralysis is reversible. Bacterio-static effect appears at the concentration of phenol higher than 0.3% in the broth. The non-flagellated H-agglutination negative cells produce protein which is immunologically indistinguishable from the flagellar protein of the bacteria.

Regeneration of flagella in cells deflagellated beforehand by phenol treatment occurs in the standard regeneration medium, which contains a 0.4% amino acid mixture and 0.2% glucose in phosphate buffer, during 3 hours at 37°C, whereas a uracil-less mutant of TM2 regenerates flagella only when uracil is supplemented to the standard regeneration medium under the same experimental condition. Cells deflagellated by sonic vibration regenerate flagella in the standard medium without uracil in uracil-less mutants as well as in prototrophic strains.

These results may be explained as follows: the multiplication of the normal flagella forming apparatus in a bacterial cell is inhibited by phenol, consequently the flagella proteins synthesized by the cell cannot be organized into a flagellum under the presence of phenol but are accumulated in the cell. For the recovery of the flagella forming apparatus, *de novo* synthesis of RNA seems to be a prerequisite.

1) This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (E-2872), Public Health Service, U.S.A.

2) Department of Biology, Faculty of Science, Tokyo Metropolitan University.

61. *Grouping of paralyzed mutants in Salmonella*¹⁾

(By Masatoshi ENOMOTO)

Sixty paralyzed mutants of independent origin were isolated from *Salmonella typhimurium*, TM2, by the method reported previously (Enomoto and Iino, Annual Report No. 12, 1961). Transductions of the motility genes (*mot*) were carried out with phage P22 in all pairwise combinations of the mutants. Production of swarm or trail on semisolid nutrient medium was recognized as the proof of allelic nonidentity or genetic complementation, respectively. The paralyzed mutants examined could be classified into three groups, that is, thirty mutants belonging to group-A, eighteen to group-B and twelve to group-C. The mutants of group-A are non-complementary with each other, but are complementary with any mutant of group-B. Similarly, a mutant of group-B is non-complementary with any other mutant of group-B but complementary with every mutant of group-A. Thus group-A and -B represent each a genetic functional unit, a 'cistron'. The behavior of the mutants in group-C is somewhat peculiar. Many mutants in this group show partial complementation with each other; a few are complementary with the mutants of both group-A and -B, while others are non-complementary with them. It may be worth noting that the absence of complementation in the latter combination is not explained as a result of deletion extending over C-region and A- or B-cistron. Syntrophic recovery of motility does not occur in the mixed culture of the representative mutants of these groups either in broth or on semi-solid medium.

Measurements of ATP content and ATPase activity of the representative mutants in these groups and of wild type were carried out. For the estimation of ATP, the energy rich phosphate esters extracted from cells with TCA were calculated as hydrolysable-P in 1N-HCl for 10 min. at 100°C. In order to search for ATP in the flagella, chromatography on column of Dowex-1 8X was used. ATPase activity was determined by measuring free inorganic-P liberated from added ATP after it was treated with crude enzyme solution. The chemical analyses have shown that there is no significant difference in ATP content and ATPase activity per cell between the mutants of the different groups. Each value is, however, slightly lower than the comparable value in the motile wild type strain, TM2. The flagella of the three paralyzed

1) This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (E-2872), Public Health Service, U.S.A. to T. Iino.

groups as well as the motile parent strains did not show any ATPase activity.

62. *Chromatography and electrophoresis of flagellin of Salmonella abortus-equi*¹⁾

(By Masatoshi ENOMOTO and Tetsuo IINO)

Flagellins of *S. abortus-equi*, SL23 and its curly flagellar mutant, SJ30 were isolated and purified. Cells incubated on nutrient agar medium for 20-25 hrs. at 30°C were collected in 0.9% saline. The saline suspensions were centrifuged at 3000 g for 15 min. and washed first with saline, then with distilled water. The cells were resuspended in distilled water to concentration of ca. $5 \times 10^{10}/\text{ml}$ and shaken (ca. 750 stroke/min., 25 mm amplitude) for 30 min. with fine glass beads and a drop of silicon defoamer. The suspension was centrifuged at 1500 g for 60 min. and the supernatant liquid was centrifuged at 90000 g for 60 min. The precipitate was disintegrated in 0.005N-HCl for 1 hr., then the suspension was centrifuged at 90000 g for 60 min. at 2°C. The supernatant was dialyzed at 2°C against 3 changes of distilled water for 5 hrs. This was used as flagellin specimen. Column chromatography was performed on DEAE-cellulose (Brown). Prior to packing the cellulose was adjusted to pH 8.0 by a KH_2PO_4 solution and equilibrated with starting buffer. The flagellin sample equilibrated with the same buffer was applied to the column (1×10 cm). A gradient was established with 100 ml of 0.005M-phosphate buffer (pH 8.0) in the mixing chamber and first 80 ml of 0.5M-phosphate buffer (pH 6.0) was added followed by 80 ml 1M-NaCl in 0.05M- KH_2PO_4 (pH 4.2) in the reservoir. The rate of flow of buffer was 20 ml/hr and 3.2 ml fractions were collected. Protein was measured spectrophotometrically at 280 m μ or/and 750 m μ after folin-phenol reaction. In the flagellin sample of SL23, only one peak (peak B) was observed in the effluent corresponding to 25 ml-50 ml. The test of H(enx)-antigenicity of this peak by means of Ouchterlony plate was positive. In some fractionations a small peak (peak A) came off before the major peak B and in this time a shoulder (peak C) always appeared behind peak B. In order to determine whether peak A and C are caused by contamination with other proteins or denaturation of flagellin, the dialyzed sample was divided into two portions and one was applied to the column immediately, while the other was stored in 0°C and applied after three

1) This work was supported by a research grant from the National Institute of Allergy and Infectious Disease (E-2872), Public Health Service, U.S.A.

days. In the chromatogram after three days, the small peak A increased, peak B decreased compared to the first fraction and the shoulder (peak C) grew to one large peak. The antigenicity of peak A was negative to both anti-H(enx) and anti-O(4, 12) serum and the other two peaks (B and C) showed positive reactions to anti-H(enx) serum. Moreover, the rechromatogram of peak B showed the fractionation pattern of three peaks. Thus it seems that peak A is a degradation product of flagellin and peak C represents a flagellin polymer. The chromatogram of flagellin sample of SJ30 showed the same profile as SL23; the fractionation pattern of one large peak or three peaks depend on the time when the dialyzed sample is applied on column. Dialyzed flagellin sample of SL23 and SJ30 was lyophilized in vacuo and used for paper electrophoresis. Electrophoresis was carried out on Toyo No. 51 paper with veronal buffer (pH 8.6, I 0.05) and 8M-urea containing veronal buffer to avoid polymerization of flagellin. In both buffers, flagellin of SL23 and SJ30 showed one band at the same distance from the original point.

63. *Further studies on the nuclei of yeasts*

(By Yoshiaki YONEDA)

Five species of yeasts (strains of *Saccharomyces*, *Schizosaccharomyces pombe*, *Lipomyces starkeyi*, *Torula utilis* and *Torula rubra*) contained single conspicuous nucleoli within the nuclei. These nucleoli stained densely with haematoxylin. Feulgen-carmin and Giemsa stainings differentiated the nucleoli from the chromatin. Such nucleoli might be comparable with those of other fungi observed by Robinow (1957) and Bakerspigel (1957, '59, '60).

In the course of nuclear divisions, a spindle-like structure was observed in *Torula utilis*. This structure appeared as a transparent zone, while the nucleoli became red by staining with aceto-carmin after fixation with alcohol-formalin-acetic acid. Since metal salt solutions were reported to fix well spindle structures in cells of higher plants (Wada and Fukunaga. 1957), the present author tested various kinds of metal salts as fixatives. Although the spindle-like bodies were not yet confirmed with these fixatives, the nuclear boundary and the nucleoli were clearly differentiated after treatment with 1/10M CuCl_2 , HgCl_2 and CdCl_2 aqueous solutions. In this case, aceto-carmin or acid fuchsin stained the nucleolus, and the chromatin was assumed to be located in the hyaline region around the nucleolus.

64. *Temperature dependence of nitrous acid effects on phage T4*(By Hiromi ISHIWA, Yonhoi YAN¹⁾ and Sohei KONDO)

Nitrous acid is one of the chemical mutagens most thoroughly studied concerning its reaction with nucleic acid and mutagenic action on phages. To obtain a better knowledge of its mutagenic action, temperature dependences were studied with respect to its lethal and mutational effects on T4 phages. Free phages were treated with nitrous acid in an acetic acid-sodium acetate buffer, 0.2M in acetate ions, at pH 4.0 or 5.0. Nitrous acid was produced by adding KNO₂ at the final concentration of 0.2M.

The surviving fraction S/S_0 , S_0 being the initial phage titer, decreased exponentially with time t of treatment and mutation frequency f increased linearly with t in the range of inactivation down to 10^{-8} :

$$\frac{S}{S_0} = e^{-\mu_l t}; \quad f = \mu_m t, \quad (1)$$

where μ_l and μ_m are, respectively, killing and mutation rates per unit time. By changing the temperature from 2°C to 27°C, both μ_l and μ_m were confirmed to follow the Arrhenius equation

$$\mu_l \propto e^{-E/kT}; \quad \mu_m \propto e^{-E/kT}, \quad (2)$$

where k is the Boltzmann constant, T absolute temperature and E denotes activation energy. Differences in E between inactivation and mutation either at pH 4.0 or 5.0 were too small to be detected significantly beyond experimental errors. Average E value was 0.71 ± 0.06 eV.

1) Department of Physics, Faculty of Science, Kyoto University, Kyoto.

E. RADIATION GENETICS IN ANIMALS

65. *Effect of radiation-induced mutations on fitness of *Drosophila melanogaster*¹⁾*

(By Yuichiro HIRAIZUMI)

Several males of *D. melanogaster* (+/cn L^2 bw ♂'s) were x-rayed in order to find out whether flies heterozygous for the induced mutations on second chromosome show any demonstrable increase in fitness. The exposure consisted of 500r given at a rate of 100r per minute for 5 minutes. The irradiated males were kept for two days together with

the females of standard, *cn bw*, stocks, and then they were mated to heterozygous females of $+/SM1$ ($SM1$: balancer) for additional two days, to isolate each irradiated and control second chromosome. Therefore most of the chromosomes thus isolated were exposed to x-rays at the stage of sperm. All the second chromosomes used in this study were originated from a single chromosome, and the genetic background other than that of the second chromosome had been replaced by that of the standard stock.

Ninety-seven x-rayed and the same number of control chromosomes were tested for their effects on female fertility, developmental rate (measured by the period from egg till eclosion) and viability (only 74 chromosomes were tested for viability). The results are summarized in the table.

Table. Effect of x-rayed second chromosomes ($=\oplus$) on several components of fitness

	$+/+$	$+\oplus$	\oplus/\oplus
Female fertility	1.000	0.981	0.957*
Developmental rate	1.000	0.998	1.003
Viability	1.000	1.003	0.972

* Significant at 5% level. The relative values shown in this table are based on the average of 97 (74 for viability) chromosome lines.

Since most of the values in the experimental classes do not deviate significantly from those in control, the results are inconclusive. Taken at face values, however, it is interesting to note that the heterozygote ($+\oplus$) shows, on the average, faster developmental rate and higher viability than the control homozygote ($+/+$), although female fertility of $+\oplus$ is reduced. This experiment is still continuing for conclusive results.

1) This work was supported by a PHS research grant No. RH-00034-01 from the Division of Radiological Health, Bureau of State Services, Public Health Service, U.S.A.

