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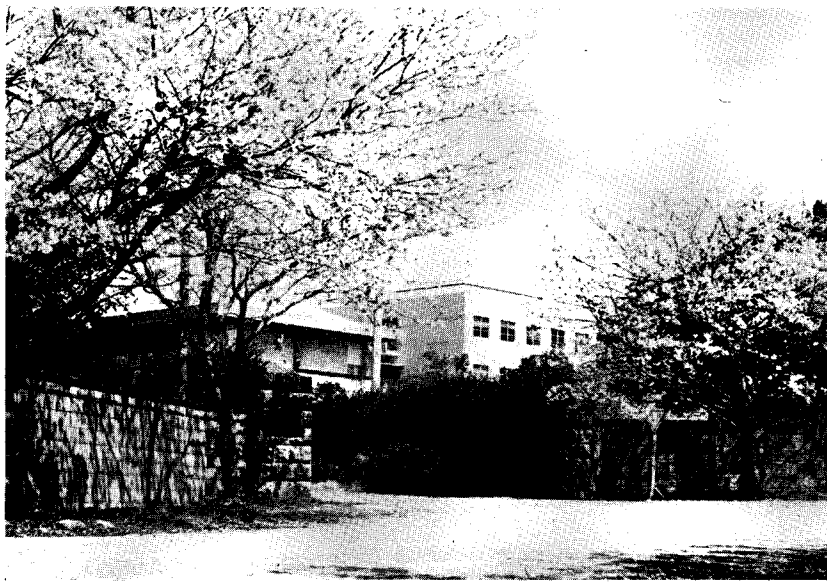
No. 16, 1965



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GENERAL STATEMENT

On the 20th of April, our Institute was honored by the visit of His Majesty the Emperor and Her Majesty the Empress. This was the second visit of His Majesty since 1954. The Emperor and the Empress have inspected our laboratories, listened to our recent progress and encouraged our staff members.

On the 20th of November we had an augural ceremony for the completion of a two-storied building which comprises on the first floor rooms for seed storage, herbarium, exhibit and research and on the second floor an auditorium with 200 seats. We are most grateful to the Rockefeller Foundation, who has given us a grant for erecting the first floor.

This year (1965) the geneticists were busy in celebrating Mendel's centennial all over the world. Our members attended various meetings as given below.

1. Impact of Mendelism on Agriculture, Biology and Medicine. New Delhi, India, February. Participant: H. KIHARA.
2. G. Mendel Memorial Symposium. Brno and Prague, Czechoslovakia, August. Participant: C. OSHIMA.
3. Mendel Centennial. Fort Collins, U.S.A., September. Participant: H. KIHARA.
4. Lectures and Exhibits in Commemoration of Mendel's Centennial. Tokyo and Kyoto, Japan, October.

In this paragraph it was our custom to mention the honors and degrees given to our members. However we will mention only the award given this year to Dr. M. KIMURA. He has received Weldon Memorial Prize from the University of Oxford for his contributions to population genetics. The prize is given every three years and is a great honor to him as well as to our Institute.

Hitoshi Kihara

STAFF

(At the End of 1965)

Director

KIHARA, Hitoshi, D. Sc., Member of Japan Academy, Emeritus Professor of Kyoto University

Members

1. Department of Morphological Genetics

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

The 1st Laboratory

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

The 2nd Laboratory

SAKAGUCHI, Bungo, D. Ag., Head of the Laboratory
SADO, Toshihiko, D. Ag.; KOBAYASHI*, Susumu

2. Department of Cytogenetics

TAKENAKA, Yô, D. Sc., Head of the Department

The 1st Laboratory

YOSIDA, Tosihide H., D. Sc., Head of the Laboratory
MORIWAKI, Kazuo, D. Sc. (in U.S.A.); OHARA*, Hiroshi, D. Sc.; IMAI*, Hirotami; FUKAYA*, Takako; TSURUTA*, Reiko

The 2nd Laboratory

TAKENAKA, Yô, D. Sc., Head of the Laboratory
YONEDA, Yoshiaki, D. Sc. (in U.S.A.)

3. Department of Physiological Genetics

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The 1st Laboratory

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WATANABE*, Takao, K.

The 2nd Laboratory

TAIRA, Toshifumi, D. Sc., Head of the Laboratory
SAKAMOTO, Sadao, D. Ag.; KATAYAMA*, Tadao C., D. Ag.; NAKAI*, Yasuo; HORI*, Tadaaki

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

4. *Department of Biochemical Genetics*

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

The 1st Laboratory

NAWA, Saburo, D. Sc., Head of the Laboratory
YAMADA, Masa-aki; UDA*, Fumiaki

The 2nd Laboratory

OGAWA, Yoshito, M. D., Head of the Laboratory
ENDO, Toru, D. Ag. (in U.S.A.)

The 3rd Laboratory

TSUJITA, Mitsuo, D. Ag., Head of the Laboratory
SAKURAI, Susumu; NARISE*, Sumiko, M. D.

5. *Department of Applied Genetics*

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

The 1st Laboratory

SAKAI, Kan-Ichi, D. Ag., Head of the Laboratory
KAWAHARA, Takatada, D. Ag.; FUJISHIMA, Tohru;
NARISE*, Takashi, D. Sc.; INOUE*, Teruo

The 2nd Laboratory

SAKAI, Kan-Ichi, D. Ag., Head of the Laboratory
IYAMA, Shin-ya, D. Ag.; SHIMAMOTO*, Yoshiya;
EL-BALAL*, Mohamed S.; HAYASHI*, Shigesuke;
MORIMURA*, Katsuyoshi; TOMITA*, Koji; TSUZAKI*, Kazuo;
WASANO*, Kikuwo

The 3rd Laboratory

OKA, Hiko-Ichi, D. Ag., Head of the Laboratory
MORISHIMA-OKINO, Hiroko, D. Ag., CHU*, Yaw-En

6. *Department of Induced Mutation*

MATSUMURA, Seiji, D. Ag., Head of the Department

The 1st Laboratory

TUTIKAWA, Kiyosi, Acting Head of the Laboratory
MUKAI, Terumi, Ph. D., D. Sc. (in U.S.A.)

The 2nd Laboratory

MATSUMURA, Seiji, D. Ag., Head of the Laboratory
FUJII, Taro, D. Ag.

The 3rd Laboratory

MATSUMURA, Seiji, D. Ag., Head of the Laboratory
IKENAGA, Mituo; MABUCHI, Tomoo

---

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

7. Department of Human Genetics

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

The 1st Laboratory

MATSUNAGA, Ei, M. D., D. Sc., Head of the Laboratory
OISHI, Hidetsune; SHINODA, Tomotaka (in U.S.A.)

The 2nd Laboratory

TONOMURA, Akira, D. Sc., Head of the Laboratory
KIKUCHI, Yasumoto, D. Sc.; OHDO*, Shozo

8. Department of Microbial Genetics

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

The 1st Laboratory

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

The 2nd Laboratory

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

9. Department of Population Genetics

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

The 1st Laboratory

KIMURA, Motoo, Ph. D., D. Sc., Head of the Laboratory
HIRAIZUMI, Yuichiro, D. Sc. (in U.S.A.)

10. Experimental Farm

MATSUMURA, Seiji, D. Ag., Head of the Farm
MIYAZAWA, Akira

Honorary Members and Part-Time Staff

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

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

LILIENFELD, Flora A., Ph. D.

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

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

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

Department of Administration

MORINAGA, Norihiro, Head of the Department
KANAMORI, Shigeru, Chief of the General Affairs Section
TANAKA, Mutsuo, Chief of the Finance Section

Association for Propagation of the Knowledge of Genetics

KIHARA, Hitoshi, President, Director of the Institute
TAKENAKA, Yô, Managing Director, Head of the Cytogenetics Department
TAZIMA, Yataro, Managing Director, Head of the Morphological Genetics Department
MATSUMURA, Seiji, Manager, Head of the Induced Mutation Department
SINOTO, Yosito, Manager, Professor of International Christian University
WADA, Bungo, Manager, Emeritus Professor of Tokyo University

COUNCIL

OKADA, Yô, Chairman, Director of National Science Museum
MORIWAKI, Daigoro, Vice Chairman, Professor of Tokyo Metropolitan University
FURUHATA, Tanemoto, Director of Scientific Research Institute of Police
IMAI, Tomizo, Director of National Institute of Agricultural Sciences
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MAKINO, Sajiro, Professor of Hokkaido University
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SAKATA, Takeo, President of T. Sakata Company
TACHI, Minoru, Director of Institute of Population Problems
TSUDA, Kyosuke, Director of Institute of Applied Microbiology, Tokyo University
TSUKAMOTO, Kempo, Director of National Institute of Radiological Sciences
WADA, Bungo, Emeritus Professor of Tokyo University

PROJECTS OF RESEARCH FOR 1965

Department of Morphological Genetics

Genetics of the silkworm (TAZIMA and ONIMARU)
Studies on dose-rate dependence of radiation-induced mutation rates (TAZIMA, SADO and ONIMARU)
RBE of radiations for induced mutation frequency in the silkworm (MURAKAMI)
Chemical mutagenesis in the silkworm (TAZIMA and ONIMARU)
Hereditary infections in *Drosophila* (SAKAGUCHI, OISHI, K. and KOBAYASHI)
Genetical and embryological studies in insects (SAKAGUCHI and SADO)

Department of Cytogenetics

Cytogenetical studies on tumor cells (YOSIDA, KURITA, OHARA, IMAI, FUKAYA, TSURUTA and MORIWAKI)
Mechanism of chromosomal abnormalities by treatment with chemicals (YOSIDA, KURITA, TSURUTA and MORIWAKI)
Study on chromosomal polymorphism of Muridae (YOSIDA, FUKAYA, MORIGUCHI and NAKAMURA)
Experimental breeding and genetics of mice and rats (YOSIDA, KURITA, SAKAKIBARA, MORIGUCHI and MORIWAKI)
Correlation between taxonomy and karyology of ants (IMAI and YOSIDA)
Interspecific hybridization in *Nicotiana* (TAKENAKA)
Genetics of *Pharbitis nil* (TAKENAKA)
Origin of *Prunus Lannesiana* (TAKENAKA)
Cytogenetics of *Oryza* species (TAKENAKA, YONEDA and CHU)
Morphological and genetical studies on some plant tumors (TAKENAKA, YONEDA and CHU)

Department of Physiological Genetics

Genetic studies on insecticide resistance in *Drosophila pseudoobscura* (OSHIMA)
Population genetics of deleterious genes in natural populations of *Drosophila melanogaster* (OSHIMA and WATANABE, T. K.)
Studies on chromosomal aberrations of natural populations of *Drosophila melanogaster* (OSHIMA and WATANABE, T.)
Eye-pigment formation in *Drosophila* (TAIRA)

Cytodifferentiation in *Drosophila* fatbody (TAIRA)
Nucleotide-pool in insects (TAIRA)
Genetical studies on isozymes in *Drosophila* (TAIRA)
Nucleus substitution in wheat and related species (KIHARA)
Comparative gene analysis with reference to the origin of wheat (KIHARA and TSUNEWAKI)
Geographical distribution of necrosis genes in wheat (TSUNEWAKI and NAKAI)
Genetic studies of wheat aneuploids (TSUNEWAKI)
Cytogenetic studies in the tribe Triticeae (SAKAMOTO)
Genetic bases of ecological differentiation in *Agropyron* (SAKAMOTO)
Collection and preservation of *Oryza* species (KIHARA)
Morphological studies of *Oryza* (KIHARA and KATAYAMA)
Investigation of photoperiodic responses of *Oryza* species (KATAYAMA)

Department of Biochemical Genetics

Studies on transformation in higher organisms (NAWA, YAMADA and TSUJITA)
Genetical and biochemical studies of pteridine metabolisms in insects (NAWA and TSUJITA)
Studies on a gene for retarded moulting (*rm*) in the silkworm (TSUJITA)
Studies on the chromogranule formation in larval hypodermal cells of the silkworm (TSUJITA and SAKURAI)
Analysis of genetic action on cell differentiation in higher organisms (TSUJITA and NAWA)
Biochemical studies on the differentiation of muscle proteins in animals (OGAWA)
Biochemical studies on the mechanism of cell division in animals (OGAWA)
Genetical and biochemical studies of plant virus (OGAWA)
Comparative studies on seed proteins of rice plant by electrophoretic analysis (SAKURAI)
Genetics on isozymes in plants (ENDO)

Department of Applied Genetics

Studies on developmental instability in poultry (SAKAI, KAWAHARA and FUJISHIMA)
Quantitative genetic studies in poultry (KAWAHARA, FUJISHIMA and INOUE)
Theoretical studies on breeding techniques (SAKAI and IYAMA)
Studies on competition in plants and animals (SAKAI, IYAMA, FUJISHIMA and NARISE)

Estimation of genetic parameters in forest trees (SAKAI, HAYASHI and TOMITA)
Developmental genetics of quantitative characters in plants (SAKAI, SHIMAMOTO, El-BALAL, MORIMURA and WASANO)
Genetic studies on developmental instability in plants (SAKAI and SHIMAMOTO)
Studies on the effects of linkage disequilibrium in *Drosophila* populations (IYAMA)
Genetic studies of isolating barriers in *Oryza* (OKA and CHU)
Survey of geographical variation in *Oryza perennis* (MORISHIMA and OKA)
Experiments on natural selection in wild and cultivated rice forms (MORISHIMA and OKA)
Analysis of sterility genes in *Oryza* (OKA and MORISHIMA)
Analysis of genetic plant types (MORISHIMA and OKA)

Department of Induced Mutation

Radiation genetics of mice (TUTIKAWA)
Population genetics of *Drosophila* (MUKAI)
Studies on the effects of irradiation on populations (MUKAI)
Radiation genetics of cereals (MATSUMURA, FUJII and MABUCHI)
Radiation genetics of *Arabidopsis* (FUJII)
Radiation genetics and its practical application (MATSUMURA and MABUCHI)
Biophysical studies of radiation genetics (IKENAGA and KONDO)
Radiation dosimetry (IKENAGA)

Department of Human Genetics

Genetics of retinoblastoma (MATSUNAGA)
Down's syndrome in Japan (MATSUNAGA, TONOMURA and OISHI, H.)
Cytogenetics in man (TONOMURA, OISHI, H. and KIKUCHI)
DNA replication in human chromosomes (KIKUCHI)
Biochemical studies on plasma proteins and enzymes (SHINODA)
Chemical modification of ribonucleic acid and their constituents (SHINODA)

Department of Microbial Genetics

Immunogenetics of *Salmonella* (IINO, ENOMOTO, YAMAGUCHI and MITANI)
Genetics of motility in bacteria (ENOMOTO)
Genetics of cellular regulatory mechanisms (SUZUKI, H., ISHIDSU and SUZUKI, Y.)

Genetic fine structure analysis on microorganisms (ISHIDSU and IINO)

Department of Population Genetics

Theoretical studies of population genetics (KIMURA)
Uses of computers in the theoretical studies of population genetics (KIMURA)
Effects of radiation-induced mutation on fitness (HIRAIZUMI)
Populational implications of meiotic drive with special reference to the *SD* locus in *D. melanogaster* (HIRAIZUMI)

FOREIGN VISITORS IN 1965

Jan.	8	LI, S., Taiwan Sugar Experiment Station, Taiwan
Jan.	8	CHENG, M. C., Taiwan Sugar Experiment Station, Taiwan
Jan.	30	BANDURSKI, R. S., Dept. of Botany, Michigan State College, Mich., U.S.A.
Feb.	9	ROBINSON, H. F., Institute of Biological Sciences, North Carolina State Univ., N. C., U.S.A.
Mar.	1-2	ZELLE M. R., Division of Biological and Medical Research, Argonne National Laboratory, Ill., U.S.A.
Mar.	5-6	GUSTAFSSON, Å., Institute of Genetics, Royal College of Forestry, Stockholm, Sweden
Mar.	5-6	KONZAK, C. F., Agronomy Dept., Washington State Univ., Wash., U.S.A.
Apr.	3	FU, T. T., Ministry of Economic, Republic of China Govenment, Taiwan
Apr.	5	SAUNDERS, J. W., Dept. of Biology, Marquette Univ., Wis., U.S.A.
Apr.	5	RUBEN, L. N., Dept. of Biology, The Reed Institute, Ore., U.S.A.
Apr.	5	STERN, H., Dept. of Botany, Univ. of Illinois, Ill., U.S.A.
Apr.	5	SCHNEIDERMAN, H. A., Dept. of Biology, Western Reserve Univ., Ohio, U.S.A.
Apr.	15	SHEBESKI, L. H., Dept. of Plant Science, Univ. of Manitoba, Manitoba, Canada
Apr.	19-20	MICHAELIS, P., Max-Planck-Institut für Züchtungsforschung, Abteilung für Plasmavererbung, 5 Köln-Vogelsang, West Germany
May	8-13	SAMPATH, S., Central Rice Research Institute, Cuttack, India

May	10	KOJIMA, K., North Carolina State Univ., N. C., U.S.A.
May	25	AFRIKIAN, E. G., General Microbiology, Antibiotics Institute of Microbiology, Academy of Sciences of the Armenian S.S.R. Charenty Street 19, Erevan, U.S.S.R.
June	8-9	MCCLUNG, A. C., International Rice Research Institute, Los Baños, Philippines
June	16	SVED, J., Dept. of Genetics, Univ. of Adelaide, South Australia
June	21	OJIMA, M., Institute Agronômico, Secaô Frutas de Clima Temperado, Sâo Paulo, Brasil
June	25	LAMBERS, H.B., Wageningen Agricultural Univ., Wageningen, Netherlands
July	8	LEE, I. S., Univ. of Seoul, Korea
July	8	LEE, S. C., Institute of Plant Circumstances, Korea
July	9	VELTMAN, P. L., Vice President, W. R. Grace & Co., Clarksville, Md., U.S.A.
July	19	OLAH, L., Biology Dept., Southern Illinois Univ., Ill., U.S.A.
July	31	NEEL, J. V., Univ. of Michigan, Medical School, Mich., U.S.A.
Aug.	7	GLASS, B., State Univ. of New York at Stony Brook, N. Y., U.S.A.
Aug.	7	GLASS, S. S., Baltimore, Md. and Stony Brook, N. Y., U.S.A.
Aug.	7	WELCK, C. A., Michigan State Univ., Mich., U.S.A.
Sep.	1	LÜERS, H., Institut für Genetik, Freie Univ. Berlin, West Germany
Sep.	1	LÜERS, T., Institut für Genetik, Freie Univ. Berlin, West Germany
Sep	10	BEACHELL, H. M., International Rice Research Institute, Los Baños, Philippines
Sep.	11	SCHICK, R., Institut für Pflanzenzüchtung, Deutsche Akademie der Landwirtschaftswissenschaften, Berlin, East Germany
Sep.	27	LI, H. W., Institute of Botany, Academia Sinica, Nangkang, Taipei, Taiwan
Sep.	29	LUGG, J. W. H., Dept. of Biochemistry, Univ. of Western Australia, Nedland, Western Australia
Oct	23	MARAMOROSCH, K., Boyce Thompson Institute for Plant Research, N. Y., U.S.A.
Nov.	9	KOU, K. E., Taipei District Agricultural Improvement Station, Taiwan
Nov.	9	POUN, T. T., Taiwan Agricultural Research Institute, Taiwan
Nov.	9	JAN, S. C., Chia-i Branch Station, Taiwan Agricultural Research Institute, Taiwan

Nov. 9	TSUAN, S. R., Tainan District Agricultural Improvement Station, Taiwan
Nov. 9	HON, C. T., Taichung District Agricultural Improvement Station, Taiwan
Nov. 12	KAIJSER, K., Paediatic Dept. of Central Hospital of Eskilstuna, Sweden
Nov. 13	LENZ, W., Institut für Humangenetik der Univ., Münster, West Germany
Nov. 15	WEICKER, H., Institut für Humangenetik der Univ., Bonn, Germany
Nov. 15	FLATZ, G., Univ.-Kinderklinik und Poliklinik, Bonn, West Germany
Nov. 16	DAY, R. I., Planned Parenthood-World Population, U.S.A.
Nov. 18	JENNINGS, P. R., International Rice Research Institute, Los Baños, Philippines
Nov. 18	TANAKA, A., International Rice Research Institute, Los Baños, Philippines
Nov. 19	HULSE, F. S., Dept. of Anthropology, Univ. of Arizona, Ariz., U.S.A.
Dec. 8	TOMES, M. L., Dept. of Botany and Plant Pathology, Purdue Univ., Ind., U.S.A.