66. *Heterozygous effects of radiation-induced mutations on viability in homozygous and heterozygous genetic backgrounds in Drosophila melanogaster*

(By Terumi MUKAI, Sadao CHIGUSA and Isao YOSHIKAWA)

It has been recognized that a unique co-adaptation system of genes

has been established within a population of *Drosophila* as a result of natural selection. In order to test the difference of heterozygous effect of radiation-induced mutations on viability between individuals having a complete coadapted genic system and hybrids originated from two unrelated populations, the following experiments were conducted.

Two isogenic lines (*AA*: Burdick's *W160*, and *BB*: Burdick's *W109*) were extracted from unrelated natural populations. *A* and *B* indicate the genomes. After X-ray irradiation at the dose of 150 r, (irradiation is indicated by superscript ['']), heterozygotes *AA'* and *AB'* were produced by the aid of *Cy/Pm Ubx/Sb* and *Xa/++* strains. The relative viability of irradiated heterozygotes with respect to the second and the third chromosomes were estimated in comparison with that of *Xa/+_A+_A*. The results are presented in terms of mean viability in Table 1.

Table 1. Heterozygous effects of radiation-induced mutations on viability (Acute 150 r)

Genotype	No. of chromosomes tested	Average viability	(irradiated) - (control)
<i>AA</i>	330	0.9843	+0.0178
<i>AA'</i>	330	1.0021	
<i>AB</i>	430	1.2831	-0.0310*
<i>AB'</i>	430	1.2531	

Viability of *Xa/+_A+_A*=1.0000

* Significant at the 5% level

From Table 1, it can be seen that radiation-induced mutations which occurred in homozygous individuals were heterozygously beneficial to their carriers, i.e., the average viability of *AA'* was larger than that of *AA* by 0.0178 viability index although it is not significantly different from 0, while those that occurred in the hybrids between individuals originated from the two unrelated populations were detrimental to their carriers, i.e., the average viability of *AB'* was smaller than that of *AB* by 0.0310.

It is a further problem to clarify whether this difference in effects of radiation-induced mutations is caused by the difference in the genic system of the genetic background or by the difference in heterozygosity *per se*.

67. *Radiation-induced mutation rates of polygenes controlling the sternopleural bristle number in Drosophila melanogaster*

(By Terumi MUKAI, Isao YOSHIKAWA and Sadao CHIGUSA)

In order to obtain a fundamental information on the genetic influence of radiation on human populations and on the estimation of spontaneous mutation rates of polygenes, radiation-induced mutation rates of polygenes controlling the sternopleural bristle number have been estimated. The experiments have been conducted since 1961, and the results obtained in 1962 are here reported.

The males of an isogenic line extracted from a wild population of Erie, Pa. (U.S.A.) were irradiated with X-rays at 250 r and 500 r. Immediately after irradiation, the irradiated males were mated in milk bottles to the females of the same line. The number of sternopleural bristles in females and males which had hatched on or before the 13th day after the mating were scored and the means and the variances were calculated to test the heterozygous effects of radiation-induced mutations. The results obtained in 1962 are presented in Table 1.

Table 1. Means and variances of sternopleural bristle numbers (1962)

	Control		250 r		500 r	
	♀	♂	♀	♂	♀	♂
Mean	17.6614	16.9815	17.6713	16.9775	17.5450**	16.9512
Variance	1.3969	1.2993	1.4488	1.2937	1.4964*	1.2999
No. of irradiated genomes	2519	2111	2422	2000	2517	1928

* Significant at the 5% level

** Significant at the 1% level

The variances of the irradiated groups have approximately linearly increased with the dose in females, but have rather decreased in males as compared with the controls. This difference might be attributed to changes of hetero-chromatic parts in Y-chromosomes.

Thus, the mutation rates were estimated by using the data for females only. A modified method for the estimation of polygenic mutation rate developed by Mukai (1961) with reference to Bateman's work (1959) was employed. For the reason that the third moment about the mean is influenced a great deal by the effect of error, it was not used in the present analysis. Accordingly, the heterozygous effects of each mutation

were assumed to be equal to one half the standard deviations (Oka *et al.* 1958). Thus, the mutation rates were estimated on the assumption of 500 loci which control the number of sternopleural bristles. This assumption may not be an underestimate considering Falconer's estimation as 100 of the number of loci controlling the abdominal bristles. The results are shown in Table 2.

Table 2. Estimation of radiation-induced mutation rates of polygenes controlling the number of sterno-pleural bristles (1962)

	250 r experiments	500 r experiments
Mean number of positive mutations per genome	0.083	0.044
Mean number of negative mutations per genome	0.066	0.241
Mean number of mutations per genome	0.149	0.285
Mutation rate per locus per r*	1.19×10^{-6}	1.14×10^{-6}
Variance increasing rate per r on heterozygote basis	$2.02 \times 10^{-4}/r$	

* Assuming 500 loci

The estimated polygenic mutation rates were of the order of 10^{-6} /locus/r both in 250 r and 500 r experiments as well as the experiments of 1961.

Thus, it may be concluded that radiation-induced polygenic mutation rates are substantially higher than those for major genes. This result may support the view that spontaneous polygenic mutation rates are higher than those for major genes (Mukai and Chigusa 1962).

68. *Radiation sensitivity of infectious particles responsible for "sex-ratio" condition in Drosophila willistoni*¹⁾

(By Bungo SAKAGUCHI and Sohei KONDO)

The abnormal condition referred to as "sex-ratio" (SR) in *D. willistoni* is characterized by extreme departure from the normal 1:1 ratio of the sexes, i.e. by either completely or nearly completely, female progenies (Malogolowkin 1957). Recently Poulson and Sakaguchi (1960, 1961) have shown that the SR agent is a spirochete-like infectious microorganism.

1) This work was supported by a grant from the National Institute of Health, Education and Welfare (GM 10238-1), Public Health Service, U.S.A.

In order to clarify its properties, and experiment on the determination of the agent's sensitive volume was carried out by γ -ray irradiation.

The dose-effect curve for inactivation of the infectious SR agent was obtained as follows. The hemolymph taken from females of *D. willistoni* in which the infectious agent is present at high level, was injected into female of *D. virilis*, which served in the place of a test tube culture of SR agent, and then the SR infected females were exposed to Cs-137 γ rays with doses from 4 to 32 kr. The hemolymph containing a large amount of the irradiated infectious agent was transferred from the irradiated flies into females of an inbred strain, Oregon-R, *D. melanogaster*, then the latter were mated with males of the same strain and observation was made on the manifestation of SR condition in the following next generation. The degree of inactivation of the infectious microorganisms was estimated from the prolongation of the latent period, which is defined as the incubation period between injection and SR manifestation.

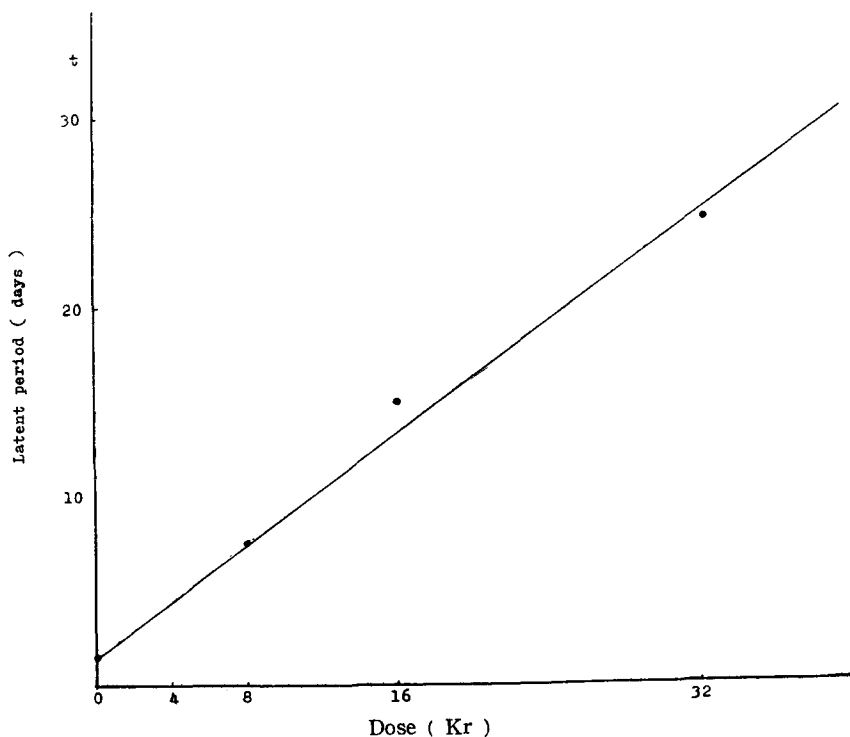


Fig. 1. Relationship between γ -ray dose and latent period of SR agent.

The experimental data are summarized in the curve of latent period t versus dose D (Fig. 1). The curve follows a clear straight line relationship expressed by the following equation:

$$t(\text{days})=1.5+0.75D(\text{kr}) \quad (1)$$

Time t required for manifestation of SR condition should depend on the concentration C of SR agent at the time of injection. The relation between t and C was experimentally determined by dilution tests of hemolymph of SR female adults of *D. willistoni*. The following empirical relation was obtained:

$$C2^{t/2.1}=\text{const.} \quad \text{or} \quad C=C_0e^{-\frac{1}{3}(t-1.2)} \quad (2)$$

where C_0 is the SR agent concentration of the control not subjected to dilution. This is interpreted to mean that the infectious microorganisms duplicate in 2.1 days and when their concentration reaches a constant threshold value SR condition appears, irrespective of the initial concentration of infectious microorganisms at the injection time.

On the other hand, if inactivation follows single hit response, then concentration C of microorganisms surviving dose D at the time of injection should be related to concentration C_0 of the control used in equation (2) as follows:

$$C=C_0e^{-\alpha D} \quad (3)$$

where α is inactivation probability per unit dose. From equations (2) and (3), we obtain

$$t=1.2+3\alpha D \quad (4)$$

Close agreement in t value at $D=0$ between equations (2) and (3) supports the validity of the assumptions used which lead to the equivalence between (1) and (4). Thus, from the identity of the two equations, we obtain $1/\alpha=4(\text{kr})$ which is the so-called inactivation dose D_{37} corresponding to 37% survival of the infectious agent.

According to the target theory, the target size in molecular weight is given by $0.7 \times 10^9/D_{37}(\text{kr})$. Hence from the experimental value of D_{37} , the molecular weight of the infectious microorganisms is estimated to be $2 \times 10^8(\text{M.W.})$. This result supports the previous experimental conclusion (Poulson and Sakaguchi 1960) that SR agent is rather spirochetal than viral in nature.

69. *Revised interpretation of the non-recovery type dose-rate dependence of radiation-induced mutation rates of silkworm gonia*

(By Yataro TAZIMA and Sohei KONDO)

As reported in our previous paper (Tazima, Kondo, Sado, 1961), two types of dose-rate dependence of radiation-induced mutation frequency were observed in early gonial cells of the silkworm. In one type (type I), mutagenic effectiveness of chronic irradiation was lower than that of acute irradiation and in the other type (type II), the situation was completely reversed. The occurrence of either type depends upon the stage of irradiated germ cells.

The type I dose-rate dependence has been interpreted in the light of the repair hypothesis proposed by Russell et al. (1958) on the grounds of their findings in the mouse. This hypothesis, however, can not explain the type II dose-rate dependence, because acute irradiation gives rise to lower mutation frequency than chronic irradiation. Therefore, we have made the following provisional assumptions. 1) Primary mutation rates differ according to the developmental stage of gonial cells, 2) highly mutable cells, in particular the secondary spermatogonia, are selectively killed after acute exposure because of their high sensitivity to radiation but can survive chronic irradiation.

Cytological studies of irradiated gonial cells, which was carried out by Sado in parallel with our work, failed to obtain clear-cut evidences in support of our hypothesis. Both acutely and chronically irradiated secondary spermatogonia were observed to undergo necrosis, although degeneration was less in the latter. Sado also found that after degeneration of primary and secondary spermatogonia, usually less in the former, young gonial cells regenerate from irradiated cells that have been presumably dormant at the time of irradiation.

Taking into account these cytological findings together with our recent results, we have modified our previous interpretation as follows. Radiation sensitivity of primary and secondary spermatogonia is much higher than we had previously assumed. Survival fraction of secondary spermatogonia depends solely upon radiation intensity; almost all cells are killed after acute irradiation within the dose range from 150 r to 1000 r but a few cells can survive chronic irradiation although the majority are killed. Dormant spermatogonia can survive both acute and chronic irradiations in the dose range used. Sensitivity of primary spermatogonia may be intermediate. The deficit, caused by killing of most of developing gonial cells in the irradiated gonad, is soon compensated by

young germ cells regenerated from the survived dormant cells. Thus change will be brought about in the proportion of cell types in the gonad after irradiation, their amount depending upon the degree of the deficit. Acute irradiation may cause more severe deficit than chronic one and hence will give rise to a higher proportion of young germ cells regenerated from dormant cells. This will result in a lower mutation frequency at acute than at chronic treatment as observed for type II dose-rate dependence in our experiment.

Thus modified hypothesis dose not seem to contradict with our previous one but explains more satisfactorily all the genetical and cytological experimental results so far obtained. Further studies, however, are necessary for obtaining direct support of the proposed hypothesis.

(Presented before the 2nd International Congress of Radiation Research, Harrogate, 1962.)

70. *Differential radiation-sensitivity of germ cells as a possible interpretation of sex difference in dose-rate dependence of induced mutation rates in the silkworm*

(By Yataro TAZIMA and Sohei KONDO)

During the course of our studies (Tazima, Kondo and Sado, 1961) on dose-rate dependence of radiation-induced mutation rates in the silkworm, we noticed that it was more pronounced in the oögonia than in the spermatogonia at an early stage at which type I dose-rate dependence was observed. The ratio of the observed mutation frequency for acute irradiation (320 r/min.) to that for chronic irradiation (0.1 r/min.) was 2:1 in the oögonia and 1.5:1 in the spermatogonia.

Our recent studies on mutation frequency versus dose curves, carried out by the specific locus method using *pe* and *re* loci, have confirmed that there was, indeed, a marked difference in mutation response between oögonia and spermatogonia, though cells of both cytologically did not show yet at that stage any appreciable difference. First, the mutation frequency was about twice as high in spermatogonia as in oögonia. Second, the shape of their dose-frequency curves was different. In oögonia, a linear relationship was clearly observed within the range from 500 to 1500 r both for acute (320 r/min.) and chronic (0.07 to 0.21 r/min.) irradiations, and acute exposures were significantly more effective than chronic ones over the whole dose range studied. In contrast, in spermatogonia the frequencies for acute and chronic exposures increased more rapidly than expected for linearity with increasing dose, and the

difference between them became smaller in the high dose range. At 1500 r, the frequency for chronic was almost equal to that for acute irradiation.

These findings seemed to indicate a close relationship between mutation induction and sensitivity of germ cells. Therefore, to evaluate the contribution of killing effect on germ cells to the mutation mechanism involved, we made an estimation of the difference in radiation sensitivity between spermatogonia and oögonia from our earlier data, by using the theoretical formula proposed by the junior author, Kondo (Ann. Rep. Nat. Inst. Genet. 11: 96, 12: 93-94).

From the experimental probability distribution of the number of mutants per mating pair, estimation was made of surviving germ cells at the time of mutative events, and survival versus dose curves were constructed for acutely irradiated spermatogonia and oögonia at various doses.

The LD₅₀ was about 1000 r and 2000 r for spermatogonia and oögonia, respectively. The difference in radiation sensitivity is clear between both sexes. Thus it was concluded that the mutation frequency is closely related to the killing effect.

These findings appear to suggest that recovery of mutational damage, if any, becomes rather difficult in the highly sensitive cells, not only after acute but also after chronic exposure. Hence, the differences in the observed mutation frequencies in the spermatogonia between acute and chronic irradiations become smaller with increasing dose, although the difference may exist to some extent. On the contrary, in oögonia, which are rather resistant to radiation, the recovery process seems to operate with less interference by the killing effect.

From these considerations, we assume that the repair process is, even if it works under normal cellular conditions, masked or eliminated to some extent by cellular radiation damage and that the degree of dose-rate dependence becomes much smaller in sensitive cells such as early spermatogonia.

(Presented before Symp. on Repair from the Genetic Radiation Damage, Leiden, 1962 and will appear on the Proceedings.)

71. *The increase in induced mutation frequency after fractionated irradiation of gonial cells of the silkworm*

(By Yataro TAZIMA and Akio MURAKAMI)

Since the dose-rate dependence of radiation induced mutation-frequency

had been observed in the silkworm, it was considered of some interest that induced mutation frequency could be modified by fractionating the administered radiation dose. For this purpose experiments have been carried out during the early larval stage from the 7th to 9th day after hatching. For the estimation of mutation frequency the specific locus method for *pe* and *re* loci was used.

The design of the experiments was as follows. In one group (Fr 1) total X-ray dose 1000 r was given in two halves separated by 48 hour interval, the first 500 r on the 7th day and the second 500 r on the 9th day. In another group (Fr 2) the same dose was given in three fractions; the first 333 r on the 7th day, the second 333 r on the 8th day, and the remaining 333 r on the 9th day, with 24 hour intervals. In the third group (Fr 3) total dose was reduced by one half to 500 r, and 10 r was given 50 times every hour during the same larval stages. For comparison's sake three single dose groups were arranged with 1000 r given in single shots at different developmental stages, i.e. on the 7th, 8th and 9th day (S1, S2 and S3) respectively. A non-irradiated group (Cont.) was simultaneously examined. The results are shown in Table 1.

Table 1. Fractionated X-irradiation of spermatogonia and oögonia

Group	Irradiated stage			Total dose	Mutation frequency ($\times 10^{-5}$)			
	days after hatching				Spermatogonia		Oögonia	
	7	8	9		<i>pe</i>	<i>re</i>	<i>pe</i>	<i>re</i>
Fr 1	500 r		500 r	1000 r	87.2	31.4	53.6	33.3
Fr 2	333 r	333 r	333 r	999 r	221.8	90.4	256.2	156.8
Fr 3	10 r \times 50	1 hour interval		500 r	57.5	35.2	43.6	26.8
S 1	1000 r			1000 r	73.6	31.5	20.7	14.2
S 2		1000 r		1000 r	31.5	9.2	37.2	32.3
S 3			1000 r	1000 r	36.7	5.6	26.9	20.9
Cont.				0 r	8.9	0.0	9.2	8.8

It may be seen from the table that fractionated groups give higher mutation frequencies than the three single shot groups; group Fr 2, in particular, gives extraordinarily high frequency. The frequency in group Fr 3 is also high when it is multiplied by two.

These findings seem to suggest that fractionated irradiation with 24 hour interval (group Fr 2) is more effective than that with 48 hour interval (group Fr 1) in increasing mutation frequency.

But shortening of the interval has a limit: fractionated irradiation with 1 hour interval showed lower mutation frequency than when 24 hour interval was applied, even when it is adjusted at 1000 r. Further experimental data, which are currently being accumulated, indicate that irradiation with 24 hour intervals is of all the most effective in increasing mutation frequency.

72. *Modification of X-ray-induced mutation rate in the silkworm by pre- or post-irradiation treatment with halogenated base analogues*

(By Akio MURAKAMI and Yataro TAZIMA)

It has been known that certain halogenated base analogues are mutagenic in bacteria when supplemented to the culture media. It has also been reported that mammalian and bacterial cells grown in the presence of halogenated thymidine analogues become highly sensitive to killing effects of X-rays and ultraviolet light. Nevertheless, for higher organisms mutagenicity of those substances was still uncertain. In order to test it a series of experiments has been carried out with the silkworm by feeding larvae on 5-Bromouracil (5BU), 5-Bromodeoxyuridine (5BdUR) or 2-Aminopurine at early instars. Thus far more than 1.5 million individuals have been tested for induced mutations at specific loci for *pe* and *re*. However, no significant increase, if any, has been observed. Therefore, the mutagenicity of those analogues has been tested in combination with X-irradiation. The results were definitely positive.

1. Experiment in the first instar.

In this series 5-BdUR was administered to wild type larvae by feeding them on leaves supplemented with the chemical (30 mg per 1500 larvae) for about one and a half day after X-irradiation at 48 hour age of the first instar. The results are given in Table 1.

It may be seen from the table that 5-BdUR treatment, when combined with X-irradiation, enhances markedly the mutation frequency above the expected level from X-irradiation only, although 5-BdUR alone has no effect on increasing mutation frequency. The enhancement is more marked in the male than in the female for both *pe* and *re* loci. Spontaneous mutation frequency in the control group is seen to be in this experiment at the ordinary level.

2. Experiment in the second instar.

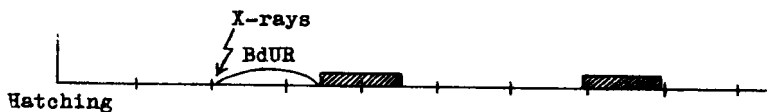
In the second series of experiments it was tested whether 5-BU is also effective in modifying X-ray induced mutation frequency. The

Table 1. Combined experiment with 5-BdUR and X-irradiation
(622, 1st instar)

Treatment	Total no. observed	<i>pe</i>			<i>re</i>		
		Mut. detd.	Mut. freq.	Conf. int.	Mut. detd.	Mut. freq.	Conf. int.
Male							
Cont.	121,800	20	16.4×10^{-5}	8.4- 25.4	2	1.6×10^{-5}	0.1- 3.9
X-rays*	101,850	45	44.2	21.7- 59.2	31	30.4	18.8-43.2
BdUR**	122,850	14	11.4	4.4- 19.4	0	0	
X+BdUR	127,400	163	127.9	107.4-149.2	89	69.9	54.6-85.9
Female							
Cont.	110,700	9	8.1	3.5- 15.8	3	2.7	0.5- 5.3
X-rays*	62,100	16	25.8	11.4- 43.0	7	11.2	3.8-14.4
BdUR**	74,250	4	5.4	0.6- 8.8	2	2.7	0.1- 3.9
X+BdUR	36,900	10	27.1	7.0- 50.0	6	16.3	1.6-30.0

* 1000 r (1 r/sec.)

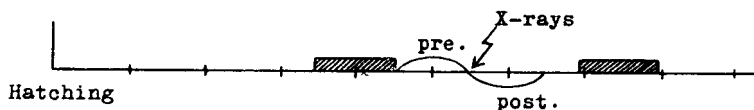
** 30 mg/1500 head.