RESEARCHES CARRIED OUT IN 1965

A. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF ANIMALS

1. Distribution and persistence of lethal genes in natural populations of Drosophila melanogaster[1)]

Chozo OSHIMA and Takao WATANABE

Many male flies were captured simultaneously in October of the past two years from large natural populations in five different graperies of the Kofu and Katsunuma territory. The frequencies of complete recessive lethal chromosomes were 17.06% and 19.46% in isolated about 700 second chromosomes from each different male fly sampled in 1963 and 1964 respectively.

A series of allelism tests between 188 lethal genes were performed by a total of 16,126 crosses and the results are given in Table 1.

Table 1. Results of allelism test between lethal genes isolated from Kofu and Katsunuma populations in 1963 and 1964.

Year	1963				1964			
Population	within KF	within KN	between KF & KN	whole territory	within KF	within KN	between KF & KN	whole territory
No. of lethal chromosomes	61	53	114	114	31	43	74	74
Allelic rate (%)	3.28	2.18	2.47	2.64	3.87	3.65	4.13	3.92
	between old and new lethals							
No. of lethal chromosomes	97							72
Allelic rate (%)	2.65							

KF: Kofu population, KN: Katsunuma population

Six lethal genes were found to be distributed over a wide area (15×3 km^2) of the Kufu and Katsunuma territory and their frequencies had been highly stable during two years as given in Table 2.

1) These works (1~5) were supported by a grant from the Japan Society for the Promotion of Science as part of the Japan-U.S. Cooperative Science Program.

Table 2. Number of persistent lethal genes in Kofu and Katsunuma populations during two years.

Symbol	Number of appearance and their linked inversion		Locus on the genetic map
	1963	1964	
l 201	13 + or *In(2R)*C	6 + or *In(2R)*C	47.9
l 202	8 + or *In(2R)*C	6 + or *In(2R)*C	33.7
l 203	6 +	3 + or *In(2R)*C	5.5
l 204 *	6 +	7 + or *In(2R)*C	32.1
l 207 *	4 + or *In(2L)*B	2 +	58.4
l 208	3 +	1 +	16.7

* allelic lethal genes were found among lethal genes isolated from the same population in 1959.

The number of such persistent lethal genes reached to about one third of the total of lethal genes and their zygotic frequencey in the population was estimated to be 0.12. One third of them were associated with inversion C on the right arm, but they were located on the left arm except for gene *l* 207.

2. *Pre-adult viability of heterozygotes for some persistent lethal chromosomes (a persistence mechanism of lethal genes)*

Takao WATANABE and Chozo OSHIMA

One double-lethal chromosome, *ll(2)*301a and three single-lethal chromosome, *l(2)*301d, *l(2)*302c, *l(2)*302d, were used as persistent lethal chromosomes, because they contained the same lethal gene as *l(2)*201 or *l(2)*202, located at 47.9 or 33.7 on the genetic map. These persistent lethal chromosomes having no inversion were picked up for the analytical experiment. On the other hand, about ten chromosomes were picked up randomly from each group of four homozygous viability classes: normal, subvital, semi-lethal and lethal. Then, the viability of heterozygotes having combination of the persistent and the various other chromosomes was estimated by *Cy-Pm* technique. The results are given in Table 3.

The persistent lethal chromosomes had a tendency to produce better heterozygous viabilities than normal heterozygotes, when they were paired with various kinds of chromosomes. On the contrary, the effect of subvital chromosomes was significantly deteriorating the viability of heterozygotes by about 4% compared with that of normal chromosomes,

Table 3. Pre-adult viabilities of heterozygotes for persistent lethal chromosomes and various kinds of chromosomes.

	Mating				Pooled basis		
	n	*sv*	*sl*	*l*	No. of counted flies	Relative viability	
n	*n*/*n*	*n*/*sv*	*n*/*sl*	*n*/*l*	103,035	1.0537±0.00929	
L	L/*n*	L/*sv*	L/*sl*	L/*l*	161,218	1.0585±0.00748	
LL	LL/*n*	LL/*sv*	LL/*sl*	LL/*l*	52,625	1.0618±0.01304	
	n	L	LL				
n	*n*/*n*	L/*n*	LL/*n*		88,278	1.0752±0.01027	**
sv	*n*/*sv*	L/*sv*	LL/*sv*		83,198	1.0321±0.01014	**
sl	*n*/*sl*	L/*sl*	LL/*sl*		68,599	1,0686±0.01156	*
l	*n*/*l*	L/*l*	LL/*l*		76,803	1.0554±0.01074	

The viability of *Cy*/*Pm* fly=1.0000.
n: normal, *sv*: subvital, *sl*: semi-lethal, *l*: lethal chromosome.
L, LL: persistent lethal chromosome.
** significant at the 1% level, * significant at the 5% level.

but other semi-lethal and lethal chromosomes expressed slight and non-significant deleteriousness by 1 or 2%, when they were paired with normal and persistent lethal chromosomes. The results suggest the existence of epistatic gene complexes in the persistent lethal chromosomes. This might be one of persistence mechanisms of the particular lethal genes in natural populations.

3. *Abnormal segregation in the offspring of persistent lethal heterozygous male flies (a persistence mechanism of lethal genes)*

Chozo OSHIMA and Takao WATANABE

When the viability of heterozygotes for each lethal chromosome: *ll*(*2*)301b, *l*(*2*)301b and *l*(*2*)301c, was examined by *Cy-Pm* technique, it was found to be strikingly better than that of normal heterozygotes. These chromosomes had the inversion, *In*(*2R*)C, on the right arm, whose frequency has been maintained in about 30% of natural populations of the Kofu and Katsunuma territory. The single-lethal chromosome has one lethal gene located at 47.9 on the genetic map and the double-lethal chromosome has in addition another lethal gene located at 33.7. In the

reciprocal matings, two kinds of offspring emerged, one having the lethal and the other the *Pm* chromosome; they were scored in five successive subcultures. The results are shown in Table 4.

Table 4. Abnormal segregation in the offspring of persistent lethal heterozygous male flies.

Mating ♀ × ♂	No. and period of subculture	No. of F_1 flies per day	no. of non-*Pm*	no. of *Pm*	Ratio of non-*Pm*/*Pm*	
Cy/*n*−*l*×*Pm*/LL	I. 3 days	69.2	6090	3664	1.66±0.035	
Cy/*n*−*l*×*Pm*/L	II. 2 days	124.0	6936	4723	1.47±0.028	**
	III. 2 days	124.7	6934	4785	1.45±0.027	
	IV. 2 days	83.7	5021	2846	1.76±0.041	**
	V. 3 days	67.7	5890	3657	1.61±0.034	**
	12 days	93.9 (mean)	30871	19675	1.57	
Cy/LL×*Pm*/*n*−*l*	I. 3 days	82.3	5223	4905	1.06±0.021	
Cy/L×*Pm*/*n*−*l*	II. 2 days	142.5	5945	5737	1.04±0.019	
	III. 2 days	130.5	5436	5268	1.03±0.021	*
	IV. 2 days	92.8	3863	3744	1.03±0.024	
	V. 3 days	77.6	4780	4762	1.00±0.021	
	12 days	105.1 (mean)	25247	24416	1.03	

L, LL: persistent lethal chromosome.
n−*l*: normal, subvital, semi-lethal and lethal chromosome.
** significant at the 1% level, * significant at the 5% level.

When the rasults of the reciprocal matings were compared, a great difference in the ratio of non-*Pm* and *Pm* flies in the offspring was noticed. The ratio of the mating in the upper part of Table 4 was on the average 1.5 times higher than that in the lower part of the same table and fluctuated significantly among the five successive subcultures. Such abnormal segregations could be brought about by some complicated factors, but it could be simply assumed that the results may be ascribed to a difference in activity (certation effect) between two kinds of sperm, one with *Pm* chromosome and the other with the persistent lethal chromosome.

On the other hand, when male flies heterozygous for the persistent lethal chromosome and one of *cn bw*, *bw*, *dp*, *cn bw*, *cl*, *ltd* or *vg* marked chro-

mosomes were mated with such mutant homozygous female flies, the great majority of emarged F_1 flies (80-90%) were wild type and mutant flies were very few. In these cases, the abnormal segregations were quite clearly detected like in the case of SD chromosome investigated by HIRAIZUMI. The paracentric inversion (*In(2R)*C) was assumed to occur in the persistent lethal chromosome as well as in SD or NH chromosomes, but the lethal gene located at 47.9 in the former was not the same as any lethal gene in the latter.

4. *Chromosomal polymorphism in the* Kofu *and* Katsunuma *natural populations*

Taishu WATANABE[1] and Chozo OSHIMA

In late October of the past three years, many female flies were captured simultaneously from the same natural populations in the Kofu and Katsunuma territory. These female flies were allowed to lay eggs individually in a vial and then the salivary chromosomes of 100 larvae

Table 5. Frequencies of inversions in the Kofu and Katsunuma natural populations during three years

Population	Kofu pop.			Katsunuma pop.		
Year	1963	1964	1965	1963	1964	1965
Second chromosome			(%)			(%)
standard (left arm)	67.0	64.0	57.5	68.0	73.0	65.0
*In(2L)*A	0.5	0.0	0.0	0.0	0.0	0.0
*In(2L)*B	32.5	36.0	42.5	32.0	27.0	35.0
standard (right arm)	73.0	74.5	70.0	79.0	74.5	74.5
*In(2R)*C	27.0	25.5	30.0	21.0	25.5	25.5
Third chromosome						
standard (left arm)	92.0	95.0	88.0	89.5	89.5	89.5
*In(3L)*E	6.5	5.0	11.5	8.5	10.5	10.0
*In(3L)*F	1.5	0.0	0.0	2.0	0.0	0.5
standard (right arm)	63.5	76.0	61.0	61.0	71.0	58.5
*In(3R)*G	11.0	9.0	20.5	18.0	12.0	26.5
*In(3R)*H	14.0	7.0	12.5	10.0	8.5	8.0
*In(3R)*I	11.0	8.0	6.0	11.0	8.5	7.0
*In(3R)*J	0.5	0.0	0.0	0.0	0.0	0.0

[1] Department of Biology, Faculty of Science, Kyushu University.

sampled from different cultures were observed. The results of the repeated experiments during three years are given in Table 5.

Individual frequencies of the common inversion: *In(2L)*B, *In(2R)*C, *In(3L)*E, *In(3R)*G, *In(3R)*H and *In(3R)*I, in both natural populations were similarly maintained, but *In(3R)*G only seemed to increase about two times in 1965.

As to the mechanism for maintaining each frequency of these common inversions in equilibrium, it was indicated in a previous report (No. 15) that total observed numbers of double inversion heterozygotes of second and third chromosomes were higher than expected.

In the present experiment, about 600 male flies, captured from a Katsunuma population simultaneously, were individually mated with several virgin female flies of Samarkand strain. The salivary chromosomes of 8 or 10 F_1 larvae of each culture were observed. Then, the chromosomal types of 300 male flies could be determined and the frequency of each kind of chromosomes was found. The expected numbers of all possible homozygous and heterozygous combinations of the standard and various inversions of second and third chromosomes were calculated, using the observed frequencies of each chromosome, on the basis of Hardy-Weinberg's

Table 6. Comparison of observed and expected numbers of various chromosomal types of second and third chromosomes.

Chromosomal type		Observed number	Total	Expected number	Total	Difference
Second chromosome	Third chromosome					
homo (+and inv.)	homo (+and inv.)	32		32.55		−0.55
single inv. hetero	homo (+and inv.)	46		46.74		
homo (+and inv.)	single inv. hetero	46	157	48.85	165.73	−8.73
single inv. hetero	single inv. hetero	65		70.14		
double inv. hetero	home (+and inv.)	21		15.82		
double inv. hetero	single inv. hetero	23		23.72		
home (+and inv.)	double inv. hetero	26	102	18.64	94.02	+7.98
single inv. hetero	double inv. hetero	21		26.79		
double inv. hetero	double inv. hetero	11		9.05		
homo (+and inv.)	triple inv. hetero	1		2.24		
single inv. hetero	triple inv. hetero	7	9	3.18	6.49	+2.51
double inv. hetero	triple inv. hetero	1		1.07		
	Total	300		298.79		

law and the observed numbers of all chromosomal types found in the samples were compared with the expected numbers as shown in Table 6. It was confirmed by the results that the equilibrium states of these common inversions could be maintained by heterosis of double or triple inversion heterozygotes.

5. *The effect of insecticide selection in experimental populations of* D. pseudoobscura

Chozo OSHIMA and Taishu WATANABE

About forty homozygous strains of four kinds of chromosomes, ST, AR, CH and PP, were used in the experiments. These strains, originated from the Mather population in California, had been established by Dobzhansky in 1963.

Three initial populations were made with given frequencies of four chromosomes represented in Table 1; further these populations were divided into two A and B. Flies in population A were exposed to insecticide (1 or 2% DDT or 0.1% Dieldrin) test paper for one hour and transferred into a new cage in every new generation. Flies in population B were transferred into a new cage without exposure. The salivary chromosomes of 150 larvae hatched from sampled eggs were observed for detecting the frequency of each chromosome in these populations. The varying frequencies of chromosomes from F_2 to F_{20} in the populations IA and IB and from F_2 to F_{12} in the populations IIA and IIB, IIIA and IIIB were observed as shown in Table 1.

The frequency of ST chromosomes has increased in both selected and non-selected populations, but after F_7 the increase in selected populations was greater than in non-selected populations. On the contrary, AR chromosomes decreased after F_7 in selected populations, but did not change so much in non-selected populations, and rather increased after F_{12} in the population IB. The frequencies of both CH and PP chromosomes have decreased gradually in all populations except the selected population IIIA. The initial frequency of PP chromosome in the population IIIA has been maintained or rather slightly increased. These results seem to suggest that the striking evolutionary changes in natural populations of *D. pseudoobscura* in California during about twenty years might be due to insecticide selection: ST chromosomes increased and AR chromosomes underwent changes in the opposite direction. CH chromosome decreased and became rare and PP chromosome emerged with spectacular frequency.

Table 1. Changing frequencies of four kinds of chromosomes in DDT or Dieldrin selected and non-selected populations.

	F_2			F_3	F_4	F_5	F_7	F_9	F_{12}	F_{15}	F_{20}
Pop. I	ST 33.3	IA (DDT selected)	ST	40.3	55.0	55.3	73.3	77.0	92.7	86.3	87.0
	AR 26.3		AR	18.3	23.0	24.3	15.7	18.7	6.3	7.7	9.7
	CH 22.7		CH	22.0	11.7	8.3	4.0	0.7	0.0	0.0	0.0
	PP 17.7		PP	19.3	10.3	12.0	7.0	3.7	1.0	6.0	3.3
		IB (non-selected)	ST	42.0	45.7	53.7	65.3	72.3	67.0	65.0	52.7
			AR	24.7	25.7	26.3	22.0	17.0	27.0	34.3	46.7
			CH	18.3	17.3	10.0	7.0	6.3	4.7	0.7	0.0
			PP	15.0	11.3	10.0	5.7	4.3	1.3	0.0	0.7

	F_2			F_3	F_4	F_5	F_7	F_9	F_{12}
Pop. II	ST 26.3	IIA (DL selected)	ST	43.7	41.0	43.0	71.0	84.3	90.3
	AR 28.0		AR	19.7	30.0	29.7	16.7	5.7	2.0
	CH 26.3		CH	15.3	9.7	10.3	4.3	6.0	4.7
	PP 19.3		PP	21.3	19.3	17.0	8.0	4.0	3.0
		IIB (non-selected)	ST	36.3	45.7	49.3	52.3	64.3	68.7
			AR	21.7	29.7	27.3	30.0	25.3	26.3
			CH	23.3	14.7	13.3	12.0	5.3	3.0
			PP	18.7	10.0	10.0	5.7	5.0	2.0

	F_1			F_2	F_3	F_4	F_5	F_9	F_{12}
Pop. III	ST 40.0	IIIA (DL selected)	ST	48.0	52.7	59.3	73.2	93.0	90.3
	AR 40.0		AR	32.7	36.7	32.0	19.7	3.0	4.7
	CH 17.0		CH	16.3	6.3	1.7	0.9	0.3	0.0
	PP 3.0		PP	3.0	4.3	7.0	6.1	3.7	5.0
		IIIB (non-selected)	ST	41.3	59.0	63.6	73.7	87.3	90.3
			AR	43.3	33.7	30.4	20.7	11.0	8.7
			CH	14.0	6.3	2.5	3.7	1.3	1.0
			PP	1.3	1.0	3.5	2.0	0.3	0.0

6. *Functional differentiation of larval fatbody cells in* Drosophila melanogaster

Toshifumi TAIRA and Taheir M. RIZKI[1)]

Physiological analyses: As mentioned in our previous report, in normal condition *Drosophila* larval fatbody consists of two kinds of functionally differentiated cells; about thousand cells in the anterior region are concerned with producing tryptophan pyrrolase and about 700 cell in the posterior region contribute to the production of pterine dehydrogenase.