Table 2. 5-BU feeding before or after X-irradiation
(623, 2nd instar male)

Treatment	Total no. observed	<i>pe</i>			<i>re</i>		
		Mut. detd.	Mut. freq.	Conf. int.	Mut. detd.	Mut. freq.	Conf. int.
BU*(pre)	120,400	15	12.4×10^{-5}	5.1- 20.8	2	1.6×10^{-5}	0.1- 3.9
X-rays**	116,900	133	113.8	93.2-135.2	75	64.2	48.4- 80.8
BU+X-rays	104,650	173	165.3	139.1-192.5	71	67.8	50.2- 85.9
Bu(post)	107,450	26	24.2	13.8- 35.6	2	1.8	0.1- 3.9
X-rays+BU	125,300	213	170.0	146.6-194.8	112	89.4	71.7-108.0

* 120 mg/1500 head.

** 1000 r (1 r/sec.)



difference between pre- and post-irradiation treatment was also studied.

Second instar larvae were raised for about one day on leaves supplemented with 120 mg 5-BU (per 1500 individuals) either before or after X-irradiation. The X-ray dose was 1000 r. The results are shown in Table 2.

Although a control plot was not used in this experiment, mutation frequency in the 5-BU group is approximately at the spontaneous level and could be treated as control. Both pre- and post-treatment groups represent higher mutation frequency than that group of X-irradiation alone, although the frequency in the pre-treatment group is slightly lower than that in the post-treatment group. These results do not allow to assume the different mechanism for both pre- and post-treatment groups in respect to modification of mutation frequencies, because the chemical administered before irradiation might have remained for some while within the insect body and become effective after X-irradiation. It may, however, be inferred with almost certainty that the enhancement is not due to the sensitizing effect of the analogues but to some sort of effect that acts upon post-irradiation recovery process.

It is tempting to speculate that the analogue is incorporated into X-rayed DNA and gives rise to a copying error in newly formed DNA. However, supporting evidences are still meagre for this conclusion.

73. *Cytological estimation of LD₅₀ X-ray dose for secondary spermatogonia of the silkworm*

(By Toshihiko SADO)

Earlier experiments have shown that the secondary spermatogonia are the most radiosensitive cell type in the silkworm testis and the maximum depression of these cells usually occurs 3 days after irradiation (Sado 1959, 1961). During the second instar, the testis contains primary and secondary spermatogonia predominantly. Therefore, when the silkworm males are irradiated during this period with doses higher than 500 r, which is generally lethal to secondary spermatogonia, mutations are induced only in primary spermatogonia. If these cells are exposed to doses much lower than 500 r, since some of the secondary spermatogonia, may not be killed, we should expect mutations induced in both primary and secondary spermatogonia. For this reason, we felt it necessary to estimate the approximate LD₅₀ dose for these cells.

Male silkworms from the C108 strain, which has been routinely used for mutagenesis work in this laboratory, were exposed to 25 r, 50 r, 100 r,

150 r, 200 r, 250 r and 500 r of acute X-rays on the first day of the second instar, or 5 days after hatching. One and three days after the exposure, the abdominal portion of the treated insects were fixed in Bouin's fluid, 7 μ thick paraffin sections were made, stained with Delafield's hematoxylin and the number of necrotic as well as surviving cells in the testis were estimated semiquantitatively.

Following irradiation with 25 r and 50 r we could find little, if any, detectable difference between irradiated and control testes. With an increase in the dose administered, the number of necrotic cells at 24 hours increased and the number of surviving cells on day 3 decreased. 100 r and 150 r were not enough to kill half of the secondary spermatogonia. When the insects were irradiated with 200 r or more more than 50% of the cells were killed.

These results suggest that LD₅₀ dose for secondary spermatogonia of the silkworm lies between 150-200 r, which is 6-8 times higher than that reported for intermediate and type B spermatogonia of the mouse (Oakberg, 1955).

74. *Inactivation of Salmonella by ³²P disintegration combined with UV¹⁾*

(By Hiromi ISHIWA, Yonhoi YAN²⁾ and Sohei KONDO)

Salmonella grown in H medium plus ³²P lost their colony forming ability during storage at -20°C and the curve of the surviving fraction versus fraction of decayed ³²P atoms incorporated into their DNA followed one hit response (e.g. Curve NO UV in Fig. 1). Calculation from experimental data gave the killing efficiency $\alpha=0.04$ per disintegration of ³²P atoms per nuclear DNA which is very close to the α value for E. coil (Fuerst and Stent 1956). This value was compared with the killing efficiency for cells surviving pre- or post-treatment with UV (Fig. 1, Curves PRE and POST). It should be noted that synergetic action of UV and ³²P disintegration is clear only for posttreatment with UV as seen from comparison of slopes of inactivation curves in Fig. 1. This work was undertaken to obtain information concerning the mechanism of inactivation by UV and ³²P disintegration.

1) This work was supported by Grant No. 91003 for Intramural Cooperative Scientific Research from the Ministry of Education.

2) Department of Physics, Faculty of Science, Kyoto University, Kyoto.

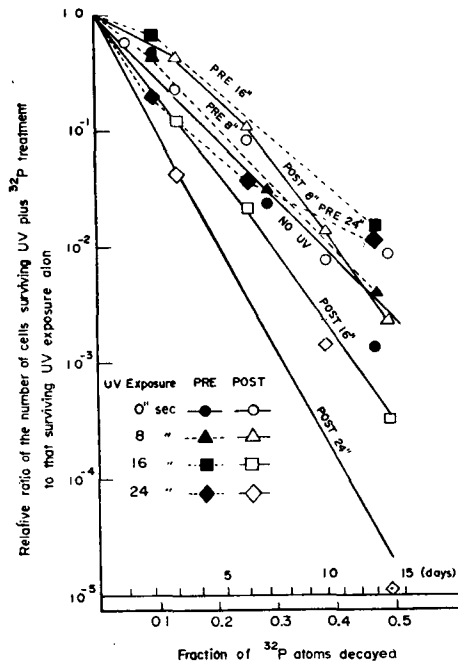


Fig. 1. Comparison of killing efficiencies of ^{32}P disintegration for cells subjected to various UV treatment at ca. 13 erg/mm²/sec; No UV— ^{32}P treatment only, PRE—UV before storage for ^{32}P disintegration, POST—UV after storage.

75. *Protective effect of sodium glucuronate on the differentiation of muscle tissue inhibited by X-irradiation*¹⁾

(By Yoshito OGAWA)

Last year, the protective effect of sodium glucuronate on abnormal formation of actin in X-irradiated *Triturus* embryos was reported²⁾. This paper deals with the influence of the same chemical on the synthesis of myosin, another important contractile protein of muscle tissue and contributes some essential data on the practical value of the protective action. The experimental method was the same as before²⁾³⁾⁴⁾.

Immediately after fertilization, the embryos were raised at 20°C in

1) This work was supported by Grant-in-Aid from Tokyo Biochemical Research Foundation.

0.01% solution of sodium glucuronate (most effective concentration to promote the growth of *Triturus* embryos⁵), and X-irradiation with 50 r, 200 r and 500 r was carried out 108 hrs. after fertilization. After irradiation, treatment with the same solution was continued and the analysis of myosin was immediately carried out. Myosin, isolated from the skeletal muscle tissue of adult *Triturus*, was injected into rabbits and the obtained anti-serum was heated with saline extracts of liver, spleen and skin tissue of *Triturus* to eliminate non-specific antibodies. The titre of the anti-serum was adjusted to 1:512 before carrying out the precipitin reaction with the saline extract of X-irradiated embryos. Non-treated but irradiated, treated and non-irradiated, and normal (non-treated and non-irradiated) groups were prepared as controls.

In normal embryos, myosin first became detectable 176 hrs. after fertilization³. In the present experiment, myosin formation in the normal group was first recognized 180 hrs. after fertilization since the examination was carried out in periods of 24 hrs. following fertilization. In the case of the non-treated but irradiated group, myosin was detected 156 hrs. after fertilization with 50 r and 200 r, and 132 hrs. with 500 r⁴) as shown in Fig. 1. Thus the synthesis of myosin was markedly promoted not only as to the rate of formation but also as to the amount, and the acceleration was proportional to X-ray dosage. In irradiated and treated embryos the tendency to promote the rate of myosin formation was markedly accelerated, though no remarkable difference was

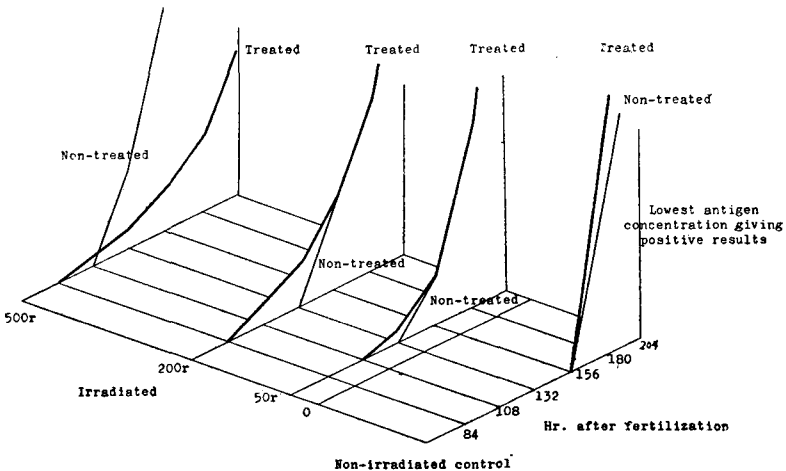


Fig. 1. Effect of sodium glucuronate on the synthesis of myosin in X-irradiated *Triturus* embryos.

found in embryos irradiated with 50 r and 200 r in the amount of synthesized myosin from that of the treated but non-irradiated group. However a heavy dose of 500 r, suppressed the amount of myosin formation. (Fig. 1).

The differentiation of muscle tissue is not accomplished before both actin and myosin formations coincide. The experimental results of both protein syntheses are summarized in Fig. 2. As shown in this figure,

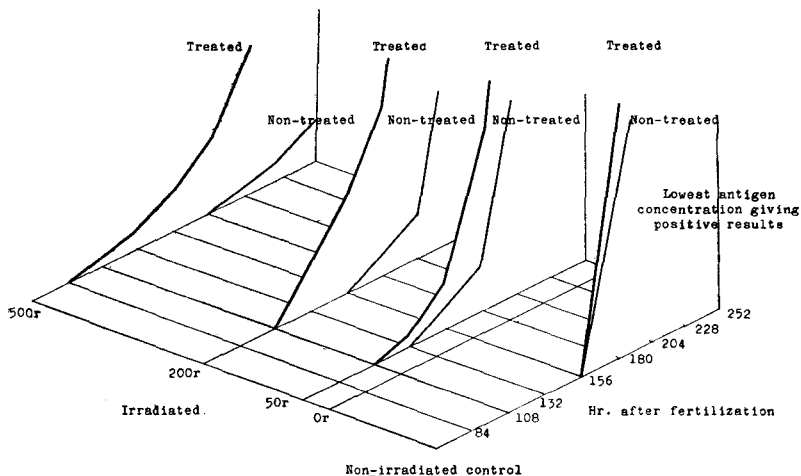


Fig. 2. Effect of sodium glucuronate on the chemo-differentiation of muscle tissue in X-irradiated *Triturus* embryo.

the chemo-differentiation of muscle tissue is markedly suppressed by X-irradiations. This suppression is due to the inhibition of actin synthesis. Sodium glucuronate markedly protects from the obstruction of actin synthesis due to X-irradiation, the main cause of the suppression of muscle tissue differentiation²⁾, and accelerates myosin synthesis during early embryonal development (Fig. 1).

Thus, it was proved that sodium glucuronate has a significant protective effect in abnormal chemo-differentiation of muscle tissue of X-irradiated embryos.