Experimental induction to produce those enzymes by feeding the respective precursors to various larval stages gave the following results (Table 1).

Table 1. Relative amounts of kynurenine and isoxanthopterin extracted from 96 hr old larvae, by feeding L-tryptophan or 2-amino-4-hydroxypteridine (AHP) to 65 hr old larvae, at 25°C.

Feeding	No. of Larvae (♀)	Kynurenine (μg)	Isoxanthopterin (μg)
Nutrients only	45	1.62	0.58
Tryptophan (0.03 M)	45	3.42	0.35
AHP (0.02 M)	45	0.82	1.39

A) L-tryptophan feeding (0.03 M, pH 8.0): Induced kynurenine cells in the posterior region were revealed only in larvae treated before the 70 hr stage. The induction of tryptophan pyrrolase in the posterior region could not be suppressed by pre-treatment with chloramphenicol (0.06 mM) fed to the larvae. At the pupation stage (108–110 hrs), however, the induced kynurenine cells in the posterior region changed into pteridine cells normally produced in this region. This replacement of cellular inclusions in an early pupa was more rapid in individuals grown on a medium rich in nutrients at the larval period.

B) 2-amino-4-hydroxypteridine feeding (0.02 M, pH 8.5): The induction of pterine dehydrogenase was clearly observed within a half hour in the anterior region, which normally contained only kynurenine cells, when the inducer was given to the larvae before the 93 hr stage. Such an induction was effectively suppressed by feeding chloramphenicol (0.06 mM). The induced pteridine cells in the anterior region changed into the normally produced kynurenine cells, at the pupation stage (108–110 hrs). Rich nutrients given through the whole larval life accelerated the replacement of cellular inclusions.

From these observations, the following conclusions may be drawn:

1) Department of Zoology, University of Michigan, Ann Arbor, Michigan, U.S.A.

(1) The inductivity of tryptophan pyrrolase is recognized in every cell of larval fatbody before the 70 hr stage. Then, it is gradually lost from the cells of the posterior region, and it can not be detected there in any cell after the 85 hr stage. This indicates that the gene which controls the production of tryptophan pyrrolase becomes inactive in the cells of the posterior region. (2) Pterine dehydrogenase can be induced in larval fatbody cells until the larvae reach nearly the pupalium formation time (96 hrs). The gene controlling the formation of pterine dehydrogenase is always active in the anterior as well as in the posterior region. This is contradictory to the actual observation that normal cells in the anterior region have no isoxanthopterin, which is converted from 2-amino-4-hydroxypteridine by the action of pterine dehydrogenase. Such an apparent loss of pterine dehydrogenase in the anterior region appears to be caused by the relative concentration of those inducers which are produced *in vivo*. (3) Larval fatbody is fully enclosed with surrounding body fluid, which contains many soluble substances. If it is so, the induction of pterine dehydrogenase should take place in every fatbody cell simultaneously. In fact, the induction occurs successively in the adjacent cells from tail to head. Along the direction from tail to head, particularly, the appearance of induced cells is faster on the dorsal than on the ventral side. These observations remind of the "bipotential hypothesis" in embryology. However, it remains to be elucidated how does the gene control the bipotentiality in the development. (4) The present induction can not fundamentally change any system of the gene action.

Fate of larval fatbody cells in pupal development: The morphological change of larval fatbody through the whole pupal period were observed in the living condition under UV-microscope. In an early pupa (108 hr stage), the histolysis of larval fatbody occurred successively starting from the front of the anterior region. Most of kynurenine in the cellular granules were converted into 3-hydroxykynurenine around the 110 hr stage. The dorsal leaf-shaped cells of the posterior region were also released by histolysis. Those detached cells migrated to the developing thorax and head. Consequently, they were incorporated into every organ tissue of the adult fly. In the process of compound eye formation, two kinds of cells, having 3-hydroxykynurenine or pteridine, were surely seen to be incorporated into eye tissue. These incorporated cells might be the initiators of the pigmentary cells of the compound eye.

Most cells of the posterior region assembled to surround the gonads. In the male fly, the number of pteridine granules in each cell of the posterior regions gradually increased, but not in the female fly. It is a question, if the activation of the pteridine enzyme might be closely related

to the sexual hormone.

7. *Further evidence supporting the optimum heterozygosity hypothesis with respect to viability in a population of* Drosophila melanogaster[1]

Terumi MUKAI

As previously reported (MUKAI 1964 Genetics 50: 1-19), spontaneous mutant polygenes controlling viability have been accumulated independently in 104 second chromosome lines which originated from a single second chromosome.

In Generation 32, the degree of dominance of accumulated mutant polygenes controlling viability was tested in heterozygous condition with a chromosome thought to be identical to the original chromosome, a different chromosome from the population from which the original chromosome was extracted, and a chromosome from an entirely unrelated population. The results were given in Annual Report 14.

Based on the above experimental results, we proposed the optimum

Table 1. Relationship between the viabilities of homozygotes and those of heterozygotes with the "original" chromosome (or a chromosome carrying very few minor mutant genes) (Pooled result of Generations 32, 60, and 78)

Range of homozygote viabilities	No. of lines* tested	Average viability**	
		Homozygote	Heterozygote
0>～.09***	14	0.0617	1.0736
.10～.19	17	0.1501	1.0783
.20～.29	17	0.2532	1.0758
.30～.39	13	0.3490	1.0921
.40～.49	15	0.4481	1.0862
.50～.59	11	0.5477	1.0917
.60～.69	7	0.6551	1.0756
.70～.79	23	0.7567	1.0416
.80～.89	46	0.8483	1.0344
.90～	33	0.9734	1.0205

* Each line is represented two or three times (Generations 32, 60 and 78).

** The viability of the original homozygote=1.00.

*** Lethal lines are not included.

[1] This work was conducted in the Department of Medical Genetics, University of Wisconsin, Madison, U.S.A.

heterozygosity hypothesis (MUKAI, CHIGUSA, and YOSHIKAWA 1965 Genetics 52: 493-501), i.e., a hypothesis according to which there is overdominance if the number of heterozygous loci is very small. But the viabilities of heterozygotes decrease with an increase in the number of heterozygous loci after the viability has reached as optimal level.

In Generation 78, the viabilities of 44 randomly chosen lines were estimated in homozygotes and in heterozygotes with the "original" chromosome (or a chromosome carrying very few minor mutant genes). The results were standardized and pooled with those of Generations 32 and 60 which were given in Annual Reports 14 and 15, respectively.

The relationship between homozygote and heterozygote viabilities is shown in Table 1. The results presented in this table clearly support the optimum heterozygosity hypothesis.

8. *Independence of optimum heterozygosity of the second chromosome on the heterozygosity of the third chromosome in* Drosophila melanogaster[1]

Terumi MUKAI

On the basis of interaction of different loci within the second chromosome, we have proposed that an optimum level of heterozygosity exists for manifestation of overdominance with respect to viability. In order to obtain information regarding the effects of interaction of heterozygosity of different chromosomes, the following experiment was conducted. Based on the Generation 78 test described in the previous article No. 7, 15 lines were selected, of which 10 lines (Group A) showed the highest amount of overdominance [magnitudes of heterozygosity were near-optimal, see Article No. 7] and the other 5 lines (Group B) exhibited very little, owing to a very small number of newly arising polygenic mutations controlling viability. Crosses were made between *Cy*/*Pm Sb*/*Ubx* (♀♀)×*Cy*/+$_i$ +/+ (many ♂♂) where i indicates line number. From the offspring, *Cy*/+$_i$ *Sb*/+ males were chosen and mated to the "original" isogenic strain (+/+ +/+, normal viability). The third chromosomes of *Cy*/+$_i$ +/+ and +/+ +/+ are homozygous and idential to each other except for recently arising mutations. In the following generation *Cy*/+ *Sb*/+, +/+$_i$ *Sb*/+, *Cy*/+ +/+, and +/+$_i$ +/+ segregate. Thus, the viability of +/+$_i$ relative to *Cy*/+ for the second chromosome can be estimated in the homozygous (+/+) and heterozygous (*Sb*/+) third chromosome genetic backgrounds.

[1] This work was conducted in the Department of Medical Genetics, University of Wisconsin, Madison, U.S.A.

The pooled results are presented in Table 1 and the analysis of variance of heterozygote viabilities is shown in Table 2.

From the results of Tables 1 and 2, the following conclusions can be drawn: (1) The manifestation of overdominance in the second chromosome is independent of the genetic background in the third chromosome. If

Table 1. Relative average viabilities of four kinds of genotypes[1]

	Genotype			
	Cy/+ *Sb*/+	+/+$_i$ *Sb*/+	*Cy*/+ +/+	+/+$_i$ +/+
Group A[2]	1	1.1589***	1	1.2325***
Group B[3]	1	0.9221	1	0.9574

[1] On the basis of counting 34,055 flies.
[2] 10 lines carrying many polygenic mutations.
[3] 5 lines carrying very few polygenic mutations.
*** Highly significant.

Table 2. Analysis of variance for the viabilities of heterozygotes with the "original" chromosome II

Source of variation	Degrees of freedom	Mean square	F
Between lines	14	0.026109	9.33***
Between genetic backgrounds	1	0.013612	4.86*
Lines × background	14	0.002798	0.88
Error	270	0.003162	
Total	299		

* Significant at the 5 per cent level.
*** Highly significant.

the second and the third chromosomes were not independent, overdominance would not be manifested in the second chromosomes under the optimum heterozygosity hypothesis, because the level of heterozygosity of the third chromosomes is probably much higher than the optimum level for the second chromosome. (2) Genetic background (the third chromosome) affects the relative viability of the second chromosomes.

9. *Suppressing system of* SD *in a natural population of* Drosophila melanogaster *in Japan*

Yuichiro HIRAIZUMI and Yukiko KATAOKA

SD was first discovered in a natural population of *D. melanogaster* in Madison, Wisconsin (SANDLER, HIRAIZUMI and SANDLER, 1959), and recently it was also discovered in a natural population (ODATE population) in Japan (HIRAIZUMI and NAKAZIMA, 1965). Since then, experiments have been carried out to study the genetic systems modifying *SD* action in that population. Mating scheme relevant to the present study is presented in Figure 1. The circled chromosome in this figure indicates that it originated from the ODATE population, and SD^{bw} is the *SD*-bearing chromosome (*SD* from the ODATE population) marked by recessive *bw* (brown eye color) mutant.

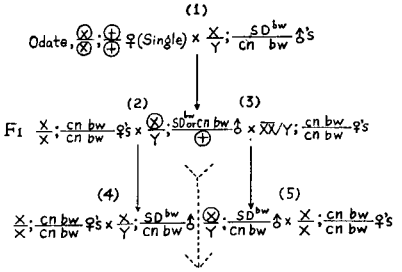

Fig. 1.

If there is any suppressing factor present in the ODATE population, some or all of the males in (2) and (3) matings will show much reduced amount of distortion (for simplicity, let $k=1$ and $k=0.5$ refer respectively to the distorting and the non-distorting segregation ratio), and the comparison between (4) and (5) matings will show in what chromosome pairs that suppressing factor locates; i.e., A) if all of the males in (4) and (5) matings show $k=1$, the suppressor must locate on the second chromosome, B) if all of the males in (4) mating show $k=1$ and all of the males in (5) mating show $k=0.5$, the suppressor must locate on the X-chromosome, and C) if the suppressor locates on the third or the fourth chromosome, half of the males in both of the (4) and the (5) matings will show $k=0.5$ (remaining half, $k=1$).

In total 19 independent sample females from the ODATE population (38 chromosomes for each pair) were tested. Of 38 X-chromosomes examined, 24 (0.63) were found to carry suppressor and of 38 second chromosomes tested, only one was found either 1) to carry suppressor or 2) to carry insensitive SD^{+} allele. None of the third and the fourth chromosomes was found to carry suppressor. The effect of suppressor was complete for all of the cases, resulting in a normal, 1:1, segregation ratio. Mappings of these suppressors are in progress.

10. *Maternal age and non-disjunction in* Drosophila melanogaster

Kyoko NAKAZIMA and Yuichiro HIRAIZUMI

Many years ago MOTTRAM (1930) and LAMY (1949) reported that the frequency of non-disjunction in female *Drosophila* increased with their age. ANDERSON (1927), on the other hand, reported a negative result. Recently, the above papers were reviewed and critisized by KELSALL (1963), and he presented data showing no correlation between maternal age and non-disjunction in *Drosophila*.

The experiment which will be described here was initially designed as one of a series of studies on the segregation distorter system of *D. melanogaster*, but during the course of experiments, we obtained results showing some relation between the frequency of non-disjunction and maternal age.

Three *y*/*y* females (*y*: yellow body color, sex-linked recessive) each less than one day old were crossed with two or three wild males from Tokyo stock, and the parents in each culture vial were transfered to a fresh food vial every three days (parents were kept for 7 days in the last, 7th, vial). Results are summarized in Table 1. Tests showed that each

Table 1. The numbers and percentages of non-disjunctional flies

Age of female in days	Total No. of flies	No. of XXY female	No. of XO male	Total No. of non-disjunctions
2	24,716	3(0.012%)	0(0.000%)	3(0.012%)
5	17,660	2(0.011%)	0(0.000%)	2(0.011%)
8	14,182	3(0.021%)	0(0.000%)	3(0.021%)
11	16,103	7(0.043%)	1(0.006%)	8(0.049%)
14	12,210	6(0.049%)	0(0.000%)	6(0.049%)
17	11,812	3(0.025%)	2(0.017%)	5(0.042%)
22	18,937	3(0.016%)	1(0.005%)	4(0.021%)
Total	115,620	27(0.023%)	4(0.003%)	31(0.027%)

of the 27 exceptional, yellow body color females (XXY) actually carried an extra-Y chromosome and all of the 4 exceptional, normal body color males (XO) were found to be sterile. Table 1 clearly shows that the maternal ageing effect is not simply linear. The frequency of non-disjunction in the females increases with their age until they reach the age of about 2 weeks, but then, beyond this age, it starts to decrease.

Since the synaptic condition of chromosome pairs during meiosis may affect the frequency of non-disjunction and of crossing over, we may

expect to have a negative correlation between them. Old studies by Bridges (1927, 1929) are available to show the relation between the frequency of crossing over and maternal age. Although Bridges's studies were on the third chromosome, we can see a clear, negative correlation between the frequency of non-disjunction and of crossing over (Fig. 1).

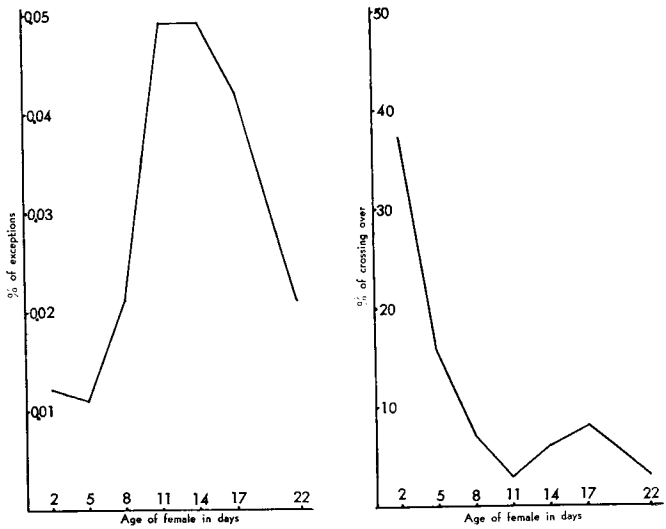

Fig. 1. Graph showing the relations between the age of female and the frequencies of non-disjunction and crossing over.

The overall frequency of exceptional females in the present study, 27/115,620, agrees quite well with that obtained by KELSALL (1963), namely, 27/123,000 but there was a big difference in the frequency of exceptional XO males (4/115,620 in the present and 64/123,000 in the Kelsall's studies). The reason for this is not yet understood, but a very reduced viability of XO male, X chromosome from the Tokyo stock, may account for this. It is interesting to note that, instead of its reduced frequency, the frequency of XO males also tends to increase with the maternal age. Further studies are in progress. Any comments to the present report should be addressed to: Y. HIRAIZUMI, Dept. of Genetics, Univ. of Hawaii, Honolulu, Hawaii, U.S.A.

11. Further report on the heterotic effect due to linkage disequilibrium in Drosophila populations

Shin-ya IYAMA

Three kinds of populations described in the 1965 issue of the Annual Report were further examined through the 30th generation (Experiment I). A similar experiment for the third chromosome bearing the ebony locus was again conducted (Experiment II). The isogenic background of those flies in this experiment was derived from the original population instead of that from the Samarkand stock used in the previous experiment. Each population included eight replicates, each batch comprising 250-300 flies in a 200 c.c. milk bottle. The number of wild and ebony flies was counted in every generation and the ebony flies were discarded before mating, artificially causing the ebony gene to be lethal. Number of pairs of $+/e$ heterozygotes initiating the population was as follows:

	Experiment I	Experiment II
Population 1	One pair of identical flies.	One pair of unrelated flies.
Population 2	One pair of unrelated flies.	Four pairs of unrelated flies.
Population 3	Six pairs of unrelated and one pair of identical flies.	Ten pairs of unrelated flies.

As shown in Figures 1 and 2, the results of the two experiments were in good accord with each other: (1) in all populations, the frequency of ebony flies was apparently higher than the theoretical expectation shown by the dotted line, which was calculated on the assumption of the same fitness for $+/+$ and $+/e$ flies, (2) the smaller was the number of initial pairs used, the slower was the decrease in the ebony fly frequency, (3) a similar rate of the decrease was observed in populations initiated with comparable number of pairs, i.e., between Population 2 of Experiment I and Population 1 of Experiment II, and between Population 3 of Experiment I and Population 2 of Experiment II.

It is concluded from this study that the manifestation of heterotic effect of $+/e$ heterozygotes depends upon the number of pairs of flies at the initial mating. This fact may support the hypothesis that linkage disequilibrium maintained between the ebony and other loci concerned with fitness in the third chromosome could be responsible for the heterotic effect. The following observation would also support the hypothesis. A part of each replicate of Population 2 of Experiment I was mixed at the 13th generation and the change in ebony fly frequency was examined. As indicated by the broken line in Figure 1, the ebony fly frequency in this population dropped rapidly, suggesting that it might have resulted from the loss of linkage disequilibrium due to mixing.

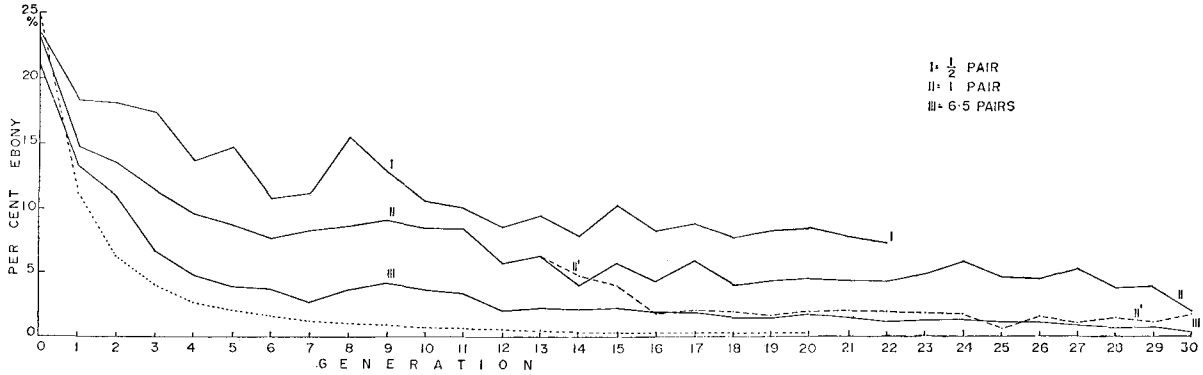
25 %
20
15
10
5
0
PER CENT EBONY
I= ½ PAIR
II= 1 PAIR
III= 6·5 PAIRS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
GENERATION

Fig. 1.

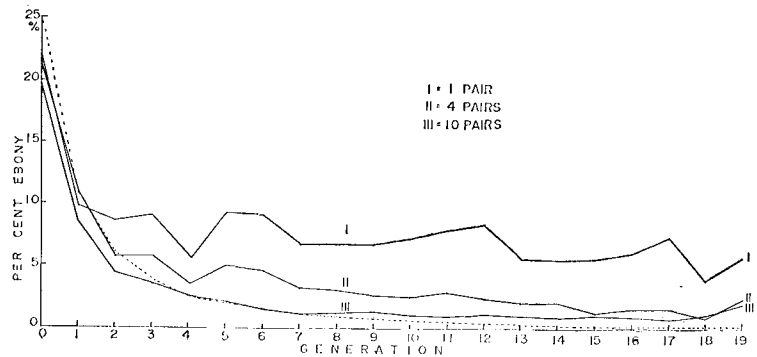
25 %
20
15
10
5
0
PER CENT EBONY
I = 1 PAIR
II = 4 PAIRS
III = 10 PAIRS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
GENERATION

Fig. 2.

12. *Effect of other species on the genetic constitution of* Drosophila melanogaster

Takashi NARISE

Frequency of genes in a closed population of an animal species is expected, after a number of generations, to be in an equilibrium which may be interrupted by genetic contributions from immigrants from other populations of the same species. How about the situation when immigration by a different species occurs as is usually expected in nature? The paper describes the result of a study conducted in order to answer this question.

Animals used in the study were two mutant strains, ss and ss^a, of *Drosophila melanogaster* and wild type flies of a different species, *D. simulans*. The experiment started with twelve half-point milk bottles with yeast-sugar-agar medium, each containing 50 male and 50 female F_1 hybrids between the two strains of *D. melanogaster*. The experiment was conducted at 25°±0.5°C. The transfer of flies into new bottles was made three times per week: Monday, Wednesday and Friday. The practice followed the method of Buzzati-Traverso (1955).

Relative frequency of ss^a gene was determined every two weeks by sampling the eggs. In 9th generation when the frequency of two genes had attained equilibrium, various numbers of flies of *D. simulans* were introduced into the *melanogaster* bottles, namly per bottle 0, 100, 200 and 400, each with three replications.

The frequency of ss^a gene at equilibrium in a pure culture of *D. melanogaster* was 0.45. The same also held in a mix-culture of 100 *simulans* and the *melanogaster* population. Mix-culture of 200 flies of *simulans*, however, brought about a decrease of relative frequency of ss^a gene, the equilibrium being at 0.39 for the three and later consecutive generations. Further decrease in gene frequency took place in the mixture of 400 flies of *simulans*, no equilibrium being attained even after 5 generations.

It is suggested from this experiment that the genetic equilibrium in a population of an animal species is subjected to change not only by immigration from other populations of the same species, but also by coexistence with other species which are not able to form Mendelian population with the former occupants.

13. Selection for bilateral asymmetry in wings of Drosophila melanogaster

Kan-Ichi SAKAI and Takashi NARISE

Wings of *Diptera* flies are usually highly symmetrical in respect of length. The same also holds for wild flies of *Drosophila melanogaster*. The wings of the vestigial strain, however, are found to be not always symmetrical. This study aimed at establishing lines with highly asymmetrical vestigial wings on the one hand, and on the other hand at finding to what extent the bilateral symmetry of wings of wild type flies could be destroyed by the genotype of the selected vestigial line.

All experiments were conducted at 25°±0.5°C. The results of a survey on wing asymmetry in three wild strains and a vestigial strain showed that irrespective of whether cultured for long or short duration in half-pint milk bottles, the wild flies were highly symmetrical in the length of their wings. Flies of the vestigial strain, however, were very variable with respect to symmetry. Sixty per cent of them were more or less asymmetrical in wing length, symmetrical flies being only 40 per cent. There seemed to be little difference between males and females. A selection experiment was conducted in those vestigial flies for and against bilateral asymmetry, the intensity of selection being 10 for higher and 10 for lower degree of asymmetry among 100 flies. Table 1 gives the

Table 1. The degree of asymmetry* in wing length of vestigial flies in succesive selection generations.

Generation		0	1	2	3	4
High	Male	0.6125	1.195	1.295	1.440	1.470
	Female	0.5250	1.570	1.845	1.940	1.905
Low	Male	0.6125	0.620	0.785	0.920	0.725
	Female	0.5250	0.785	0.955	1.160	0.650

The unit is 0.1 mm.

* The degree of asymmetry is expressed in terms of the absolute difference between right and left wing length on an individual basis.

result of selection experiment, from which we find that the selection for higher asymmetry was quite successful, whereas the selection for symmetry was ineffective. Females in the selected lines were higher in bilateral asymmetry than males.

It was observed in the course of the selection experiment that bilateral asymmetry in wings is likely to be accompanied by lower fecundity, lower hatching ability and low viability of larvae and adult flies.

14. *Deoxyribonucleic acid from SR spirochetes in* Drosophila[1]

Bungo SAKAGUCHI, Saburo NAWA and Donald F. POULSON[2]

It has been demonstrated by POULSON and SAKAGUCHI (1961) that the infectious agents responsible for the maternally transmitted "Sex-ratio" condition (SR) in *D. willistoni* and *D. nebulosa* are treponema-like spirochetes. Furthermore, POULSON and SAKAGUCHI (1961, '63) found that they can be easily transferred from either of the two species, *D. willistoni* and *D. nebulosa*, to the Oregon strain of *D. melanogaster* where become stable and persistent.

Female flies of a SR line of *D. melanogaster*, Oregon strain, with *nebulosa* SR spirochetes were used for isolation of spirochete DNA. The SR spirochetes from a large number of the host flies were partially purified by differential centrifugation. The procedure of MARMUR (1961) for the isolation of DNA was followed, except that the alcohol precipitation steps in the procedure were omitted. This was to avoid loss of the small amount of DNA of the SR spirochetes. The chemicals used for the isolation of DNA were removed by dialysis against sodium chloride and sodium citrate solution.

The DNA isolated was analysed by CsCl density gradient centrifugation in the Spinco model E analytical ultracentrifuge. The CsCl solution was at a mean density of 1.70 g/cm^3 and contained about 2 μg of the test DNA and 1 μg E. coli DNA used as the density reference. After 20 hrs centrifugation at 44,770 rev./min. at 25°C, UV absorption photographs of the cell were taken.

Examination of DNA obtained from homogenates of normal whole flies by the analytical density gradient centrifugation reveals two peaks in the densitometer tracing of the UV photographs; one peak shows the major DNA derived from the flies and the other shows the minor DNA (Figure 1, A). The minor DNA does not constantly appear and its orgin is not yet known. The major DNA has a density of 1.704 and the minor one has 1.689. When the DNA obtained from whole cells of the homogenate of SR flies was examined, three peaks were shown in the densitometer tracing of the density gradient UV photographs (Figure 1, B). Two peaks out of the three were identical with the DNA from normal flies, but the other minor peak was shown specifically only in the DNA isolated from the SR flies. The minor peak has a density of 1.681. From this density value, the guanine-cytosine content of the DNA is estimated to be 21

[1] This work has been supported by U.S. Public Health Service Research Grant GM 10238 from the Division of General Medical Sciences.

[2] Department of Biology, Yale University, U.S.A.

per cent. The G-C content is rather low compared with that of most other organisms. The major DNA peak from the partially purified SR

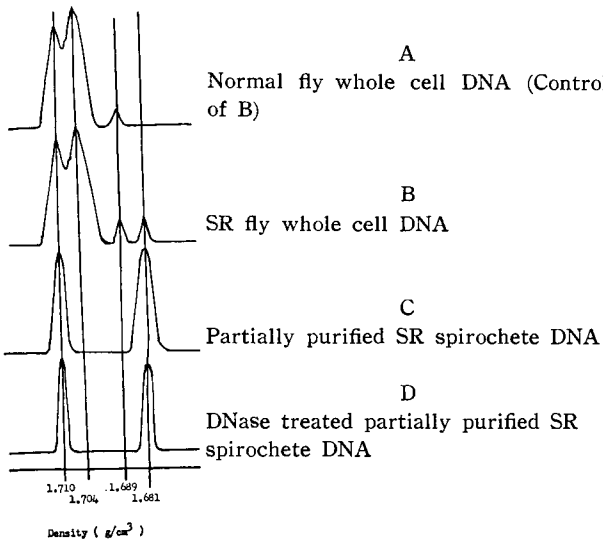

Fig. 1. Densitometer tracings of UV photographs made in the Spinco model E ultracentrifuge. DNA's obtained from the fractions indicated had been centrifuged in CsCl for 20 hours at 44,770 rev./min. E. coli DNA of density 1.710 was the marker. The densities of host fly DNA, host fly minor DNA and partially purified SR spirochete DNA are 1.704, 1.689 and 1.681 respectively.

spirochete fraction (Figure 1, C) has a density of 1.681, identical with the peak seen in whole cell DNA of the SR flies (Figure 1, B).

The UV absorption spectrum of the material of the SR specific peak isolated by CsCl gradient using SW 39 rotor of the Spinco centrifuge was shown to be typically that of nucleic acid; the maximum absorption was at 260 mμ. This maximum absorption disappeared after DNase treatment. However, after the treatment with DNase the peak (density of 1.681) in the CsCl gradient by the analytical ultracentrifuge was still present (Figure 1, D). The material remaining in this peak contained abundant hexose, presumably glucose. These facts suggest the possibility that the SR specific peak may represent an overlap between the SR DNA and polysaccharide in the position corresponding to density of 1.681.

More detailed examination of the nature and properties of the SR spirochete DNA using the method of the CsCl density gradient is now underway.

15. *Effect of DNA treatment on the wing scale in* Ephestia

Saburo NAWA

The wild type of *Ephestia* (NCR) has a pattern of black scales (about 20 per cent) on its fore wing, while a recessive multant *ml* has a uniform type of scales without pattern. As previously reported, the appearance of black scales on the fore wings of *ml*/*ml* was observed, when larvae were treated with DNA prepared from wild animals. The black pattern scales constitute nearly 100 per cent of the total number of scales on the fore wing of a mutant *b*/*b*. Thus, it might be expected that treatment of *ml*/*ml*; *b*/*b* with NCR-DNA produces about five times as many black scales as does treatment of *ml*/*ml*; $+^{b}/+^{b}$ with the same DNA. Three black scales were found in 58 *ml*/*ml*; *b*/*b* animals treated (1 black scale/39 wings). In the case of *ml*/*ml*; $+^{b}/+^{b}$, the frequency was 1/37 wings (19/712). It appears that the frequencies are the same in both cases, although the number of *ml*/*ml*; *b*/*b* animals treated was small. This may mean that any scale, wherever it is located on the wing, is able to be affected with DNA. Effect of DNA from various sources was also examined. So far, no black pattern scales have been found in the experiments using calf thymus DNA or *E. Coli* DNA. The appearance of black scales was observed on the wings of *ml*/*ml* animals treated with Bombyx DNA, although the frequency was low (2/226). This may represent a case of interspecies-transformation.

16. *Genetic and biochemical studies of chromogranules in the hypodermal cells of the silkworm*

Mitsuo TSUJITA and Susumu SAKURAI

A large amount of spherical chromogranules that contain pteridine compounds such as isoxanthopterin and sepiapterin, and uric acid are found in the larval hypodermis of the silkworm. Owing to the presence of those chromogranules, normal larvae exhibit a non-transparent hypodermis. Since the chromogranules of the hypodermal cells of normal larvae contain mainly the pteridine compound isoxanthopterin, and uric acid, their presence gives white color to the larval skin, but the yellow chromogranules of the *lem* mutant which contain mainly the pteridine compound sepiapterin, and uric acid give yellow color to the larval skin.

The present experiments were undertaken to examine the RNA contents of chromogranules.

In order to gather the granules, larvae at 4-5th days of 5th instar

were cut open at their dorsal side, all their inner organs were removed and the cytoplasm of the hypodermal layer which contained the granules was scraped and gathered. The raw fresh material of cytoplasm thus obtained was mixed with 2.2 M sucrose solution (volume ratio of sample to sucrose solution 1:10) and then centrifuged at 14,000 g for 60 minutes. The supernatant and floating cell components were discarded and the precipitate that contained the chromogranules and nucleus was placed in 20 ml of 3 M sucrose solution in a test tube and centrifuged at 40,000 g for 60 minutes. Four layers could be discriminated in the test tube, namely, 1st upper layer containing floating cell components, 2nd transparent layer, 3rd layer containing a suspension of chromogranules and 4th layer of precipitated chromogranules. In order to precipitate all of the chromogranules the 3rd layer of the chromogranule suspension was centrifuged at 54,000 g for 90 minutes.

Thus separated chromogranules were lyophilized. On the other hand, the larval hypodermis of each of the strains was dried and ground to powder. The isolated chromogranules and powdered, dry hypodermis were used for the determination of the RNA amount. RNA, protein and lipid fractions were prepared from purified chromogranules according to the procedure of Schmidt-Thannhauser and Schneider (1946), and RNA was determined by the orcinol method of Mejbaum (1939).

Protein and lipid fractions were dried to constant weight and estimated quantitatively by weighing. Experimental results are given in Table. 1.

Table 1. Biochemical composition of chromogranules

Granules	*E-lem*	*d-lem*	C-124	Hypodermis	*E-lem*	*d-lem*	C-124	Daizo	w^{oz}
RNA μg/mg P.G.	70.4	74.7	87.2	RNA μg/mg d.m.	51.9	49.4	65.5	56.9	79.6
Lipid μg/mg P.G.	10.9	13.4	8.8	Lipid μg/mg d.m.	—	—	—	—	—
Protein μg/mg G.	364	276	274	dry matter μg/mg H.	297	324	296	290	280

Abbreviation, d.m.: dry matter
G.: granule
P.G.: protein in granules
H.: hypodermis

In the table the amount of RNA is shown by μg RNA/mg protein of chromogranules, the amount of lipids is shown by μg lipid/mg protein of chromogranules and the amount of protein is given by μg protein/mg

chromogranules.

For the study of the effect of ribonuclease on RNA contained in the chromogranules and in the hypodermal cells, 50 mg of chromogranules of the *lem* larvae or 100 mg of powdered dry hypodermis of C-124 and w^{oz} were treated with 300 μg RNase at 37°C for 1.5 hrs. and then the amount of remaining RNA in each of three sample was determined. Experimental results are given in Table 2.

Table 2. The loss of RNA in the granules and hypodermis treated with RNase.

E-lem granules	70.4 μg/mg P.G.
RNase treated *E-lem* granules	28.3 μg/mg P.G.
C-124 hypodermis	65.5 μg/mg d.m.
RNase treated C-124 hypodermis	23.5 μg/mg d.m.
w^{oz} hypodermis	79.6 μg/mg d.m.
RNase treated w^{oz} hypodermis	16.5 μg/mg d.m.

Abbreviation, P.G.: protein in granule
d.m.: dry matter

17. Location of silkworm rm *gene on the 3rd chromosome*

Mitsuo TSUJITA and Susumu SAKURAI

YD_4 is a silkworm in which strain the lem^l gene is located at 22.3 units on one of the 3rd chromosomes. However, a gene for retarded moulting (gene symbol: *rm*) arose by spontaneous mutation at a locus near $+^{lem}$ gene on the other of the 3rd chromosomes. Consequently, in each batch of the next generation produced by sib-mating of individuals with normal larval phenotype segregated from YD_4 strain, the following segregation was found; about one fourth of the larvae became lethal yellow immediately after the 1st moulting, about another fourth had retarded moulting owing to slow growth, and the remaining two fourth showed the normal phenotype. It may be safely said that larvae having the normal phenotype had the genotype $rm +/+ lem^l$.