- 2) OGAWA, Y.: Ann. Rep. Natl. Inst. Genet., **12**: 36, 1962.
- 3) OGAWA, Y.: Nature, **182**: 1312, 1958.
- 4) OGAWA, Y.: Nature, **186**: 77, 1960.
- 5) OGAWA, Y.: Ann. Rep. Natl. Inst. Genet., **10**: 150, 1960.

F. RADIATION GENETICS IN PLANTS

76. *Radiation-induced variegations in einkorn wheat and their inheritance*¹⁾

(By Seiji MATSUMURA)

Seeds of *Triticum monococcum flavescens* were exposed to X- and γ -rays by ⁶⁰Co, thermal and fast neutrons (14 MeV) and β -rays by ³²P- and ¹³¹I-solution. In the treated X₁ and later generations white and/or yellow stripes were often observed and their mode of inheritance was studied. The striping was divided into the following types. 1) Most of white- or yellow-striping found in X₁ was maternally or cytoplasmically inherited and was due to plastid mutation. The first appearance of this type occurred seldom in the X₂-generation. 2) In the X₂ head progeny segregating *albina* in a simple Mendelian ratio, white-striped leaves were often observed in a few plants. They might be mostly due to a somatic mutation in a heterozygous plant concerning *albina* (*Aa*). 3) Some white stripes which could be distinguished from others by the fine nature of striping occurring in all leaves of a plant were controlled by a mutated recessive gene. 4) Special variegation was found in three X₁-plants. Their progeny contained some white seedlings. The *basi-viridis* (or *virido-albina*) seedlings, other than *albinas*, invariably grew up to be variegated; they were controlled by a mutated recessive gene. Evidence indicated that the recessive *basi-viridis* gene for variegation, which was stable, stimulated plastids to mutate (irreversibly) from green to white. Plastid "exomutation", or mutation from green to white was affected by environmental factors, such as temperature. This type of variegation was already reported for "Okina-barley by IMAI (1929).

77. *Effects of chronic irradiation on einkorn wheat*

(By Seiji MATSUMURA and Tarô FUJII)

The facility for chronic γ -irradiation of growing plants (γ -field) was built first in 1961 by the Ministry of Agriculture and Forestry in the National Institute of Radiation Breeding, Hitachi-Omiya, Ibaragi-ken, Japan. The radiation source there is radio-cobalt *ca.* 2,000 curies. Our

1) This work was done under Research Contract No. 27 with the International Atomic Energy Agency.

experiment was carried out with seedlings of einkorn wheat (*Triticum monococcum flavescens*), planted at the stage of 3~4 foliage leaves on Feb. 23rd in several distances from the source and irradiated with varying dose rates and dosages. The plants were irradiated during their growth and harvested June 21st. Regular irradiation followed the routine of the γ -field, namely it was applied 20 hours per day starting from April 1st, but before that time, irradiation was given occasionally for measurements of dosage or dose rates and checks of the apparatus. Therefore, total irradiation time from planting to harvest amounted 1,843 hours. And total dosages were calculated as 369 r at minimum and 4,608 r at maximum, intensity being 4~50 r/20 hr. Survival rate was clearly decreased at the highest dosage, while there was no marked difference between the other five irradiated lots and the non-irradiated one. On the other hand, seed fertility was non-linearly (gradually at higher dosages) decreased with increasing dosage and reached to about one half of that of the control lot at the highest dosage.

For comparison, seedlings of the same material were irradiated by 0.5 and 1 kr of acute γ -rays (1 kr/hr) 15 days after sowing. Survival rate and fertility were markedly decreased at 1 kr irradiation. The results from these lots were not easily comparable with those of chronic irradiation because acute irradiation was done only in early seedling stage, but some information may be obtained on the difference of effects between acute and chronic irradiation. Acute 1 kr irradiation showed severe effects and a similar effect was seen at the highest dosage of chronic irradiation (4,608 r). Thus, the plants must be more tolerant to higher dosage when radiation intensity is low.

78. *Relation of chlorophyll mutations to dose rates in γ -irradiated einkorn wheat*¹⁾

(By Seiji MATSUMURA and Tomoo MABUCHI)

Dry seeds of *Triticum monococcum flavescens* were exposed to γ -rays at 4~15 kr. For acute and chronic irradiation the dose rates, 10,000 r/hr with ¹³⁷Cs and 35.6 r/hr with ⁶⁰Co, were used, respectively, as shown in the diagram of Fig. 1. The germination rate, seedling height 20 days after sowing, seed fertility in X₁ and chlorophyll mutations in X₂ were investigated.

Chronic irradiation was slightly more effective in inhibiting seedling

1) This work was supported by Grant from the Toyo Rayon Foundation for the Promotion of Science and Technology.

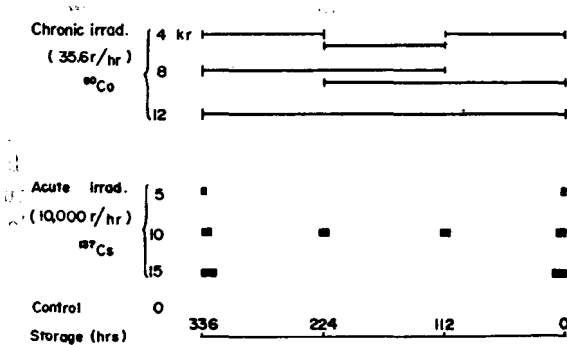


Fig. 1. Diagram of acute and chronic Irradiations

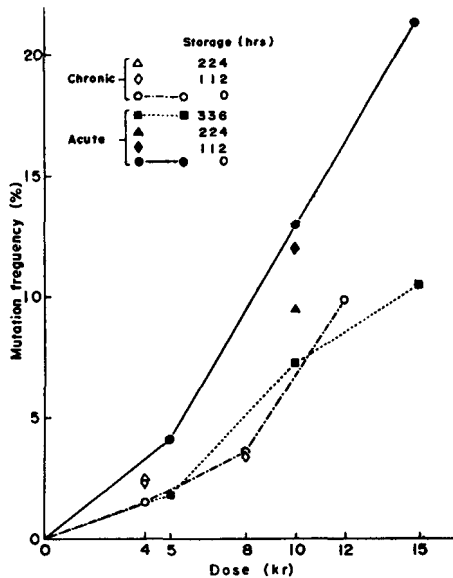


Fig. 2. Relation between chlorophyll mutations and doses or dose rate in einkorn wheat

growth than the acute one applied at the beginning of the chronic one, and clearly less effective than that applied at the end of the latter. The later was the irradiation at the same dose, the more reduced was seedling growth, especially at acute irradiation. Thus, almost no intensification of radiation damage due to storage effects was found. It is supposed that recovery phenomena were involved.

The frequency of chlorophyll mutations in the X_2 -generation is shown in Fig. 2. For acute irradiations which were applied just before sowing, it increased roughly in a linear relation to dose. Acute irradiation showed clearly a higher mutation rate than the chronic ones, which were terminated just before sowing. This indicates a dose rate dependency in the frequency of chlorophyll mutations. On the other hand, there was no marked difference between chronic and acute irradiation at the beginning of the chronic 12 kr irradiation after the longest storage. Unexpectedly, the later was the irradiation at acute 10 kr, the higher was the mutation rate. It is supposed that recovery phenomena were involved.

79. *Relation of radiation effects to dose rates of γ -radiation in rice*¹⁾

(By Seiji MATSUMURA and Tomoo MABUCHI)

A series of experiments with rice were initiated to expand and verify the earlier studies (cf. Ann. Rep. No. 11: 100 and No. 12: 106). Dry seeds of upland rice were irradiated by γ -rays from a ^{60}Co or ^{137}Cs source at the doses of 10, 20, 30 and 40 kr. For acute and chronic irradiations the dose rates, 10,000 r/hr with ^{137}Cs and 20 r/hr with ^{60}Co , were used, respectively. All acute and chronic 10 kr treatments were given in three ways; namely at the beginning, in the middle and at the end of the chronic 40 kr irradiation. Also the chronic 20 and 30 kr irradiations were done in two ways; namely at the beginning and at the end of the chronic 40 kr treatment. The germination rate, seedling height 13 days after sowing, seed fertility in X_1 and chlorophyll mutations in X_2 were investigated.

The results are shown in Fig. 1 for seedling height. An intensification of radiation damage due to storage effects was clearly observed. For instance, at 20 and 30 kr acute irradiations only the batches given the treatment at the end of the chronic ones showed moderate germination while others did not germinate. The earlier was the irradiation with 10 kr, the more reduced were seedling growth and seed fertility at acute and chronic irradiations. Furthermore, chronic irradiations were clearly more effective in radiation damage than the acute ones administered at the end of the chronic ones, and less effective than those given at the beginning of the chronic ones. Thus it is supposed that almost

1) This work was done under Research Contract No. 27 with the International Atomic Energy Agency.

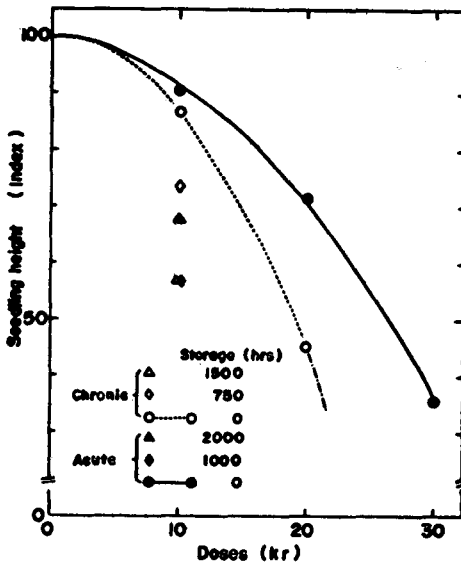


Fig. 1. Relation between seedling height and doses or dose rates in upland rice (1961)

no recovery phenomena were involved.

As to chlorophyll mutations, a slight increase of mutation rate due to storage was also found. Further, the acute irradiations showed mostly a higher mutation rate at 10 kr than the chronic ones, indicating probably a dose rate dependency in the frequency of chlorophyll mutations.

80. *A red-stem mutant in einkorn wheat induced by X-rays*

(By Tarô FUJII)

In 1957, a mutant with red stem was found in the X₂-generation of *Triticum monococcum flavescens* after 10 kr irradiation by X-rays. It appeared among 8 X₂-plants, and 50 normals and 22 red-stem plants were segregated in the X₃-generation from the green X₂ heterozygotes. They showed almost normal green color at the seedling stage. The red pigment appeared around the middle of April in the main veins of the 3rd or 4th leaf and successively in the veins of the following leaves and stems. The red color of the stems was more distinct within the leaf sheaths than in the non-sheathed upper parts of the stem which develop

much more chlorophyll. According to the microscopical examination of cross sections of stems, the pigment is found in the cell walls of the stem epidermis. Some mutant seedlings were placed in the greenhouse; the red pigment appeared from the 3rd or 4th leaf like in those grown in the field during the winter. The appearance of red pigment was not influenced by temperature.

The plant height was measured at heading time in 1959; it was 115.8 cm in mutants and 126.9 cm in normals. Germination and fertility of normal and mutant plants were examined; they were slightly lower in mutant strain than in the normals. Crossing experiments with normals and several other mutants were carried out. All F_1 plants showed normal green color and relatively high fertility, showing no marked difference from the normal strain. Segregation ratios were examined in the F_2 -generation. Linkage relationship between chlorophyll mutant genes and the gene for red stem could not be determined because of high mortality in winter of chlorophyll mutant seedlings, which made it impossible to recognize the red-stem mutant. But genes for irregular ear and red stem may be linked.