From the results of linkage experiments between *rm* and *Ze* gene, it was concluded that the *rm* gene locates relatively near the $+^{lem}$ gene, but an accurate crossing-over value could not be determined.

It is assumed from various cross experiments that the penetrance of the *rm* gene is considerably changed by the presence of suppressors affecting its manifestation, although the suppressing genes have not yet

been sufficiently analysed. It seems that there is some relation between the *rm* gene and the moulting hormone. As a means to explore its biochemical action, an electrophoretic study of soluble proteins contained in the body fluid is now under way with polyacryl amide as supporter.

18. Experimental induction of androgenesis, gynogenesis and polyploidy, in Bombyx mori *by treatment with CO_2 gas*

Yatarô TAZIMA and Akio ÔNUMA

Experimental induction of parthenogenesis and dispermic merogony has been investigated in the silkworm by many workers. The most powerful agents used so far were hot hydrochloric acid, thermal stimuli and X- or γ-rays. Quite recently, it was found in this laboratory that CO_2 gas is also a very effective agent for the induction of androgenesis, gynogenesis as well as polyploidy. The immediate effect of this gas permitted us the estimation of the progress in time of the maturation division, syngamy and cleavage.

When eggs from wild type females, mated with double recessive *pe re* males, were subjected to CO_2 gas soon after deposition, a surprisingly high incidence of *pe* eggs as well as mosaics for *pe* and + was observed. Although most of *pe* eggs died during embryonic development, some hatched out and attained maturity, all giving males with pink eyes. The genetic test revealed that they were homozygous for *pe* and *re*. Taking these results into consideration, together with those of HASIMOTO (1934) and ASTAUROV *et al.* (1956), those *pe* eggs were assumed to be the product of dispermic merogony. Following this experiment, it was soon confirmed that CO_2 gas is also effective for the induction of parthenogenesis as well as polyploidy (detected by non-disjunction of two genomes). Nitrogen gas was completely ineffective.

By marking each parental genome by a gene for a different egg colour, detailed analyses have been made of the changes in frequency in the experimental induction of those exceptional cases, which escaped fertilization, in relation to the time that elapsed since deposition of the eggs. Female moths were allowed to deposite eggs for 20 minutes. After collection those eggs were divided into several batches and each was placed according to the experimental design in a glass vial, through which a stream of CO_2 gas was passed for a period of definite duration at various ages of the eggs. Except time of the treatment, the eggs were kept at 25°C. The cross combinations used were; +/+ ♀ × *re*/*re* ♂ for the detection of androgenesis, *re*/*re* ♀ × +/+ ♂ for gynogenesis and *pe*+/+*re* ♀ × *pe re*/*pe re* ♂ for non-disjunction of two genomes.

The incidence of non-disjunction of homologous genomes declined soon after deposition and disappeared after 50 minute egg age. Then a brisk incidence of androgenesis came into appearance representing two cyclic peaks, a major one at egg age of 70 minutes followed by a minor peak

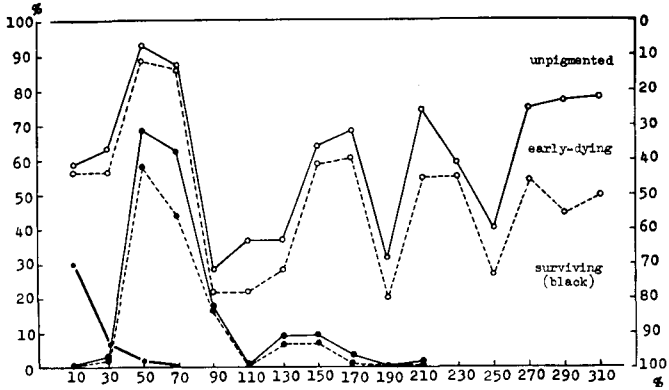

Fig. 1. Changes in the incidence of unpigmented (unfertilized) and early dying eggs and androgenic *re* eggs with the progression of egg age. CO_2 gas treatment for 180 minutes. (Expt. 653) Abscissa: egg age in minutes, Ordinate: frequency, Broken line with black circle: androgenic *re* eggs, Solid line with black circle: mosaics for *re* and +, Heavy solid line with black circle: non-disjunction, obtained from another experiment.

at egg age of 150 minutes. An almost similar type of relation was obtained for gynogenesis, although the frequency was very low. From the observed survival of the treated eggs the net survival for the egg nucleus has been calculated by subtracting the frequency of androgenic eggs. The net survival curve declined immediately after oviposition and reached the first minimum at 90 minutes, then went up again to 150–170 minutes. Thereafter, it showed cyclic changes in 50 minute periods.

These results furnish a very good clue for the estimation of the progress in time of the maturation and further developments. For instance, the net survival decline during 10 to 90 minutes seems to indicate the duration of maturation division and the cyclic changes after 170 minutes appear to correspond with cleavage cycles. Furthermore, the finding that non-disjunction ceased to occur by the egg age of 50 minutes suggests that the reduction division takes place in this insect at meiosis I.

19. Autoradiographic studies of silkworm spermatogenesis using tritiated thymidine

Toshihiko SADO

Informations on the timing of spermatognesis are essential for studying the effects of physical or chemical agents on various phases of the spermatogenic cycle. In the silkworm, a rough estimate of this cycle has been made previously by myself (SADO 1959). This estimation was based on a time table recording the first appearance of cells of one stage, then those of the next and so forth in both normal and irradiated testes. In order to furnish a more complete time table of the spermatogenic cycle of this insect, further experiments have been carried out with the use of H^3-thymidine (H^3-Tdr) as cell marker.

In one experiment, 10 day old male larvae were injected with 0.6 μc H^3-Tdr into the dorsal vessel, using a microinjection apparatus devised by RITZKI (1953). At intervals, testes were fixed with Carnoy's fluid, sectioned at 6 μ and autoradiographed using SAKURA NR-M2 emulsion and exposed for 4 weeks. As expected, the last stage at which H^3-Tdr was incorporated was that of the youngest spermatocytes (64 cell stages) just following the last spermatogonial mitosis. 24 hours later many of the spermatocytes at synapsis (or zygotene) were found to be labeled. The labeled spermatocytes in metaphase I—anaphase II were for the first time observed nine days after injection of the isotope. A few young spermatids were also found to be labeled at this time. On the 17th day there were many labeled spermatids but no labeled spermatozoa. On the 18th day, 30 to 40% of the spermatozoa cysts were found to be labeled. Thus it is clear that both meiotic prophase I and spermatid stage (spermiogenesis) last for nine days. This means that it takes 18 days from the last DNA synthesis to the completion of spermatozoa.

Spermatogonia of this insect can be classified into two categories; *primary* and *secondary* spermatogonia. Using (a) the time in which the number of primary spermatogonia doubled during early larval stages, (b) half-time of the mean grain number/cell as a function of time after a single pulse injection of H^3-Tdr and (c) colchicine method (Leblond 1959), the generation time of primary spermatogonia was estimated to be approximately 34 hours. The secondary spermatogonia comprise 6 generations and the total duration of this stage (sum of 6 generations) amounts to 5 days as seen from the fact that the testis of a newly hatched larva contains no secondary spermatogonia whereas it contains early spermatocytes 6 days later. The time table of spermatogenesis of this insect is summarized in Table 1, in which the earlier estimates are also given for comparison.

Table 1.

Spermatogenic stages	Present result	Sado (1959)
Primary spermatogonia	34 hours	A few days
Secondary spermatogonia (Sum of 6 generations)	5–6 days	
Spermatocytes		
Resting stage	≪1 〃	—
Meiotic prophase I (Leptotene to diakinesis)	9 〃	10–11 days
Metaphase I—Anaphase II	≪1 〃	Within 1 day
Spermatid stage	9 〃	5–6 〃
Spermatozoa	(1–14 days)*	—
Total spermatogenesis	Approx. 26 days	Approx. 20 days

* Storage time in the testes.

In another experiment where H^3-Tdr was injected into larvae 19 days after hatching, or 17 days prior to eclosion of the moths, no spermatozoa were found to be labeled at the time of emergence. However, when the isotope was injected 16 and 17 days after hatching, approximately 6 and 0.4%, respectively, of the spermatozoa bundles in the seminal vesicles of newly emerged moths were found to be labeled. Maximum labeling of spermatozoa bundles was obtained when H^3-Tdr was injected between 8 and 12 days after hatching. In these instances, as many as 25% of the spermatozoa bundles were found to be labeled. This indicates that in any attempt to test a transmutation effect of an isotope incorporated into DNA or to induce transformation by external DNA using spermatogenic cells the administration of these agents must be done in this insect during this period.

20. Inter-breed competition in growing chickens

Tohru FUJISHIMA

Three breeds of demestic fowl, White Leghorn, Barred Plymouth Rock and Rhode Island Red, were crossed by each other following the scheme of 3×3 diallel crosses, and the progeny were investigated with regard to intergenotypic competition.

At one week of age, the hybrid males and females obtained from those crosses were divided into three paternal strain groups and two intermingled groups involving three different paternal strains. Each paternal strain group contained twenty seven birds, comprising 9 birds of each of three different dams in a paternal strain, while each intermingled group con-

tained a total of 27 birds consisting of 3 birds of each of three different dams in every parternal strain. The experiments were repeated three times. Competitive ability for each paternal strain was estimated on the basis of mean superiority or inferiority of body weight in intermingled groups as compared with body weight in the paternal strain groups. The experiments were conducted under restricted and full feeding conditions in the intermingled groups, but only under restricted conditions in the paternal strain groups. The same amount of feed consumed by the full-fed groups in the previous week on a per bird basis was fed to the restricted group every week during the experimental period. The restricted group always received the same quality of food as the full-fed group, the only difference being in the quantity of feed received.

The results of the present experiment, as shown in Table 1, indicate

Table 1. Inter-breed competitive abilities in body weight at 5 weeks of age.

Dam \ Sire		White Leghorn			Rhode Island Red			Barred Plymouth Rock		
		Inter-mingled group	Paternal strain group	Competitive ability	Inter-mingled group	Paternal strain group	Competitive ability	Inter-mingled group	Paternal strain group	Competitive ability
		Mean gr.	Mean gr.	Mean gr.	Mean gr.	Mean gr.	Mean gr.	Mean gr.	Mean gr.	Mean gr.
Male	W.L.	233.4	244.7	−11.3	233.8	248.8	−15.0	248.3	231.7	16.6
	R.I.R.	217.8	231.5	−13.7	180.0	200.9	−20.9	206.8	204.1	2.7
	B.P.R.	245.6	245.4	0.2	246.2	250.9	−4.7	202.6	191.1	11.5
	Mean	232.3	240.5	−8.2	220.0	233.5	−13.5	219.2	209.0	10.2
Female	W.L.	229.2	232.6	−3.4	219.4	238.8	−19.4	225.9	230.7	−4.8
	R.I.R.	239.4	234.8	4.6	200.9	207.9	−7.0	204.8	218.8	−14.0
	B.P.R.	256.2	244.4	11.8	244.2	264.4	−20.2	190.1	211.5	−21.4
	Mean	241.6	237.3	4.3	221.5	237.0	−15.5	206.9	220.3	−13.4

that there are general combining abilities for competitive ability in the domestic fowl, because the male progeny from B. P. R. used as parental breed have generally statistically significant superiority in competitive ability to those from the other breeds ($P<0.01$), while in female progeny, those from W. L. have significant superiority to those from the other breeds ($P<0.01$), and there are statistically significant sire X dam interactions in competitive ability of female progeny ($P<0.05$), *i.e.*, non-additive genetic effects in inter-breed competitive ability.

21. *Variance and covariance analysis of egg production and related characters in the domestic fowl.*

Takatada KAWAHARA and Teruo INOUE

Heritability of and genetic and phenotypic correlations among egg production (up to 120 days after first egg), egg weight, egg width, egg length, body weight and sexual maturity were investigated in a closed flock of White Leghorns. Data were collected from 907 pullets originated from 65 sires and 284 dams. The estimates of heritability from an analysis of variance are given in Table 1.

Table 1. Heritability estimates of egg production, egg weight, egg width, egg length, body weight and sexual maturity.

Heritability	Egg production	Egg weight	Egg width	Egg length	Body weight	Sexual maturity
h^2_s	0.309	0.386	0.392	0.506	0.895	0.407
h^2_d	0.189	0.465	0.292	0.339	0.346	0.483
h^2_{s+d}	0.249	0.426	0.342	0.396	0.620	0.445

Table 2. Genetic and phenotypic correlations between egg production and related traits.

Traits	Genetic correlation	Phenotypic correlation
Egg production—Egg weight	−0.217	−0.140**
Egg production—Egg width	−0.090	−0.064*
Egg production—Egg length	−0.232	−0.216**
Egg production—Body weight	−0.132	−0.023
Egg production—Sexual maturity	−0.113	−0.048

* Significant at the 1% level.
** Significant at the 5% level.

Table 2 represents the estimates of coefficients of genetic and phenotypic correlations between egg production and related traits.

Both genetic and phenotypic correlation coefficients between egg production and the related characters are all negative. Interesting is the finding that the genetic correlation between egg production and egg length was −0.232 and that between egg production and egg width was −0.090.

This indicates that the generally accepted negative relationship between egg number and egg weight could be rather due to a change in egg length than in egg width.

22. Estimation of genetic parameters of the productive traits of Japanese *quail.*

Takatada KAWAHARA and Teruo INOUE

Heritability of and genetic and phenotypic correlations among egg production index (up to six months after hatching), age at sexual maturity, egg weight (average first three eggs) and body weight (at sexual maturity) were investigated in a closed population of the Japanese quail. Data were collected from 235 female offspring originated from 47 pair matings of one male and one female each. The estimates of heritability from an analysis of variance are given in Table 1.

Table 1. Heritability estimates of various productive traits in Japanese quail.

Traits	Heritability ± S.E.
Egg production index (P)	0.203 ± 0.093
Age at sexual maturity (S)	0.329 ± 0.104
Egg weight (E)	0.542 ± 0.124
Body weight (B)	0.422 ± 0.113

Table 2 represents the estimates of coefficients of genetic and phenotypic correlations between various characters.

Table 2. Genetic and phenotypic correlations between various traits in Japanese quail.

Traits	Genetic correlation	Phenotypic correlation
P—S	−0.514 ± 0.198	−0.109 ± 0.065
P—E	−0.387 ± 0.195	−0.261 ± 0.061
P—B	−0.118 ± 0.123	+0.153 ± 0.064
S—E	+0.390 ± 0.163	+0.393 ± 0.055
S—B	+0.542 ± 0.149	+0.220 ± 0.062
E—B	+0.872 ± 0.042	+0.394 ± 0.055

23. Further study on chromosomes of plasma cell tumors of BALB/c mice[1]

Tosihide H. YOSIDA, Hirotami T. IMAI
and Michael POTTER[2]

Chromosomes of 7 mouse plasma cell tumors had been already observed by us (YOSIDA *et al.* 1964). All of them were characterized by a near-tetraploid chromosome distribution. These tumors were remarkable by producing several kinds of specific γ-globulins. Two important question

Table 1. Karyotypes of 9 plasma cell tumors in BALB/c mice

Tumor lines	Variation of chrom. number (mode)	No. of marker chroms. (mode)	No. of cells observed	Protein specificity	Transpl. generation.
RPC-6A positive	64-176 (68)	M[1] 0-5 (1) SM[2] 0-1 (0)	50	γA serum protein	56
RPC-6A negative	53-162 (73)	M 0-2 (2) SM 0-2 (2)	50	—	70
RPC-9	53-148 (75)	M 0-1 (1) SM ST[3] 0-2 (2)	50	Kappa chain	13
RCP-20 positive	67-154 (79)	SM 0-2 (1) M 1-3 (1) m[4] 0-2 (1)	50	Lambda chain	69
RPC-20 negative	69-142 (72)	SM 0-1 (0) M 1-3 (2) m 0-1 (0)	20	—	70
MOPC-88	48-69 (67)	M 0-2 (1)	53	γA urinary protein	66
MOPC-31C	40-90 (44)	M 0-2 (1) SM 0-1 (0)	50	γF serum protein only	48
MOPC-70A	37-97 (73)	M 0-1 (0)	50	γF serum protein + excess Kappa chain	12
MOPC-70A·10A	57-68 (66)	SM 0-1 (0) m 0-1 (0)	50	〃	69

1) M = median metacentrics. 2) SM = submedian metacentrics.
3) ST = subtelocentrics. 4) m = minutes.

[1] This work was supported by a research grant from the National Cancer Institute (CA 07798-02), Public Health Service, U.S.A.

[2] National Cancer Institute, Bethesda, Md., U.S.A.

arose; (1) Why did they have tetraploid karyotypes? Was our investigation carried out in some exceptional material? (2) Is there a correlation between karyotype and protein specificity of tumors?

To answer those questions, the chromosomes of 9 plasma cell tumors (RPC-6A positive, RPC-6A negative, RPC-9, RPC-20 positive, RPC-20 negative, RPC-88, MOPC-31C, MOPC-70A and MOPC-70·10A) were examined again. They had been induced in BALB/c mice by one of the present authors, M. Potter, in National Cancer Institute, Bethesda, by injection of mineral oil, and the materials were sent to this institute. The karyotypes and protein specificity of these tumor cells are given in Table 1. As may be seen from the table, only MOPC-31C showed a hyperdiploid stemline. The other 8 tumors were remarkable by having hypotetraploid karyotypes. In general, the range of distribution of chromosome numbers was narrower in the present materials than in the previous examinations of the same strains. Two sublines of protein positive (protein producing) and protein negative (not producing protein) were compared using two strains (RPC-6A and RPC-20). The chromosomes of the negative sublines were markedly different from those of the respective positive lines. No constant relationship between karyotype and protein specificity could be found until now.

24. Chromosomes of normal plasma cells of mouse[1]

Tosihide H. YOSIDA and Hirotami T. IMAI

Almost all plasma cell tumors of BALB/c mice induced by injection of mineral oil or mineral oil adjuvant and those of C3H mice developed following ovariectomy were characterized by near-tetraploid karyotypes including a few marker chromosomes (YOSIDA *et al.* 1964). What are the karyotypes of normal plasma cells of mice was an important question. On this point, however, no information had been yet obtained. In order to observe chromosomes of normal plasma cells, an adjuvant-vaccine emulsion was injected into the sole of the feet of the animals. One to two weeks after the injection hypertrophic lymphnodes were observed in the inguinal region. A remarkable increase followed of macrophages, lymphocytes and plasma cells characterized by endoplasmic reticulum in the cytoplasm and mitotic cells in the lymphnodes. In metaphase cells, however, recognition of plasma cells among other cells was difficult, because the characteristic figures observed in their cytoplasm had disappeared. Under such con-

[1] This work was supported by a research grant from the National Cancer Institute (CA 07798-02), Public Health Service, U.S.A.

ditions, it was very hard to identify the karyotype of plasma cells. About 5 percent of all interphase cells in the lymphnodes were examined 16 days after injection. From that information at least 5 percent of metaphase cells in the lymphnodes should be plasma cells. However, 579 mataphase cells obtained from hypertrophic lymphnodes were all characterized by a strictly diploid karyotype. Tetraploid cells were never observed in this cell population. The above investigation strongly suggests that normal plasma cells are characterized by a normal diploid chromosome complement.

25. *Further study on chromosomes of sensitive and drug resistant sublines of mouse lymphocytic neoplasm, P388, growing* in vitro[1]

Tosihide H. YOSIDA, Hiroshi OHARA, Robert A. ROOSA[2] and Takako FUKAYA

Chromosomes of sensitive sublines and 8-azaguanine and amethopterin resistant sublines of mouse lymphocytic leukemia, P388, growing *in vitro* had been previously observed. Those materials were fixed in National Cancer Institute, Bethesda, U.S.A. in 1962. We found at that time that the chromosome pattern of sensitive lines differed markedly from those of resistant sublines. In order to reinvestigate this problem, we have newly obtained in November 1965, sensitive and drug resistant sublines of P388 growing *in vitro* from Wistar Institute, Philadelphia, namely, 3 sensitive lines, 5 8-azaguanine resistant, one FUDR resistant and one amethopterin resistant lines. Although the total chromosome number (44) in the sensitive parental line growing in a suspension culture was less than that (47 to 49) of the attached culture lines, the number of maker chromosomes was quite similar to that of the three sensitive lines. They were characterized by 8 to 9 metacentric chromosomes and 4 to 5 submetacentric markers. These findings were similar to those obtained by us previously.

On the other hand, several drug resistant lines showed alterations in the constitution of their biarmed chromosomes as well as in the total chromosome number. In an 8-azaguanine resistant line, AZG^{r-3} (1×10^{-4} M AZG), the number of metacentric chromosome was decreased. A $FUDR^{r-3}$ resistant line (5×10^{-8} M FUDR) was characterized by a marked decrease of the total chromosome number and a marked increase of biarmed

[1] This work was supported by a research grant from the National Cancer Institute (CA 07798-02), Public Health Service, U.S.A.

[2] Wistar Institute, Philadelphia, Penn. U.S.A.

chromosomes. In the amethopterin resistant line, AMT^{r-5} (8×10^{-6}M AMT), a slight decrease of metacentric chromosomes was noted. From the above investigations, it can be said that the chromosome patterns of cultured lines varied according to the culture method employed, and those of sensitive lines were different from those of drug resistant lines.

26. Chromosome study of survived sublines of mouse fibroblastic in vitro *line L-5 after treatment with 8-azaguanine*[1)]

Hiroshi OHARA, Takako FUKAYA and Tosihide H. YOSIDA

The chromosome pattern of a parental mouse fibroblastic *in vitro* line L-5 and of the survived sublines developed by treatment with various concentrations of 8-azaguanine was examined in order to find the relationship between the development of 8-azaguanine resistance and the change of karyotype. The cells were propagated in static closed bottles and in petri dishes in a CO_2 incubator in MEM medium supplemented with 10 to 15% of whole calf serum. For the experiment, 8-azaguanine was dissolved in the above medium, in which the cells were kept for 7 days; then it was changed to drug free medium in order to permit a rapid and abundant growth of the survived cells. From the treatment with the chemical the following sublines were obtained; 5 clonal lines (treated with 5 μg/ml), 5 lines (treated in a bottle with 5 μg/ml), 3 lines (treated with 0.1, 1 and 10 μg/ml, respectively) and 10 lines (treated 5 times with 5 μm/ml). ID_{50} of parental L-5 line to 8-azaguanine was approximately 0.75 μg/ml. However, some of the lines developed by the drug treatments, showed no perceptible growth when the medium contained the above concentrations. Only one repeatedly treated line showed a slow growth at the drug concentration of 2 μg/ml. Therefore, they were not true resistant lines, and have probably developed a false resistance by physiological adaptation. The majority of the parental line cells were characterized by 53 to 55 chromosomes including 10 metacentrics and 5 submetacentrics, among the latter a strikingly large submetacentric D-chromosome. At the end of 1965, however, the karyotype of the parental line was slightly changed. The chromosome pattern of the cells of the survived lines developed by treatment with various concentration of 8-azaguanine was almost similar to that of the parental line concerning the chromosome number and karyotype. It may be reasonably assumed that these survived lines are

1) This work was supported by a research grant from the National Cancer Institute (CA 07798-02), Public Health Service, U.S.A.

not yet established true resistant lines which would be eventually developed by further succession of mutation and selection.

27. *The effect of 8-azaguanine on DNA synthesis of Yoshida sarcoma cells*[1)]

Tosihide H. YOSIDA, Yoshinori KURITA
and Reiko TSURUTA

The anti-cancer agent, 8-azaguanine, inhibits the growth of a variety of living cells. The present studies deal with the effect of the chemical on the chromosomes and DNA synthesis of YOSHIDA sarcoma cells. After incubation of the cells with 10 μc of H^3-thymidine and 70 mg 8-azaguanine *in vitro*, they were injected intraperitoneously into rats. Sampling was made every 2 hours thereafter up to 24 hours after incubation. The results were as follows:

In a total of 1,000 cells treated with the chemical DNA synthesis was not affected. The generation time of a YOSHIDA sarcoma cell observed in the present study was about 14 hours, and the duration of each phase of the mitotic cycle was G_1=3 hrs., S=8 hrs., G_2=2 hrs. and M=1 hr. The results here obtained differ from those of the preceding non-treated experiment (KURITA, *et al.* 1964), in which one generation time of the cell cycle was 18.5 hours.

28. *Chromosome aberrations induced in mouse bone marrow cells by 20-methylcholanthrene*[1)]

Reiko TSURUTA, Yoshinori KURITA, Tosihide H. YOSIDA
and Katsumi SAKAKIBARA

Since the chromosomes of chemically induced mouse leukemias generally deviate from a normal complement, it is of particular importance to detect whether carcinogens may affect chromosome rearrangements. In order to throw a light on this problem, the types and frequency of chromosome abnormalities induced in bone marrow cells of RF strain were examined after treatment with 20-methylcholanthrene.

No chromosome aberrations were observed 8 hours after treatment with 10^{-3} M of the chemical. 24 hours after treatment, however, structural chromosome abnormalities became evident. The aberrations observed

1) This work was supported by a research grant from the National Cancer Institute (CA 07798-02), Public Health Service, U.S.A.

were only of chromosome type, namely, interstitial deletions and minute chromosomes which were never found in the control specimens. The frequency of those aberrations was 7 percent. This seems to indicate that 20-methylcholanthrene may weakly affect the chromosomes of bone marrow cells of mice.

29. *Autoradiographic analysis of the effect of Mitomycin C on chromosomes of Ehrlich ascites tumor cells*[1)]

Yoshinori KURITA, Tosihide H. YOSIDA
and Reiko TSURUTA

Mitomycin C, a chemically reactive antibiotic derived from *Streptomyces caespitosus*, is known as a selective inhibitor of DNA synthesis in *E. coli*. The effect of the chemical on plant and human chromosomes has been noted by several investigators. The present study was undertaken in order to carry out an analysis of the types and frequencies of chromosome aberrations under the influence of the chemical. Ehrlich mouse tumor cells served as the material under particular consideration of the relation between the type of induced aberration and the mitotic cycle.

Ehrlich ascites tumor cells transplanted into SL strain mice were treated with 10^{-3} M and 10^{-4} M Mitomycin C, and simultaneously with 10 μc of H^3-thymidine *in vivo*. Treatment with 10^{-3} M Mitomycin C resulted in an inhibition of DNA synthesis and mitosis. On the other hand, with 10^{-4} M treated cells showed a one hour lag of G_2 phase. It was also observed that the duration of S phase was greatly prolonged following the treatment. Although it is not clear whether this period represents the first or the second post-treatment mitosis, almost all cells with chromosome aberrations observed within 36 hours after treatment were labeld with H^3 thymidine. Moreover, it was noted that chromatid breaks appeared mostly 8 hours after treatment (0.35/cell), but the first chromatid exchanges were seen 14 hours after treatment and their frequency gradually increased from 14 to 22 hours. The frequency of chromatid exchanges observed 22 hours after treatment was 6.5/cell. These results may indicate that Mitomycin C induces a cross-link in twin molecular chains of double stranded DNA and then inhibits their separation which is essential for cell division.

1) This work was supported by a research grant from the National Cancer Institute (CA 07798-02), Public Health Service, U.S.A.

30. *Alteration of karyotype in a transplantable granulocytic leukemia (chloroleukemia) in the rat*

Takao SAKAI, Tosihide H. YOSIDA and Mitsuyuki SHIMIZU[1)]

A transplantable granulocytic leukemia (chloroleukemia) used in the present study was transferred to this institute from Showa University, Medical College, Tokyo, where the material was obtained from Dr. Harry Shay, Fels Institute, Philadelphia, Penn. U.S.A. This tumor was originally induced in a male Wistar rat by gastric instillation of methylcholanthrene in 1951. In 1961 the chromosomes of this tumor were studied by Norwell *et al.* (Norwell *et al.* 1963). According to them, the tumor showed a persistent chromosome change, namely in the stemline cells with 43 chromosomes an extra chromosome was added to the group of median size acrocentrics.

The present observations revealed that the chromosome numbers varied from 37 to 44 with the mode at 43 (80%). Karyotypes of the tumor stemline cells were slightly different from those observed by Norwell *et al.* In the present material, an extra-long telocentric and one small subtelocentric chromosomes were usually observed in the stemline karyotypes. An extra chromosome in the group of medium size acrocentrics was noticed by Norwell *et al.* From the above observations it was assumed that a distal part of an acrocentric element was translocated to one of No. 2 acrocentrics resulting in the extra-long element, while its other part with the centromere remained as the small subtelocentric. Cells with 43 and 45 chromosomes were sometimes observed. From the idiogram analysis it is assumed that those karyotypes were developed by nondisjunction of one of No. 14 submetacentric chromosomes.

31. *Population survey of Japanese and Korean black rats with chromosomal polymorphism*

Tosihide H. YOSIDA, Takako FUKAYA, Yung Sun KANG, Yukuo MORIGUCHI, Kyoko OHARA and Akira NAKAMURA

The black rat, *Rattus rattus* L., is characterized by polymorphism of the largest No. 1 chromosome pair (YOSIDA *et al.* 1965). According to their morphology, three types were distinguished: telocentric homomorphic pair (T/T), subtelocentric homomorphic pair (S/S) and telo- and subtelocentric heteromorphic pair (T/S). The animal occurs almost everywhere in Japan, Korea and other countries, especially in the southern areas.

1) Showa University, Medical College, Tokyo.

We collected 124 animals from 7 places of Japan and 9 animals from Seoul, Korea. Among 124 animals, 94 (75.8%) wers T/T type, 29 (23.3%) were S/T type and the remaining only one animal (0.8%) was S/S type. All animals collected in Sugadaira, Nagano-ken, and Obama, Fukui-ken, were T/T. The S/S animal was collected in Hamamatsu population. The frequency of the three types varies considerably in Japan. Among 9 animals collected in Seoul 3 were T/T type, 5 were T/S type and the remaining one was S/S type. In Japan, S/S and T/S animals are scarce, while in the Korean population their frequency seems to be higher. These differences between Japan and Korea populations will be further investigated with more material.

32. Crosses between black rats characterized by chromosomal polymorphism

Tosihide H. YOSIDA, Yukuo MORIGUCHI, Takako FUKAYA and Akira NAKAMURA

The black rat *Rattus rattus* L., is characterized by polymorphism of the largest No. 1 chromosomes (YOSIDA *et al.* 1965). As stated in the foregoing report, animals with subtelocentric homomorphic pair (S/S) were very few in the natural population of Japan. In order to find out why this particular type is so scarce in Japan, animals with different karyotypes were crossed by each other in our animal rooms, and then the chromosomes of the F_1 hybrids were observed with the following results: segregation from hybrids between T/T and T/S was into 3 T/T and 4 T/S animals. The ratio of segregation closely approaches the theoretical ratio 1:1. 28 F_1 hybrids between T/S parents which were collected in Hamamatsu segregated into 9 T/T : 15 T/S : 4 S/S. The theoretical ratio (1:2:1) would be 7 T/T : 14 T/S : 7 S/S. 7 F_1 animals between the Korean T/S animals segregated into 1 T/T : 4 T/S : 1 S/S : 1 ?. 10 animals obtained from T/T parents showed all the T/T homomorphic chromosome pair. From this study, it seems that animals with the subtelocentric homomorphic pair (S/S) were born and could survive in the animal room. A further problem is to find out, why S/S animals are so few in the natural population though they are expected to be born in numbers comparable to those of T/T animals.

33. *Chromosomal polymorphism of the rat*, Rattus norvegicus, *and its segregation*

Tosihide H. YOSIDA, Reiko TSURUTA
and Yukuo MORIGUCHI

The third chromosome pair of laboratory bred and wild rat, *Rattus norvegicus*, is characterized by polymorphism (YOSIDA and AMANO 1965). The differences in the character of the polymorphic chromosomes are very striking especially in the segregating offspring of hybrids between animals with different karyotypes. YOS-strain rats were characterized by No. 3 subtelocentric homomorphic pair (T/T), while WIS-strain had a telocentric homomorphic pair (S/S). 20 F_1 hybrids between YOS- and WIS-strain rats showed all a heteromorphic telo- and subtelocentric No. 3 pair (T/S). 16 back-cross hybrids between F_1 and WIS-strain segregated into 7 T/T, 8 S/T and 1 (?) according to the ratio of 1:1, as expected.

The karyotype in a new strain of rats, Toma, obtained from Toma Institute, Tokyo, was examined. It was of WIS-type (T/T type) concerning the No. 3 chromosome. This strain may have originated from WIS-strain rats which are widely spread in this country.

34. *Chromosomes of male ants* Aphaenogaster osimensis *produced by parthenogenesis of workers*

Hirotami T. IMAI and Tosihide H. YOSIDA

It is well known that normal males develop from unfertilized eggs produced by the queen, and male haploidy was cytologically proved in *Monomorium* by Smith and Peacock (1957). It is believed that unfertilized eggs laid by the workers develop also into males, but very few cytological observations are available.

Recently it was found that the vernal workers of *Aphaenogaster osimensis* could lay eggs when they were isolated from the inhibition exercised by the queen (IMAI 1966). Such eggs produced by parthenogenesis of vernal workers were cultured and all developed into morphologically normal males. The chromosomes of both their germ and somatic cells were studied.

The chromosome number in spermatogonia and spermatocytes was haploid ($n=16$), *i.e.* the same as in normal males (IMAI 1965). No reduction division was observed. The brain cells had also the haploid chromosome number. Those males have developed from haploid eggs laid by the workers. The eggs are assumed to have undergone two normal matura-

tion divisions reducing the chromosome number. Therefore those males have a haploid chromosome number both in somatic and germ cells like normal males.

35. *Polyploid cells observed in male and queen ants of* Aphaenogaster osimensis

Hirotami T. IMAI and Tosihide H. YOSIDA

Polyploid cells in somatic tissues of worker ants have been already reported by Hauschteck (1961). The present authors report polyploidy in both somatic and germ cells of *Aphaenogaster osimensis*. The normal chromosome number of this species is $n=16$ and $2n=32$ (IMAI 1965). This species is very convenient for the observation of ploidy, because large submetacentric and subtelocentric chromosomes stand out in their karyotype and therefore ploidy can easily be determined by counting the number of those marker chromosomes.

One example of polyploidy was found in a male prepupa, in which the prepupal stage was considerably delayed but sperms had been already formed. In this stage a few spermatogonial cell divisions are usually observed and spermatogenesis begins only after an early pupal stage (IMAI 1966). Among 55 somatic cells observed in the brain 39 were haploid, 14 were diploid, and 2 cells were tetraploid. The chromosomes of a testis in the same individual were observed in 285 cells, of which 280 had the normal haploid number, but three were diploid and two were tetraploid. In addition ploidy was also found in the ovary of a queen pupa. Among 56 oogonial cells 8 were found to be tetraploid. It is interesting that ant gonad contain polyploid cells, because ant eggs can develop by parthenogenesis, and the occurrence of polyploid individuals is possible in this animal.

36. *Nuptial flight and multiple mating observed in the formicine ant,* Lasius niger

Hirotami T. IMAI

The author had the opportunity to observe the nuptial flight of *Lasius niger* and to take a photograph of the copulation. This observation was made in August of 1964 at Kinugawa. It was a muggy and windless evening. More than 10 thousand winged queens and males suddenly appeared in a swarm around a street lamp. The flight was observed from p.m. 7 to p.m. 9. Although the pairing flight could not be observed,

some interesting events were seen.

Most ants soon landed and were creeping around. The size of the male in this species is about one half of the queen, but the number of males seemed to be larger than that of the queens. They were lively and followed eagerly the queens. The photograph (Fig. 1) represents one event. It was found that most of the queens copulated with many males one after another. This multiple mating was already reported by Kerr (1961) in attine ants, *Mycocepurus*. It is strange that a few queens copulated with 3 males at the same time. This multiple mating suggests that the fertilized eggs of the queens might be genetically very heterogeneous.

Fig. 1. Copulation of *L. niger*.

37. Ovary development of workers and queens in the ant Aphaenogaster osimensis

Hirotami T. IMAI

It is generally recognized that the worker ants have a vestigial ovary whose function is completely reduced or inhibited by the queen in natural condition. It can be, however, recovered in spring, and the workers oviposite when they are isolated from the queen's inhibiting influence.

This report deals with the ovariole number of the workers of *Aphaenogaster osimensis* as compared with that of the queens. Each ovary of 9 normal queens of this species contained 12 to 18 (mean 13.6) ovarioles in the pupal stage. On the other hand, only two ovaries having each one ovariole were observed in the 20 adult workers (head width ranging from 1.00 mm to 1.33 mm, while that of queens varied from 1.54 mm to 1.62 mm), as already observed by Brian (1954) in *Myrmica*.