81. *Multi-subunit target model for RBE versus LET relationship for radiation damage to higher forms¹⁾*

(By Sohei KONDO)

Assume that RBE depends only on LET. Then, the RBE of radiation A_i to A_0 is expressed by (Quastler: 1960) $RBE(A_i : A_0) = (\bar{L}_0 \bar{p}_i / \bar{p}_0 \bar{L}_i)$ where \bar{L} and \bar{p} are, respectively, the mean LET (energy E divided by charged-particle range R) and the mean reaction probability to give rise to a given type of biological response when a target molecule is traversed by a particle. Since \bar{p} is expressed as $R^{-1} \int p(L) dR$ where $p(L)$ is the reaction probability of a particle at stopping power L , assuming $R \propto E^{1.8}$ we have

$$p(L) = \bar{p}(\bar{L}) - 0.44 \bar{L} (d\bar{p}/d\bar{L}) . \quad (1)$$

A multi-subunit target model is assumed for $p(L)$ as follows:

$$p(L) = (1 - e^{-Lt/w})^m , \quad (2)$$

where $w (=75 \text{ eV})$ is mean energy per primary ionization, m the number of subunits per target each of which must be inactivated by one or

1) This work was supported by Grant from the Toyo Rayon Foundation for the Promotion of Science and Technology.

more primary ionizations and t the average diameter of each subunit. In other words, chromosomes are assumed to be made up of m or more than m subunits of t in diameter. All the curves of \bar{p} versus \bar{L} calculated from the published aerobic RBE values with respect to radiation damages to mice and *Tradescantia* show striking similarity. By adjusting m and t , equation (2) for the intermediate LET can be fitted very well to the empirical p versus L curves obtained by applying (1) to these \bar{p} versus \bar{L} curves. In conclusion, all the RBE versus LET curves in the intermediate LET can be explained by the assumption that chromosomes in higher forms are made up of ca. 10 or more subunits and that primary lesions of radiobiological damages to higher forms are due to chromosomal damage whose production requires one or more primary ionization in each subunit of ca. 60Å in diameter.

G. HUMAN GENETICS

82. *Embryonal sex ratio in the Japanese determined by the sex-chromatin test*

(By Ei MATSUNAGA, Akira TONOMURA, Naomichi INUI and Takeo HONDA)

Fresh epithelial tissues obtained from 218 medical abortions in apparently normal early pregnancy (about 4 to 18 weeks) were investigated. Preparations for the sex-chromatin test were made according to the ordinary histological method, after fixation in alcohol-acetic (3:1), and stained by Feulgen technique. One hundred cells from each specimen were examined by the use of a standard microscope at a magnification of 2000 X. Specimen encountered with 30 to over 90 per cent of sex-chromatin positive nuclei were classified as female, while those with less than 10 per cent were regarded as male.

In the first examination of the 218 specimens, 14 were discarded from the present study, since the staining of tissue cells was unfavorable for the detection of sex. Of the remaining 204, 76 specimens were easily identified as male and 73 as female. The remaining 55 specimens exhibited some discrepancy in the percentages of sex-chromatin positive nuclei between the investigators, so that these were re-examined in an additional hundred cells from each specimen. The result of the second examination showed that 19 specimens were male, 14 female, and in the remaining 22 the percentages of sex-chromatin positive nuclei ranged from 11 to 29. In the third examination 14 out of the 22 specimens had 11 to 20 per cent of sex-chromatin positive nuclei and 5 ranged from 21

to 29 per cent. The remaining 3 specimens showed 30 per cent of sex-chromatin positive nuclei and were determined as female. The results of the three successive examinations are summarized in the following table.

Table. Result of sex-chromatin test in 204 human embryos

Examination	Percentage of sex-chromatin positive cells				Total	Sex ratio
	1-10	11-20	21-29	30-		
First	76	—	—	73	149	104.1:100
Second	19	—	—	14	33	
Third	—	14	5	3	22	
	95			90	(185)	105.6:100
Total	95	14	5	90	204	

In the present investigation 185 out of 218 specimens (84.9%) were available for the determination of sex; namely 95 specimens were classified as male and 90 as female. Disregarding the remaining 19 cases which failed to provide unambiguous results in sex determination, this gives a ratio of 105.6 males: 100 females. Though the material here examined is too small to be conclusive, the result of our preliminary investigation may approximate the early embryonal sex ratio in the Japanese.

83. *Studies on selection in ABO blood groups*¹⁾

(By Ei MATSUNAGA, Yuichiro HIRAIZUMI, Toshiyuki
FURUSHO and Hisajiro IZUMIYAMA)

In order to investigate the selective mechanism operating on *ABO* blood groups, an extensive survey was carried out in the City of Ohdate, in Akita Prefecture, in collaboration with the local government and the Ohdate Health Center. About 3,400 couples with wife ranging in age from 30 to 40 years at the time of the investigation, were located irrespective of whether they had children or not. With the aid of some twenty trained midwives who were acquainted with the individual families, information with respect to reproductive histories was obtained by interviewing the wives. An effort was made to examine the blood groups

1) This work was supported by a grant (RF 61113) from the Rockefeller Foundation.

Table 1. Summary of pooled data, showing segregation of blood groups in children.

A. Compatible matings

Mating ♀ × ♂	No. of matings	Childless couples		No. of living children					
		No.	Proportion	O	A	B	AB	Unexamined	Total
A × A	256	10	0.039	103	531	—	—	26	660
B × B	159	2	0.013	70	—	325	—	19	414
AB × AB	18	0	0	—	8	16	18	3	45
A × O	287	13	0.045	289	401	—	—	29	719
B × O	204	6	0.029	211	—	308	—	14	533
AB × O	75	1	0.013	—	96	99	—	11	206
AB × A	67	2	0.030	—	84	44	41	9	178
AB × B	48	0	0	—	33	63	24	8	128
O × O	275	7	0.025	692	—	—	—	23	715
Total	1389	41	0.030	1365	1153	855	83	142	3598

B. Incompatible matings

O × A	274	5	0.018	258	435	—	—	20	713
O × B	215	7	0.033	214	—	304	—	18	536
O × AB	78	4	0.051	—	98	91	—	7	196
B × A	162	3	0.019	78	108	81	126	14	407
A × B	202	7	0.035	93	123	115	190	21	542
A × AB	81	6	0.074	—	117	29	57	8	211
B × AB	44	2	0.045	—	28	49	32	2	111
Total	1056	34	0.032	643	909	669	405	90	2716

of as many couples and as many children as possible. Thus, appropriate data were collected from about 2,500 couples including some six thousand children. Some results of preliminary nature are summarized in Tables 1 and 2.

As to segregation ratio of the blood groups of children, comparisons between two reciprocals in $A \times O$, $B \times O$, $AB \times A$ and $AB \times B$ matings, respectively, revealed no significant deficit of incompatible children. There were no differences in the proportion of childless couples or in the mean number of living children between compatible and incompatible mating groups. The mean number of postnatal deaths of children was almost the same in these two mating groups.

As was anticipated from the general trend toward a sudden increase

Table 2. Summary of pooled data, showing mortality of children.
Proportions refer to the mean number per mating

A. Compatible matings

Mating	Mean no. of living children	Mortality of children				Mean no. of pregnancies, excluding induced abortions
		Postnatal		Prenatal		
		No.	Proportion	No.	Proportion	
A × A	2.578	57	0.223	50	0.195	2.996
B × B	2.604	61	0.384	43	0.270	3.258
AB × AB	2.500	3	0.167	7	0.389	3.056
A × O	2.505	108	0.376	70	0.244	3.125
B × O	2.613	72	0.353	51	0.250	3.217
AB × O	2.747	37	0.493	30	0.400	3.640
AB × A	2.657	16	0.239	17	0.254	3.149
AB × B	2.667	17	0.354	17	0.354	3.375
O × O	2.600	105	0.382	69	0.251	3.233
Total	2.590	476	0.343	354	0.255	3.188

B. Incompatible matings

O × A	2.602	75	0.274	59	0.215	3.091
O × B	2.493	81	0.377	62	0.288	3.153
O × AB	2.593	24	0.308	18	0.231	3.051
B × A	2.512	55	0.340	43	0.264	3.117
A × B	2.683	62	0.307	53	0.262	3.252
A × AB	2.605	26	0.321	23	0.284	3.210
B × AB	2.523	10	0.227	8	0.181	2.932
Total	2.572	333	0.315	266	0.252	3.139

of induced abortions during the past ten years in this country, the present survey revealed that the local area here concerned was not an exception in this respect. If the answers of the wives are taken at their face value, the average frequency of such abortions was about 1.02 per wife. In most cases the wives had experienced induced abortions after they had given birth to two or more children. However, the distribution of induced abortions was homogeneous in different matings, so that this was omitted from our calculation and the numbers of prenatal deaths presented in Table 2 included only spontaneous miscarriages and stillbirths. Because of the uncertainty of marital histories these figures are

less reliable than the postnatal mortality data, but it is evident that there was no significant difference in the mean number of prenatal deaths recorded between compatible and incompatible mating.

To sum up, the statistical analysis of the pooled data so far indicated no evidence of elimination of children due to *ABO* incompatibility. The result shows a remarkable contradiction to the previous ones obtained either by segregation studies of family data published by various investigators or by fertility surveys carried out in Hokkaido. The reason for this contradiction is at present not clear: the statistical analysis provides no hints of interference of the frequent occurrence of induced abortions with a possible effect of maternal-fetal incompatibility.

Analyses of the data are in progress by means of more rigorous methods.

84. *Spontaneous and induced chromatid aberrations
in human cells cultivated in vitro*

(By Akira TONOMURA)

Breakages in chromosome regions induced by 5-bromodeoxyuridine (BUDR) treatment were analysed in cultured human cells, and compared with those occurring spontaneously. The cells obtained from a three-month-old fetus were cultured in McCoy's synthetic medium supplemented with 20% of calf serum. The three-day culture cells were treated with BUDR (25 $\mu\text{g}/\text{ml}$) for 3 and 5 days. In scoring the chromatid aberrations, the chromosomes were classified according to the generally accepted Denver system of nomenclature.

1) *Spontaneous aberrations*: In the control culture spontaneous chromatid aberrations occurred with a frequency of 0.06 breaks/cell. Most of these aberrations were found on the large chromosomes of group I. Single chromatid breaks were rarely detectable in the medium-sized submedian chromosomes of group III; some were found probably in one of the three pairs of chromosomes nos. 8-10. No single chromatid or isochromatid deletion was observed in the chromosomes of groups II and IV.

2) *Induced aberrations*: Following the treatment with BUDR for 3 and 5 days, the aberration rates increased to 0.40 to 0.63 breaks/cell. One of the most striking features of the chromosomes was the lengthening of the secondary constriction and centromeric region. In control cells a secondary constriction located in the long arm near the centromere can be detected in chromosome no. 1. In the BUDR-treated series, the secondary constriction of this chromosome was exaggerated in a large

number of cells. Similar responses were also noted in the centromeric regions of the short arm of chromosome no. 1 and of the long arm of no. 2. Single chromatid breaks were characteristic of the long arm of chromosome nos. 1, 4, 8, 9 and 11. Furthermore, an obvious chromatid breakage was observed at the middle part of the long arm in one of the three pairs of group IV.

If the specific regions with high frequency of breaks are closely associated with incorporation of BUDR into the DNA, the chromosome analysis, using such base analogues, may help in the investigation of the structure of the human chromosomes in more detail.