Egg development in the ovaries of 20 vernal workers kept 17 days after isolation from the queen was also observed. The two ovarioles of each worker contained one to ten oocytes, on the average 4.5. These oocytes were linked together along the ovariole in a row from a large basal one of almost mature size (0.58 mm long) to the smallest distal one (0.17 mm long). Oviposition by vernal workers was observed in other ants. The development of the eggs was comfirmed.

From the above investigations it is clear that workers' ovaries are fully

functional or at least have the potential to produce eggs by parthenogenesis: they differ only in ovariole number from the queens' ovaries.

B. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF PLANTS

38. Hereditary characteristics of three cytoplasms revealed by long-time substitution experiments

Hitoshi KIHARA and Koichiro TSUNEWAKI

Nuclei of eight wheat species, five hexaploid and three tetraploid, were introduced by successive backcrosses into three different cytoplasms of *Aegilops caudata*, *Ae. ovata* and *Triticum timopheevi*. The main characteristics of those nucleus-substitution lines are summarized in Table 1.

Table 1. Hereditary characteristics of three cytoplasms revealed by substitution experiments

Nucleus donor	Cytoplasm		
	Ae. caudata	*Ae. ovata*	*T. timopheevi*
Hexaploid wheat			
T. v. e.	male-sterile, germless grains	male-sterile	male-sterile
P. 168	partially male-fertile	completely male-fertile	male-sterile
Salmon	male-sterile, haploid and twin seedlings	male-sterile	male-sterile
Comp. 44	partially male-fertile	male-sterile	male-sterile
spelta duh.	male-sterile, reduced female fertility	male-sterile	completely male-fertile
Tetraploid wheat			
durum reich.	pistillody, abortive ovules	male-sterile, delayed heading	partially male-fertile
polonicum vest.	pistillody	male-sterile, delayed heading	completely male-fertile
dicoccoides spont.	pistillody	male-sterile, delayed heading	slightly male-fertile

The response of a nucleus to the three cytoplasms was not the same. The cytoplasm of *Ae. caudata* was, when functioning with the nucleus of a hexaploid wheat, characterized by frequent formation of germless

grains, haploid or twin seedlings, and by pistillody when the nucleus belonged to emmer wheat. In some cases, female fertility was reduced. P. 168 and Comp. 44 acted as partial fertility-restorers. *Ovata* cytoplasm induced male sterility with the nuclei of most wheat varieties and extremely prolonged vegetative growth with the nucleus of emmer. No pistillody was caused. P 168 restored completely male fertility. *Timopheevi* cytoplasm was different from the above two, because with the nuclei of *T. spelta* and all emmer varieties male fertility was restored to a varying level. This cytoplasm did not exhibit any other remarkable effect.

Hereditary characteristics of different cytoplasms are revealed only when a series of nucleus substitutions have been carried out and various different nuclei of more or less closely related species have replaced their own. This kind of approach provides a useful mean for the study of cytoplasmic differentiation which was taking place in the course of plant evolution.

39. Preliminary study on free amino acids in the anthers of nucleus-substitution lines

Hitoshi KIHARA

Nucleus-substitution lines with the cytoplasm of *Aegilops caudata*, *Ae. ovata* or *Triticum timopheevi* have been developed in many varieties of 6x and 4x wheats. In this experiment, those of six 6x wheats were employed, *i.e.*, *Triticum vulgare* var. *erythrospermum* (*T. v. e.* in abbrev.), P 168 (a derivative of *T. v. e.*), *T. compactum* var. No. 44 (Comp. 44), *T. vulgare* var. Salmon (Salmon), *T. spelta* var. *duhamelianum* (Spelta), and a synthesized hexaploid (ABD 13). The mature anthers were collected before anthesis and the content of free amino acids was analyzed according to the method described by FUKASAWA. An automatic amino acid analyzer was used. No replication was made. The analysis was carried out by Mr. S. SAKURAI of the Department of Biochemical Genetics, whose technical help is greatly appreciated.

As the investigation is incomplete, I want to give only a short résumé of the results hitherto obtained.

The normal strain of P 168 is characterized by an accumulation of a large quantity of free tyrosine. Among various nucleus-substitution lines, no consistent tendency was observed to accumulation or deficiency of amino acids due to a particular cytoplasm. However, in a majority of cases (4 out of 6), proline was deficient in the substitution lines with *ovata* cytoplasm. On the other hand, serine was accumulated in many substitution lines. No clear relation was found between the content of free amino acids and the restoration of fertility.

40. Comparative gene analysis of common wheat and its ancestral species. II. Waxiness

Koichiro TSUNEWAKI

For the study of origin and differentiation of cultivated plants, the new method of "Comparative gene analysis" has been applied to common wheat and its relatives.

According to this method, gene analysis is carried out in a given cultivated species and in parallel in its relatives in the same genetic background. Due to the results obtained, the origin of genes which are found in a cultivated species can be traced, and its coming into existence can be based on the origin of those genes.

Adopting this working plan, a comparative gene analysis of common wheat and its direct ancestors, emmer wheat and *Aegilops squarrosa*, and, incidentally einkorn wheat, has been carried out for foliage waxiness. The analysis consisted of three steps, *i.e.*, (1) monosomic and conventional gene analyses of common wheat in order to clarify the genic system controlling this character, (2) monosomic analysis of various synthesized 6x wheats, from which the genotypes of their components, *i.e.*, emmer wheat and *Ae. squarrosa* were determined in comparison with those of common wheat, and (3) survey of the distribution of those genes in a large number of varieties of common wheat and its relatives by customary crossing experiments or by simply observing their phenotypes.

Waxiness of common wheat is controlled by genes located in four loci, *i.e.*, W_1 on chromosome 2A (formerly XIII), W_2 in D genome (chromosome remains unidentified), I_1-W in A or B genome and I_2-W on chromosome 2D (formerly XX). The W loci are for waxy genes and the I-W loci for their epistatic inhibitors.

Almost all varieties of common wheat possess W_1 and, probably, W_2 but lack both I_1-W and I_2-W. In emmer wheat, almost all cultivated varieties have W_1 and i_1-W except *Triticum pyramidale* that possesses I_1-W together with W_1. On the other hand, wild emmer contains w_1 in addition to I_1-W. All varieties of einkorn wheat, including both wild and cultivated species, have w_1 but not I_1-W. Waxy *Ae. squarrosa* strains have the genotype, $W_2\, i_2$-W, while waxless strains have $W_2\, I_2$-W.

The presumable emmer and *squarrosa* parents of common wheat must have been of the genotype $W_1\, i_1$-W and $W_2\, i_2$-W, respectively. *T. dicoccoides* and non-waxy *squarrosa* can be excluded from the list of probable parents of common wheat. The progenitor of common wheat must have had the genotype $W_1\, W_2\, i_1$-$W\, i_2$-W. Since the occurrence of *Ae. squarrosa* with the genotype $W_2\, i_2$-W is restricted to north-western Iran, the birth-

place of common wheat is assumed to have been in this region.

41. Comparative gene analysis of common wheat and its ancestral species. III. Growth habit and awnedness

Koichiro TSUNEWAKI

A comparative gene analysis has been carried out for two characters, *i.e.*, growth habit and awnedness. The results are summarized as follows:

Growth habit of common wheat is mainly controlled by genes belonging to three loci, Sg_1, Sg_2 and Sg_3, located on chromosome 5D (formerly XVIII), 5A (IX) and 2A (XIII), respectively. Some multiple alleles are found in these loci; Sg_1, Sg_2 and Sg_3 are alleles for typical spring habit, Sg_1^e, Sg_2^e and sg_3 for semi-spring habit, and sg_1 and sg_2 for typical winter habit. The sg_1 allele is much more effective than sg_2 for the induction of winter growth habit. Most of those homologous alleles are found in the ancestors of common wheat, *i.e.*, Sg_2, Sg_2^e, sg_2, Sg_3 and sg_3 occur in emmer wheat and Sg_1^e and sg_1 in *Ae. squarrosa*.

All waxy strains of *Ae. squarrosa*, which is the D-genome donor to common wheat, have sg_1. This fact suggests that common wheat received the most powerful gene for winter habit, sg_1, from the *squarrosa*, parent, acquiring a better adaptability to high latitudes than that of the emmer parent. As the geographical distribution of the sg_1 gene in *squarrosa* populations is mainly concentrated in northern Iran, the probable birthplace of common wheat should be sought in this region.

Sg_2 alleles of emmer wheat are epistatic over Sg_1 alleles of *Ae. squarrosa*, when brought together in synthesized 6x wheats. On the other hand, such a relation of Sg_2 to Sg_1 alleles is not observed in common wheat. Since the two pertinent loci are considered to be duplicated loci; their genetic diploidization seems to have caused the loss of epistasis in the natural polyploid.

Awnlessness or awnlettedness of common wheat is confirmed to be mainly controlled by three epistatic inhibitors, *Hd* on chromosome 4B (formerly VIII), B_1 on 5A (IX) and B_2 on 6B (X). Awn promotion is caused by three genes, a_1 on chromosome 2B (II), a_2 on 2D (XX) and a_3 on 2A (XIII). All varieties except Red Bobs have the three promoters. More than 30% of common wheat varieties have one or more epistatic inhibitors.

Almost all emmer varieties have the promoters, a_1 and a_3, but no epistatic inhibitors. Apparently, all three inhibitors have been originated at the hexaploid level. a_2 of common wheat must have been derived from the *squarrosa* parent.

42. Comparative gene analysis of common wheat and its ancestral species. IV. Glume hairiness

Koichiro TSUNEWAKI

Monosomic and conventional gene analyses indicated that glume hairiness of common wheat is controlled by a single dominant gene, *Hg*, located on genome A chromosome, 1A (formerly XIV). A similar analysis of a synthesized 6x wheat, ABD-VI, revealed that its glume hairiness is also controlled by a single dominant gene, derived from *Triticum durum* var. Golden Ball, which is located on chromosome 1A, indicating that the *Hg* gene of emmer is the same as that of common wheat. A dominant gene controls also glume hairiness of einkorn, the A genome wheat. Thus, it is reasonable to assume that the *Hg* gene of einkorn wheat is the same as that found in the homologous chromosomes of the ubiquitous A genome of the tetra- and hexaploid wheats.

In common wheat both *Hg* and *hg* (allele for non-hairiness) are present, but there is undoubtedly a significant difference between their frequencies in various parts of the world. Primitive populations, both geographically and chronologically, contain more varieties with the dominant *Hg* than do advanced populations. Apparently owing to selection against hairy glume the *Hg* allele is gradually being eliminated.

In emmer wheat hairy and hairless glumes are common both in wild and cultivated species. Therefore, the responsible alleles found in common wheat must have been obtained from emmer when the first 6x wheat was produced or, later, through formation of pentaploid hybrids between the already existing hexaploid and emmer wheats.

T. boeoticum, a wild einkorn wheat, has *Hg*, while cultivated einkorn contains only *hg*. It is, therefore, assumed that the gene *Hg* of emmer wheat was derived from wild einkorn wheat when the first tetraploid wheat was produced. Since introgression of genes from einkorn to emmer wheat through the formation of triploid hybrids is very difficult, the origin of *hg* in emmer wheat can hardly be traced back to cultivated einkorn wheat.

Taking all these considerations into account, we may assume that *T. dicoccoides*, the wild emmer, was produced from a cross between *T. boeoticum*, a wild einkorn, with *Hg* and some species of Sitopsis (most likely *Ae. speltoides*), followed by amphidiploidization of the hybrid. In this way, *Hg* of wild einkorn wheat was introduced into the wild emmer wheat, in which mutation from *Hg* to *hg* took place. Both alleles were transferred to cultivated emmer wheat during its differentiation from the wild form, and later incorporated into common wheat.

43. *Awn inhibitor in Redman wheat*

Koichiro TSUNEWAKI

Based on the F_1 data of a series of crosses between Redman monosomics and Prelude, Campbell and McGinnis (1958) concluded that the common wheat variety, Redman, carries two complementary genes for awn suppression on chromosomes 5A (formerly IX) and 1D (XVII). One of them located on chromosome 5A seems to be allelic to B_1 gene.

In order to confirm their result, Redman and two other awnless varieties, Elgin and Jones Fife, which are known to carry only the B_1 gene, were crossed to two awned varieties, Prelude and S-615. Actual segregation ratios of all six crosses fitted the 1:3 ratio, disproving the 7:9 ratio of awned vs. awnless.

Mono-5A of Redman was crossed to S-615 as male parent. In the F_1 generation, a cytological examination was carried out in order to distinguish the disomic from the monosomic hybrids. Their selfed progenies were tested for the segregation of awn types. Similarly to the result of Campbell and McGinnis, all disomic F_1 plants were awnless, while all F_1 mono-5A's were awned. In the F_2 generation of the disomics, however, 1:3 ratio of awned vs. awnless was obtained instead of 7:9 ratio expected from segregation of two complementary genes. No awnless plants were found in the offspring of F_1 mon-5A.

From these results it can be said that the gene system for awnlessness of Redman is not different from that of Elgin and Jones Fife, indicating only one dominant inhibitor, B_1. Person (1956) has already pointed out that univalent shifts and other cytological changes may occur in monosomics due to occasional meiotic irregularities. Therefore, it is necessary to carry out a monosomic analysis, at least, until the F_2 generation, and to compare the F_1 record with the segregation data in F_2. By such procedures a wrong conclusion due to an occasional cytological change that occurred in a certain monosomic line may be avoided.

44. *Genome analysis of the genus* Eremopyrum

Sadao SAKAMOTO

Morphological and cytogenetical studies of *Eremopyrum*, a genus of the tribe Triticeae, were carried out in 13 different interspecific hybrids among three strains of two diploid species, *Er. buonapartis* (Spreng.) Nevski and *Er. triticeum* (Gaertn.) Nevski and nine strains of two tetraploid species, *Er. buonapartis* and *Er. orientale* (Linn.) Jaub. et Spach.

The main characteristics of the hybrids were as follows: (1) vigorous

growth of hybrids, and normal tillering and flowering; (2) intermediate spikelet characters of the hybrids between parents; (3) very high pollen sterility and complete seed sterility in all hybrid combinations.

Hybrids' average chromosome pairing per cell at MI of PMCs is shown

Table 1. Average chromosome pairing per cell in the PMCs of interspecific *Eremopyrum* hybrids

Cross combinations (♀ × ♂)	No. of cells observed	Average chromosome pairing per cell		
		III	II	I
Er. buonapartis (4x) × *Er. buonapartis* (2x):				
7034 × 7035	189		6.95	7.11
Er. buonapartis (4x) × *Er. triticeum* (2x):				
7031* × *Er. trit.*-1	156		1.37	18.27
7032 × 〃	306	0.01	1.35	18.30
7033 × 〃	183		0.97	19.06
7034 × 〃	408	0.02	1.13	18.70
7034 × *Er. trit.*-2	139	0.02	1.73	17.48
7036 × *Er. trit.*-1	115		0.97	19.05
7038 × 〃	425	0.01	1.35	18.28
7042 × 〃	273		0.76	19.48
7043 × 〃	346		1.01	18.70
Er. buonapartis (4x) × *Er. orientale* (4x):				
7034 × 7037	276		6.22	15.55
Er. orientale (4x) × *Er. buonapartis* (4x):				
7037 × 7042	445	0.00	6.62	14.76
Er. orientale (4x) × *Er. triticeum* (2x):				
7037 × *Er. trit.*-1	725	0.00	6.82	7.34
Er. distans (2x) × *Er. triticeum* (2x)**:				
7041 × *Er. trit.*-2	263	0.01	0.70	12.59

* As to spikelet morphology this strain closely resembles *Er. orientale.*

** SAKAMOTO and MURAMATSU (1963).

in Table 1. From these data, the following conclusions are drawn: (1) the genome of diploid *Er. buonapartis* is found in tetraploid *Er. buonapartis*; (2) the genome of diploid *Er. triticeum* occurs in *Er. orientale*; (3) a common genome is shared by tetraploid *Er. buonapartis* and *Er. orientale.*

The morphological characteristics and chromosome pairing of F_1 hybrids between the diploid species *Er. distans* (C. Koch) Nevski and *Er. triticeum* had been previously examined (SAKAMOTO and MURAMATSU 1963). Also a karyotypical comparison had been previously carried out for diploid and

tetraploid *Er. buonapartis*, *Er. distans* and *Er. orientale* (SAKAMOTO and MURAMATSU 1965). From those and the present studies, it is assumed that the tetraploid *Er. buonapartis* has originated from an amphidiploid between diploid *Er. buonapartis* and *Er. distans*, while the tetraploid *Er. orientale* was derived from an amphidiploid between *Er. distans* and *Er. triticeum*.

45. *An intergeneric hybrid between* Eremopyrum orientale (*Linn.*) *Jaub. et Spach. and* Aegilops squarrosa *Linn.*

Sadao SAKAMOTO

An intergeneric triploid hybrid between *Er. orientale* (2n=28; Iran) and *Ae. squarrosa* (2n=14; material received from Dr. H. KAPPERT) was obtained in 1964.

The shape of the F_1 spikes was intermediate between the parents but the spikelets showed characters of *Eremopyrum*. Pollen- and seed-sterility were complete. Average chromosome pairing per cell of the F_1 was $0.1_{II} + 20.8_I$. All bivalents observed were associated loosely by a terminal chiasma. This indicates that the genomes of *Er. orientale* are quite different from the D-genome of *Ae. squarrosa*.

46. *Intergeneric hybrids between two* Eremopyrum *and* Agropyron *species*

Sadao SAKAMOTO

The following three intergeneric pentaploid hybrids were produced in 1964: (1) *Er. buonapartis* (Spreng.) Nevski var. *buonapartis* (2n=28; Iran) × *Ag. tsukushiense* (Honda) Ohwi (2n=42; Japan), (2) *Er. buonapartis* var. *sublanuginosum* (Drob.) Melderis (2n=28; Afghanistan) × *Ag. tsukushiense* and (3) *Er. orientale* (Linn.) Jaub. et Spach. (2n=28; Iran) × *Ag. tsukushiense*.