85. Sex-chromosome conditions in four cases of true hermaphroditism

(By Akira TONOMURA and Takeo HONDA)

The importance of sex-chromosome conditions in chromatin-negative true hermaphrodites has aroused specific interest not only in relation to the clinical features but also to the specific role of the sex-chromosomes in sex differentiation. During our current survey of human chromosomes in various types of congenital disorders and sex anomalies, the authors examined the chromosomes in four cases of true hermaphroditism whose nuclei of the oral mucosa cells showed no sex-chromatin bodies. The clinical and histopathological examinations were carried out at the Department of Urology, Faculty of Medicine, University of Tokyo. The results are summarized briefly in the following table 1.

The number of chromosomes and karyotypes from each case were examined in cultured leucocytes from peripheral blood. All of the cells here examined showed 46 chromosomes. They were found to contain 5

Table 1. Some clinical and pathological data in four patients under study

Case no.	Age	Rearing Sex	Urethra	Vaginal opening	Uterus	Gonad	
						Right	Left
1	12	M	perineoscrotal hypospadias	present separately	present	testis	ovary
2	13	M	penoscrotal hypospadias	"	well-developed	"	"
3	18	M	"	in the posterior urethra	rudimentary	ovo-testis	testis
4	6	M	"	no opening	small	ovary	"

small acrocentric chromosomes in group VII and 15 medium-sized submedian chromosomes in group III. The *Y* chromosome was readily identified as the largest one in group VII. It is, therefore, beyond doubt that the present four true hermaphrodites have male chromosomal constitutions with an *XY* complex.

So far as we know, the presence of cells with *XY* complex in true hermaphrodites has been recorded only in *XO/XY* and *XX/XY* mosaics. The latter case, however, showed a chromatin-positive pattern and was interpreted as another possibility of *XX/Xx* mosaicism (Waxman *et al.*, 1962). One of the *XO/XY* mosaics revealed a partial deletion of the *Y* chromosome (Conen *et al.*, 1961). It is, therefore, a possibility of mosaicism in tissues from different parts of the body but the possibility of undetectable small deletion on the *Y* chromosome is not eliminated in the present cases. On morphological basis, however, it does not seem possible to prove the latter possibility. Consequently, the present four cases may be concluded to be the first examples of chromatin-negative true hermaphroditism in which cells with *XY* complex are predominant.

86. *Character of modified human hemoglobin*

(By Tomotaka SHINODA);

In order to obtain a fundamental information on the biological function of the prosthetic groups in native protein in relation to its chemical structure and reactivity to chemical agents, native human hemoglobin (*Hb-A*) was used as the model substance.

Crystallized *Hb-A* was treated with trinitrobenzenesulfonic acid (TNBS), which was previously found by the author to be an effective modifying agent of primary amino group in aqueous solution. Under several conditions employed, three steps of the reaction were observed. At the first step, β -*di-TNP-HbA* was produced, at the second α , β -tetra *TNP-HbA* and at the third step α , β octa-*TNP-HbA* appeared. Thus modified hemoglobin has a specific *UV*-absorption spectrum of trinitrophenylated protein (λ max 355 $m\mu$, pH 6.8) in addition to that within the range of 500 $m\mu$ to 600 $m\mu$ which corresponds to absorption normally observed in native hemoglobin.

However, this modified substance was homogeneous in agar-gel and starch-gel electrophoresis or *CM*-cellulose column chromatography. Its oxygen affinity was decreased, and it was unstable in acidic medium, being easily changed into methemoglobin. In the alkaline denaturation test, it was also less stable compared with untreated hemoglobin. By

dissociation-recombination experiment of the *TNP-HbA* with normal one, formation of new hemoglobin molecules, which were assumed to be $\alpha_2^A\beta_2^{TNP}$ and $\alpha_2^{TNP}\beta_2^A$, was observed electrophoretically. From structural analyses, the following results were obtained.

i) β -*di-TNP-HbA* corresponded to hemoglobin in which lysine No. 144 placed in the β -chain was modified with TNBS.

ii) α , β -tetra *TNP-HbA* was the substance in which the terminal amino group of the α -chain and lysine No. 144 in the β -chain were modified.

iii) α , β -octa-*TNP-HbA* was the substance in which additional trinitrophenylation occurred on lysine No. 60 or 61 in α -chain and No. 65 or 66 in β -chain.

To follow up this conclusion, an experiment on hemoglobins from different species is now in progress. Details of this work will be published elsewhere.

BOOKS AND PAPERS PUBLISHED IN 1962
BY STAFF MEMBERS

- CHIGUSA, S. and T. MUKAI 1962 Two cases of heterosis associated with the recessive genes *sepia* (*se*) and *expanded wing* (*ew*) in *D. melanogaster* (in Japanese). Jap. J. Genet. **37**: 381-382
- ENDO, T. 1962 Inheritance of anthocyanin concentration in flowers of *Torenia fournieri*. Jap. J. Genet. **37**: 284-290
- 1962 Biochemical genetics of flower color variation (in Japanese). Nucleus and Cytoplasm **4**: 30-35
- FUJII, T. 1962 Radiosensitivity in plants. V. Experiments with several cultivated and wild rices. Jap. J. Breed. **12**: 63-68
- 1962 Chlorophyll mutations in einkorn wheat induced by radiation. Seiken Zihô **13**: 138-143
- 1962 Chlorophyll mutations induced by radiation in einkorn wheat and their occurrence in clusters. Seiken Zihô **14**: 1-11
- 1962 A red-stem mutant in einkorn wheat induced by X-rays. Seiken Zihô **14**: 51-53
- HINATA, K. and H. I. OKA 1962 A survey of hybrid sterility relationships in the Asian forms of *Oryza perennis* and *O. sativa*. Jap. J. Genet. **37**: 314-328
- and ——— 1962 Some considerations on the evolutionary dynamics of cultivated rice. Jap. J. Genet. **37**: 329-342
- HIRAZUMI, Y. 1962 Low viability induction by the segregation-distorter (SD) locus; preliminary note. D. I. S. **36**: 77
- 1962 Distorted segregation and genetic load. Jap. J. Genet. **37**: 147-154
- NOVITSKI, E., ——— and E. MATSUNAGA 1962 Meiotic drive. Science **137**: 861-862
- IINO, T. 1962 Curly flagellar mutants in Salmonella. J. Gen. Microbiol. **27**: 167-175
- ISHIWA, H., E. RYO and S. KONDO 1962 Synergetic action of ³²P transmutation and radiations on lethal effect in Salmonella (in Japanese). Jap. J. Genet. **37**: 389-390
- KATAYAMA, T. C. 1962 Some considerations on rice cultivation in New Guinea. Jap. J. Trop. Agr. **5**: 80-86
- KIHARA, H. 1962 Comparative gene analysis in wheat and its relatives. Jap. J. Genet. **37**: 363-373
- and K. TSUNEWAKI 1962 Polyploids and aneuploids of *Triticum dicoccum* var. Khapli produced by N₂O-treatment. W. I. S. **14**: 1-3
- and ——— 1962 Use of an alien cytoplasm as a new method of producing haploids. Jap. J. Genet. **37**: 310-313
- , T. C. KATAYAMA and K. TSUNEWAKI 1962 Floating habit of 10 strains of wild and cultivated rice. Jap. J. Genet. **37**: 1-9
- KIMURA, M. 1961 Some calculations on the mutational load. Proc. Symp. Genetic Effect of Radiation. Jap. J. Genet. **36** (Suppl): 179-190

- 1962 On the probability of fixation of mutant genes in a population. *Genetics* **47**: 713-719
- 1962 A suggestion on the experimental approach to the origin of supernumerary chromosomes. *Amer. Nat.* **96**: 319-320
- KONDO, S. 1961 Response of silver-activated phosphate glass to α -, β - and γ -rays and neutrons. *Health Phys.* **7**: 25-35
- 1962 Physical aspects of radiation biology (in Japanese). *Tanpakushitsu Kakusan-Koso* **7**: 94-102
- KURITA, Y. 1962 Viren als Ursache von Leukämien (in Japanese). *Iden* **16**: 15-19
- LILIENFELD, F. A. 1962 Plastid behavior in reciprocally different crosses between two races of *Medicago truncatula* Gaertn. *Seiken Zihô* **13**: 3-38
- MATSUMURA, S. (ed.) 1962 Environment-controlled growth rooms in Japan. *Comm. Environ.-Cont. Growth Rooms*, Tokyo: pp. 67
- 1962 Radiation genetics in wheat. VII. Comparison of radiation effects of beta- and gamma-rays on diploid wheat. *Radiation Botany* **1**: 155-165
- 1962 Radiation-induced variegations in Einkorn wheat and their inheritance (Jap. with Eng. summ.). *Seiken Zihô* **13**: 75-88
- 1962 RBE and dose rate for radiation effects in wheat. *Seiken Zihô* **13**: 155-159
- , T. FUJII and T. MABUCHI 1962 Relation between radiation effects and dose rates in rice (in Japanese). *Proc. 3rd Conf. Radioisotopes*, Tokyo: 1064-1067
- , S. KONDO and T. MABUCHI 1962 Boron effects upon gamma-ray and thermal neutron irradiations in einkorn wheat; RBE of heavy particles from $^{10}\text{B}(n, \alpha)^7\text{Li}$ reaction. *Seiken Zihô* **14**: 86-92
- and T. MABUCHI 1962 Relation between radiation effects and dose rates of gamma-rays in rice and einkorn wheat. *Seiken Zihô* **14**: 66-73
- OHTA, T. and ——— 1962 Cytological observations on chemically induced male sterility in sugar beet (Jap. with Eng. summ.). *Seiken Zihô* **14**: 53-56
- MATSUNAGA, E. and Y. HIRAIZUMI 1962 Prezygotic selection in ABO blood groups. *Science* **135**: 432-434
- 1962 Selective mechanisms operating on ABO blood groups. *HOMO* **13**: 73-81
- 1962 Some evidences of heterozygote advantage in the polymorphism of MN blood groups. *Proc. 8th Congr. Int. Soc. Blood Transf.*, Tokyo 1960: 126-130
- and K. MURAI 1962 Genetic study of haptoglobin types in a Japanese population. *Proc. 8th Congr. Int. Soc. Blood Transf.*, Tokyo 1960: 397-400
- 1962 The dimorphism in human normal cerumen. *Ann. Human Genet.* **25**: 273-286
- 1962 Selective mechanisms operating on ABO and MN blood groups with special reference to prezygotic selection. *Eugen. Quart.* **9**: 36-43
- 1962 Population genetics on the selection due to ABO incompatibility between mother and fetus (in Japanese). *Jap. J. Human Genet.* **7**: 1-9
- 1962 An inert allele Hp^0 at the Hp locus. *Jap. J. Human Genet.* **7**: 133-136

- , K. MURAI and E. MATSUDA 1962 Inheritance of haptoglobin types in 51 Japanese families. *Acta genet.* **12**: 262-280
- MORISHIMA, H., K. HINATA and H. I. OKA 1962 Comparison between two cultivated rice species, *Oryza sativa* L. and *O. glaberrima* Steud. *Jap. J. Breed.* **12**: 153-165
- , ———, and ——— 1962 Floating ability and drought resistance in wild and cultivated species of rice. *Indian J. Genet. and Pl. Breed.* **22**: 1-11
- MORIWAKI, K. 1962 Activity change of phosphatases in the skin of hairless mutant mice during development. *Physiol. Zool.* **35**: 193-200
- MUKAI, T. 1962 A study of the genetic structure of natural populations in *Drosophila melanogaster* by means of spontaneous polygenic mutation rates 1. Theoretical and statistical. *Proc. Jap. Acad.* **38**: 741-746
- 1962 On the mutation rates (in Japanese). *Iden* **16**: 19-23
- and A. B. BURDICK 1962 Behavior of sex-linked recessive lethals in populations. *Seiken Zihô* **14**: 35-44
- and S. CHIGUSA 1962 Radiation-induced mutation rates of polygenes controlling the number of sternopleural bristles in *D. melanogaster*. *D. I. S.* **36**: 93-94
- and S. CHIGUSA 1962 Spontaneous mutation rate of polygenes controlling the viability, and the genetic structure of natural populations in *D. melanogaster* (in Japanese). *Jap. J. Genet.* **37**: 400
- and ——— 1962 A study of the genetic structure of natural populations in *Drosophila melanogaster* by means of spontaneous polygenic mutation rates 2. Experimental. *Proc. Jap. Acad.* **38**: 747-752
- , ——— and I. YOSHIKAWA 1962 A radiation genetical consideration concerning the genetic structure of natural populations in *Drosophila melanogaster*. Abstracts of the papers presented in the 4th meeting of The Japan Radiation Research Society: 41-42
- , I. YOSHIKAWA and S. CHIGUSA 1962 Radiation-induced mutation rate of polygenes controlling the sternopleural bristle number in *Drosophila melanogaster* (in Japanese). *Jap. J. Genet.* **37**: 400
- NARISE, T. 1962 Studies on competition in plants and animals, X. Genetic variability of migratory activity in natural populations of *Drosophila melanogaster*. *Jap. J. Genet.* **37**: 451-461
- NAWA, S. and H. S. FORREST 1962 Synthesis of the yellow pteridine, isopepiapterin. *Nature*, **196**: 169-170
- and ——— 1962 Structures of sepiapterin and isosepiapterin. *Nature* **196**: 372-373
- NISHIKAWA, K. 1962 Hybrid lethality in crosses between Emmer wheats and *Aegilops squarrosa*, II. Synthesized 6x wheats employed as test varieties. *Jap. J. Genet.* **37**: 227-236
- 1962 Hybrid lethality in crosses between Emmer wheats and *Aegilops squarrosa*, III. Gene analysis of Type 2 necrosis. *Seiken Zihô* **14**: 45-50
- HIRAYOSHI, I., ——— and T. FUJII 1962 Studies on homologous inhibitors, III. Growth inhibitors extracted from culture solution of pea, upland- and paddy-

- rice plants. Res. Bull. Fac. Agr. Gifu Univ. **17**: 99-103
- OGAWA, Y. 1962 Temperature and development of muscle protein in *Triturus* embryos (in Japanese). Kagaku **32**: 34
- 1962 Growth, differentiation and regeneration XXVIII. Influence of temperature on the synthesis of myosin in early *Triturus* embryo (in Japanese). Med. and Biol. **62**: 4-6
- 1962 Growth, differentiation and regeneration XXIX. Effect of sodium-glucuronate on the synthesis of contractile protein, myosin, in the X-irradiated *Triturus* embryo (in Japanese). Med. and Biol. **62**: 33-36
- 1962 Abnormal taste response to phenyl-thio-carbamide in Japanese (in Japanese). Med. and Biol. **62**: 116-119
- 1962 Protective action of sodium glucuronate in abnormal differentiation of skeletal muscle tissue in the X-irradiated *Triturus* embryo. Proc. of the 7th Symp. on Glucuronic acid 128-137
- and H. WATANABE 1962 Growth, differentiation and regeneration XXX: Effects of *p*-hydroxy-benzyl-methyl-ether, a component of *Citrullus colocynthis*, on the growth and differentiation of *Triturus* embryo (in Japanese). Med. and Biol. **63**: 1-5
- 1962 Synthesis of skeletal muscle proteins in early embryos and regenerating tissue of chick and *Triturus*. Exptl. Cell Research **26**: 269-274
- 1962 Biochemical relation between actin and myosin syntheses during the differentiation of skeletal muscle tissue of *Triturus*. Exptl. Cell Research **26**: 526-533
- 1962 Hyper sensitive and sweet taste response to phenyl-thio-carbamide (PTC) (in Japanese). Med. and Biol. **63**: 57-60
- 1962 Growth, differentiation and regeneration XXXI. Similarity of the response to X-ray of myosin synthesis in early embryo and regenerating limb tissue of *Triturus* (in Japanese). Med. and Biol. **63**: 112-115
- 1962 Influence of temperature on the syntheses of contractile proteins in *Triturus* embryos. Nature **194**: 978-979
- OKA, H. I. 1961 A review of rice culture in Tropical America (in Japanese). Jap. J. Trop. Agr. **5**: 32-35
- and W. T. CHANG 1962 Rice varieties intermediate between wild and cultivated forms and the origin of the Japonica type. Bot. Bull. Acad. Sinica **3**: 109-131
- and Y. DOIDA 1962 Phylogenetic differentiation of cultivated rice, XX. Analysis of the genetic basis of hybrid breakdown in rice. Jap. J. Genet. **37**: 24-35
- OSHIMA, C. 1962 The persistence of some recessive lethal genes in natural populations of *Drosophila melanogaster*. II. Proc. Jap. Acad. **38**: 278-283
- 1962 The persistence of deleterious genes in natural populations of *D. melanogaster*. D. I. S. **36**: 105
- and S. MINAMORI 1962 Study of deleterious genes in natural populations of *Drosophila melanogaster*. Jap. J. Genet. **37**: 408
- 1963 The persistence of some recessive lethal genes in natural populations of *Drosophila melanogaster*. III. Proc. Jap. Acad. **39**: 125-130

- 1963 The persistence of deleterious genes in natural populations of *D. melanogaster*. *D. I. S.* **37**: 115
- SAKAGUCHI, B. and D. F. POULSON 1962 Distribution of "sex-ratio" agent in tissues of *D. willistoni*. *Genetics* **46**: 1665-1676
- 1962 Cytoplasmic factors of abnormal "sex-ratio" condition in *Drosophila* (in Japanese). *Nucleus and Cytoplasm* **3**: 27-34
- SAKAI, K. I. 1962 Recommendations for the coconut palm investigations. *The Indian Coconut Journal*. **15**: 111-112
- SAKAI, K. I. and S. IYAMA 1962 Genetics. In "Comprehensive Dictionary for Statistical Science" (ed. by NAKAYAMA, I.) (in Japanese). Toyô Keizai-Shimpô-Sha, Tokyo: pp. 536-540
- SAKAMOTO, S. 1961 *Arisaema triphyllum*, Jack-in-the-Pulpit, in Minnesota, especially at the Cedar Creek Natural History Area. *Proc. Minn. Acad. Sci.* **29**: 153-168
- SHINOHARA, M. 1962 Variation in the number of nucleoli in rice species having the AA genome (in Japanese). *Jap. J. Genet.* **37**: 243-247
- TAKENAKA, Y. 1962 Cytogenetic Studies in *Nicotiana*, XV. Reduction divisions in five interspecific hybrids between sections *Alatae* and *Suaveolentes* (in Japanese). *Jap. J. Genet.* **37**: 80-85
- 1962 Cytogenetic studies in *Nicotiana*, XVI. Reduction divisions in six interspecific hybrids between *N. tabacum* and other six species (in Japanese). *Bot. Mag. Tokyo* **75**: 237-241
- 1962 Cytogenetic studies in *Nicotiana*, XVII. Reduction divisions in five interspecific hybrids (in Japanese). *Jap. J. Genet.* **37**: 343-347
- 1962 Cytogenetic studies in *Nicotiana*, XVIII. Reduction divisions in three interspecific hybrids between autotetraploid *N. tabacum* and three other species (in Japanese). *Jap. J. Breed.* **12**: 275-277
- 1962 Cytogenetic studies in *Nicotiana*, XIX. The reduction divisions in four hybrids between *N. paniculata* and other species of section *Alatae* (in Japanese). *Jap. J. Breed.* **12**: 278-280
- 1962 Studies on the genus *Prunus*, I. The origin of *Prunus yedoensis* (in Japanese). *Bot. Mag. Tokyo* **75**: 278-287
- TATEOKA, T. 1962 Starch grains of endosperm in grass systematics. *Bot. Mag. Tokyo* **75**: 377-383
- TAZIMA, Y. 1962 The genetics of the silkworm. Logos press, London (in press)
- and S. KONDO 1962 Differential radiation-sensitivity of germ cells as a possible interpretation of sex difference in dose-rate dependence of induced mutation rates in the silkworm. Proceedings of a symposium on "Repair from genetic radiation damage and differential radiosensitivity in germ cells". Pergamon Press, Oxford (in press).
- TONOMURA, A., Y. TOYOFUKU and E. MATSUNAGA 1962 The frequency of so-called drumsticks in the polymorphonuclear neutrophil leucocytes of Japanese females. *Jap. J. Human Genet.* **7**: 60-66
- , T. HONDA and T. KURITA 1962 Chromosome abnormalities in a child with Down's syndrome and in its father. *Proc. Japan Acad.* **38**: 526-530
- TOYOFUKU, Y. 1962 *Drosophila* survey of Hokkaido, XVII. A study of chromo-

- somal polymorphism in natural populations of *Drosophila nigromaculata*. Jap. J. Genet. **37**: 291-301
- TSUJITA, M. 1962 Changes in the amount of isoxanthopterin with developmental stages of the silkworm larvae (Abst). J. Seric. Sci. Jap. **13**: 212
- 1962 Emission mechanism of alkaloids from tobacco plants (I) Changes in the amount of alkaloids with the growth of two "Bright yellow" strains, ordinary and cherry red, and emission of alkaloids from their leaves (Abst). Proc. Ann. Meet. Tokai Branch Seric. Soc. Jap. **10**: 25-26
- 1962 Emission mechanism of alkaloids from tobacco plants (II) The amount of alkaloids obtained from fresh leaves by different distilling method (Abst). Ibid: 26
- 1962 Tumour formation by multiplication of abnormal small cells on the mid-gut wall of the silkworm larvae (Abst). Ibid: 29-30
- 1962 Manifestation mechanism of yellow larval color in the silkworm, with special regard to *d-lem* gene. Jap. J. Genet. (in press)
- TSUNEWAKI, K. 1962 Monosomic analysis of synthesized hexaploid wheats. Jap. J. Genet. **37**: 155-168
- 1962 Functional differentiation among the homoeologous chromosomes of common wheat. Seiken Zihô **13**: 112-118
- 1962 Production of polyploid wheat by nitrous oxide, II. N₂O-treatment during fertilization with reference to culture media. Seiken Zihô **14**: 12-20
- and H. KIHARA 1962 Comparative gene analysis of common wheat and its ancestral species, I. Necrosis. Jap. J. Genet. **37**: 474-484
- YAMADA, Y. 1962 Genotype by environment interaction and genetic correlation of the same trait under different environments. Jap. J. Genet. **37**: 498-509
- YOSIDA, T. H., K. MORIWAKI, Y. MATANO and K. UTSUMI (1961) Cytological studies on the effect of radiation, II. Modification of frequency of X-ray induced chromatid breaks in Ehrlich tumor cells by pretreatment with dinitrophenol. Jap. J. Genet. **36** (Suppl.): 94-104

ACKNOWLEDGMENT

The editor wishes to acknowledge Dr. F. A. LILIENFELD for reading the manuscripts (Sohei KONDO).

国立遺伝学研究所年報 第13号

昭和38年8月26日 印刷

昭和38年8月31日 発行

発行者 木 原 均

国立遺伝学研究所内

編集者 近 藤 宗 平

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 国際文献印刷社
会社

東京都新宿区山吹町184

発行所 国立遺伝学研究所

静岡県三島市谷田1,111

電・(三島)(5) 0771, 0772, 3492