All F_1 plants were perennial, and the shape of their spikes and spikelets was of *Agropyron* type. Complete pollen sterility was observed in all hybrid combinations. Average chromosome pairing per cell of the F_1 was in (1) $0.0_{III}+0.7_{II}+33.6_I$, in (2) $0.8_{II}+33.5_I$ and in (3) $0.9_{II}+34.0_I$. Bivalents and trivalents were all loosely associated the partners connected only terminally. It was concluded that there is no genomic homology between the two *Eremopyrum* species and *Ag. tsukushiense*.

Fifteen backcrossed plants were obtained when *Ag. tsukushiense* was crossed as male parent to the third hybrid, *Er. orientale* × *Ag. tsukushiense*. To its tillering clones colchicine solution was applied and two well-developed seeds were obtained in 1965.

47. Photoperiodic response of various Oryza *species, VIII*

Tadao C. KATAYAMA

A classification of *Oryza* strains into photoperiodically "sensitive" or "insensitive" to short day treatment was given in a previous report (KATAYAMA 1964). In the present article, further studies are reported on 114 strains belonging to 10 species, which were recently collected from several tropical countries, Dutch New Guinea, Brazil, Australia, Borneo, Africa and Madagascar.

The seeds were sown on April 20 and the seedlings were transplanted on May 20, 1965. They were grown under natural day light until June 22 (summer solstice) and thereafter, one half of the plants were exposed to short day condition (12 hours light+12 hours dark cycles) and the other half were kept under natural day length (control). The heading dates observed in those two plots were compared. Plants whose heading was earlier by 20 days or more under short day treatment than the control were classified as photoperiodically "sensitive", and those whose heading was earlier by less than 10 days were classified as "insensitive".

Both sensitive and insensitive strains were found among 56 strains of the cultivated species (*Oryza sativa*) collected from Borneo and Madagascar, namely 26 were sensitive (46.4 per cent of the total).

Also 58 strains belonging to 10 wild species were tested, i.e., *O. sativa* f. *spontanea*, *O. perennis*, *O. barthii*, *O. breviligulata*, *O. officinalis*, *O. punctata*, *O. eichingeri*, *O. tisseranti* and *O. perrieri*, collected from Dutch New Guinea, Brazil, Borneo, Australia, Africa and Madagascar. Among them 57 strains (98.3 per cent) were photoperiodically sensitive. Only one strain of *O. barthii* from South Africa was insensitive. Summarizing the present data and those reported in the previous paper (KATAYAMA 1964), 143 strains (70.4 per cent) of cultivated species were classified as sensitive and the remaining 60 as insensitive, while 348 strains (90.9 per cent) of wild species were sensitive and only 35 were insensitive.

Thus the great majority of wild *Oryza* species were photoperiodically sensitive. This finding indicates that sensitive strains may have had in the process of selection an advantage over the insensitive ones under natural condition. On the other hand, many insensitive strains were found in cultivated species, *O. sativa* and *O. glaberrima*. This is probably due to that insensitive strains often carry such useful agronomic characteristics as a short growing period, hence their wide occurrence among the cultivated species resulting from breeders' selection.

48. *Diallel crosses among Sikkimese rice types, II*

Tadao C. KATAYAMA

In Sikkimese rice, the occurrence of *indica* and *japonica* types was ascertained in the recent three years from the examination of several characters and behavior in crosses. One of the most important characters for the classification into *indica* and *japonica* types is the hybrid sterility between them. Therefore, diallel crosses were carried out using sixteen strains, namely, fourteen of Sikkimese rice, one *indica* type and one *japonica* type strain (KATAYAMA 1965). Most of the crosses were successful and 2,072 hybrid seeds were obtained during the last two years. Some of them were sown in April, 1965. Several characters of the hybrids, such as germination rate, vigor, number of tillers, plant height, heading date, length of panicles, number of rachises, pollen- and seed-fertilities, number of seeds, grain weight, were compared with those of the parents.

The results hitherto obtained indicate the occurrence of both *indica* and *japonica* types in Sikkimese rice. Of 14 strains studied, 10 strains, C7716, C7717, C7718, C7725, C7727, C7729, C7732, C7734, C7754 and C7757, were of *indica* type, while, C7707, C7719, C7722 and C7735 were of *japonica* type. The average pollen fertilities of 20 *japonica*×*japonica*, 110 *indica* ×*indica*, 55 *indica*×*japonica* and 55 *japonica*×*indica* hybrids were 85.5, 89.8, 72.0 and 68.3 per cent, respectively. The pollen-fertility of the latter inter-sterile two groups was considerably lower than of the former intra-fertile two groups.

From the present results, it is assumed that the differentiation of cultivated *Oryza sativa* into *indica* and *japonica* types might have taken place in the Indian subcontinent, particularly in Sikkim or adjacent regions, and that both types have been cultivated together in the Sikkimese paddy fields.

49. *Embryo transplantation in the genus* Oryza

Tadao C. KATAYAMA

In order to investigate the compatibility relation between embryo and endosperm of *Oryza* species, embryo transplantations between rice species were made. The method was described in the previous paper (KATAYAMA 1965).

Nine strains of five species, namely *O. sativa* (two *indica* and two *japonica* types and a strain of *O. sativa* f. *spontanea*), *O. perennis*, *O. barthii*, *O. glaberrima* and *O. breviligulata*, were used. Seedlings from intact seeds developed besides the coleoptile, the 1st, 2nd and 3rd leaves

and one to four roots. Embryos alone without endosperm produced only the coleoptile, no root appeared except in "Kyoto Asahi", a *japonica* type of *O. sativa*.

Seedlings from homogeneous transplantation (embryo transplanted to their own endosperms) produced the coleoptile, 1st, 2nd and 3rd leaves and three roots. In heterogeneous transplantation, embryos of "Kyoto Asahi" (KA) were grafted upon the endosperms of other strains of *O. sativa*, *O. sativa* f. *spontanea*, *O. perennis*, all Asian species. In this case the coleoptile, 1st and 2nd leaves and a root have developed. In other heterogeneous transplantations, between "Kyoto Asahi" (KA) and African species, KA/*barthii*, KA/*glaberrima* and KA/*breviligulata*, only the coleoptile grew but no root was found. In all reciprocal transplantations, *sativa*/KA, *spontanea*/KA, *perennis*/KA, *barthii*/KA, *glaberrima*/KA and *breviligulata*/KA, the coleoptile, 1st leaf and a root developed.

In heterogeneous transplantation, "108" (*indica* type of *O. sativa*) to other strains of *O. sativa*, only the coleoptile and 1st leaf appeared. However, in other heterogeneous transplantations, 108/*spontanea*, 108/*perennis*, 108/*barthii*, 108/*glaberrima*, 108/*breviligulata*, only the coleoptile grew. In the reciprocal combinations, *sativa*/108, *spontanea*/108, *perennis*/108, *barthii*/108, *glaberrima*/108, *breviligulata*/108, coleoptile, 1st and 2nd leaves developed.

From these results, the following conclusions were drawn. The growth of embryos grafted upon endosperms differs according to the combination. Embryos of *japonica* type grafted upon endosperms of Asian species were more vigorous than the same embryos grafted upon the endosperms of African species. Such differences were not found with embryos of *indica* type. Nutrient absorption from endosperms of *indica* type by embryos of other species, was more remarkable than that from endosperms of *japonica*.

50. *Anatomical changes in the seminal root of* Oryza sativa *after the removal of adventitious roots*

Tadao C. KATAYAMA

It has been observed in several Gramineae crops that the seminal root dies after the development of many adventitious roots. However, it was found in rice that the seminal root alives very long and exhibits structural and developmental changes. To examine them, the following experiment was made. A common variety of Japanese rice, "Kyoto Asahi", was used as experimental material.

The plants were cultured in WAGNER'S pots. In order to keep the

materials alive by the seminal root alone, all adventitious roots developed from the nodes were cut off in succession. During the period from 30 to 100 days after sowing, the seminal roots were fixed 24 hours with formalin-acetic acid mixture, dehydrated with ethyl alcohol and N-buthyl-alcohol and embedded in paraffin. Ten to 20 μ thick transverse and longitudinal sections were made and Delafield's haematoxylin was used for staining.

The first change in the seminal root was an enlargement of root diameter due to a remarkable secondary growth and an increase in the xylem and phloem strands. Under normal condition, six strands of xylem and phloem were observed as shown in Fig. 1, but more than seven of

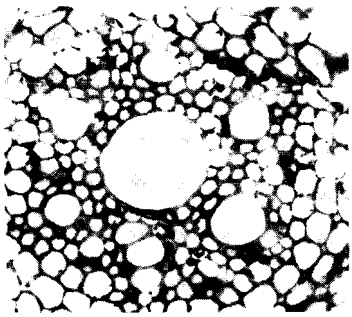

Fig. 1.

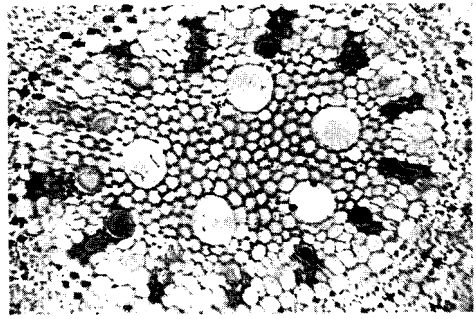

Fig. 2.

both were found in the present experiment. Thirteen strands of Fig. 2 are the extreme case observed.

Further, more lateral roots developed as compared with the control.

The secondary growth of the seminal root is an adaptive change of this root performing in my experiment the function of the normal root system.

51. Variations in the breeding system of a wild rice, Oryza perennis

Hiko-Ichi OKA and Hiroko MORISHIMA

A number of genetic stocks of *Oryza perennis* Moench, collected from various tropical countries of the world, were investigated regarding a variety of characters related to the breeding system of the species. *O. breviligulata* and cultivated rice species, *sativa* and *glaberrima*, were also observed for comparison. Two variation axes were assumed, one concerning the pollination system, and other concerning the relative extent

of seed and vegetative propagation. The former was expressed by a combination of measurements for the time interval from flowering to pollen emission, stigma size and pollen-grain number per anther; latter from the regenerative ability of stem cuts, seed number per plant, "awn-development index" and "seed-dormancy index". Variations in all these characters were correlated, and it was concluded from the correlations that strains propagated mainly by seeds tend to be relatively autogamous. For vegetative propagation were found three methods, cespitose growth, rhizome formation and tiller separation. Most African and a few Asian as well as Oceanian strains were rhizomatous, while some Amazonian strains showed tiller separation. Not only the Asian forms were differentiated into perennial (*perennis*) and annual (*spontanea*) types as previously pointed out by the writers, but *O. perennis* as a whole seemed to be differentiated in the same manner. (Evolution, in press)

52. *Estimation of indices showing genetic plant types and their correlations with yielding capacity in a segregating population of rice*

Hiroko MORISHIMA, Hiko-Ichi OKA and Te-Tzu CHANG

A genetic plant type was defined as a genetically conditioned association of morphological features. A number of F_3 lines obtained from a cross between two rice varieties, Peta (tall)×IGT (short), were measured regarding various agronomic characters, in the wet and dry crop-seasons, at the International Rice Research Institute, Philippines. As the parents differ in a major gene controlling height which gives a 3:1 F_2 ratio, F_3 lines homozygous for the tall and the short alleles were used for observation. The major height gene seemed to pleiotropically modify various characters bringing about a contrast between "tall" and "short" types. We assume that polygenes may also bring about axes of genetic variation in plant type. Regarding polygenic variations, genetic correlations of characters within the tall and short line-groups were analyzed by using the technique of principal component analysis. After rotation to "simple structure" of component vectors, two axes of variation in plant type were obtained, one (α axis) showing the variation between "panicle-number" and "panicle-length" types, and the other (β axis) showing the variation between "internode-length" and "internode-number" types. The panicle-number type has a larger number of shorter panicles and smaller leaves than the panicle-length type. The internode-length type has a smaller number of longer internodes and more erect leaves than the internode-number type. These axes of genetic variation in plant type

were considered as showing latent phases of character association due to polygenic effects. Potential yielding capacity of the F_3 lines in an intensive cultural condition was estimated from the same data in terms of total panicle length per plant and light transmission ratio (—K. LAI). Short lines with the major gene for short stature were more productive than tall plants. Within the tall and short line-groups, plants of "panicle-number type", as well as of "internode-length type", seemed to have higher yielding potential than those of the opposite types. These plant-type scores had relatively high heritability values, and were considered to be useful criteria for selection.

53. Seasonal change in genetic plant type of rice

Hiroko MORISHIMA, Hiko-Ichi OKA and Te-Tzu CHANG

Genotype-season interactional variations in various agronomic characters and in plant types were investigated using the data for F_3 lines of a cross between two rice varieties, Peta×IGT, which were grown in duplicate in the wet and dry crop-seasons at the International Rice Research Institute, Philippines. As mentioned in our previous paper, the α and β axes of genetic variation in plant type were obtained from genetic correlations (due to polygeness) of characters, by rotation to "simple structure" of vectors of principal components. The α axis shows the variation between "panicle-number" and "panicle-length" types, the former having a larger number of shorter panicles and shorter leaves than the latter. The β axis shows the variation between "internode-length" and "internode-number" types, the former having a smaller number of longer internodes and more erect leaves than the latter. The results of variance analysis showed that in leaf and stem characters as well as in the α and β plant-type scores, the variance due to genotype-season interaction was significantly large, indicating that their response to growing seasons is genetically controlled. Variations in the magnitude of seasonal differences in various characters were estimated by using a response index, $R=\bar{X}_{\text{wet}}-\bar{X}_{\text{dry}}/\bar{X}_{\text{wet}}+\bar{X}_{\text{dry}}$ (in%). Regarding seasonal change in plant type, those along the α and β axes of genetic variation and those of the axes were distinguished. It was found that the complex of co-variations of characters due to genotype-season interaction could be for a greater part accounted for by seasonal changes in the α and β plant-type scores. It was concluded that so far as the data of the present study are concerned, selection of "panicle-number type" in the wet season and of "internode-length type" in the dry season may bring about not only a stability of growth in different seasons but also a high yielding potential.

54. *Analysis of genes controlling heading time in Taichung 65 and other rice varieties*

Kuo-Hai TSAI and Hiko-Ichi OKA

A representative Ponlai variety of Taiwan, Taichung 65, and an extremely early variety from Northern China, Tatong-tsailai, were compared regarding their differences in genes controlling the heading date; both varieties belong to the Japonica type and are insensitive to photoperiod. After a biometrical survey by Mather's method, genic analysis was made by selecting certain homozygous progeny lines from the original cross and crossing them with the parental strains or with one another, so that the effects of invidual genes could be observed in a relatively uniform genetic background. In addition to the earliness gene, *E*, which was carried by our isogenic lines of Taichung 65, at least two modifiers promoting flower initiation, M_1 and m_2, were found to be involved. Under the high temperature of the second crop-season, M_1 and m_2 exert their effect only when *E* is present, but in lower temperature of the first crop, they promote flower initiation without *E*. They were considered to be genes lowering the temperature response in respect to flower initiation. It was found further that polygenes would exert a buffering action on the effect of major genes, accelerating the heading of late lines and delaying that of early lines. The earliness gene *E* was found to be distributed in various early varieties of the Japonica type, even in a photoperiod sensitive variety, Kissin. (Bot. Bull. Acad. Sinica, 7 [2]: 54–70, 1966)

55. *Weakness of F_1 hybrids in the* Sativa *rice group*

Yaw-En CHU, Hiko-Ichi OKA and Yô TAKENAKA

Various crosses within the Sativa group containing *series sativa* and *series glaberrima* were observed. In general, the frequency of occurrence of F_1 weakness was low within *series sativa* and high within *series glaberrima* as well as between the two *series*. The F_1 plants of three different crosses were used for culturing separately shoots and roots in agar and liquid medium, respectively. The F_1 between two *sativa* varieties (414×419) was characterized by leaf necrosis which appeared 19 days after germination. The F_1 between an Asian *perennis* and a *barthii* strain (W106×Af111) showed a reduced plant weight; shoot elongation seemed to be retarded, though the roots grew normally. In another cross between a *glaberrima* and a *barthii* strain (W025×W031), both shoots and roots were poor, showing unbalanced growth.

56. *Deterioration of F_1 embryos in hybrids between* Oryza sativa *and* O. perennis *subsp.* barthii

Yaw-En CHU and Hiko-Ichi OKA

Anatomical studies of deteriorating F_1 embryos in the cross *sativa*×*barthii* were reported last year. Reciprocal cross experiments showed that when *sativa* was used as the female parent, the cross ability was almost normal (53.8% on the average), but germination was as low as 16.9%. In contrast, when *barthii* was used as the female parent, the cross ability dropped to 9.7%, while germination was normal (100%). From anatomical observations of the embryos at early stages of development (1-3 day after pollination) fertilization was found to take place normally in both directions of the cross. It then seems that the reciprocal crosses differ in the stage at which deterioration occurs. In *sativa* ×*barthii* deterioration occurs 5–7 days after fertilization, while in *barthii* ×*sativa* it occurs 3 days after fertilization. The deterioration of embryos was always accompanied by that of the endosperm. These observations suggest that the inviability of F_1 seeds was primarily due to the degeneration of the endosperm.

57. *F_1 pollen and embryosac sterilities in the* Sative *rice group*

Yaw-En CHU and Hiko-Ichi OKA

The F_1 plants between *series sativa* and *series glaberrima* generally show a high pollen sterility. The F_1 plants showing various degrees of pollen sterility were investigated regarding the frequencies of chiasmata, lagging chromosomes and chromosome bridges. Some crosses with high pollen sterilities showed a reduced number of chiasmata (*e.g.* 16.9 per cell in W042×W106 and 23.3 in the parents). But no significant correlation was found between chiasma frequency and pollen sterlity. Some F_1 plants showed at low frequency lagging chromosomes and bridges (3.2% in F_1 plants and 1.5% in parents).

Further, the embryosacs of F_1 hybrids were observed. The pollen sterile F_1 hybrids between *sativa* and *glaberrima* had morphologically normal embryosacs at a high percentage (26.9–82.4%). Traces of degenerating cells found in the embryosacs suggested that the degeneration was taking place after meiosis.

58. Developmental instability in leaves, panicle and culm internodes of rice

Kan-Ichi SAKAI and M. S. BALAL

The present report includes some results of investigations on developmental instability in the length of several organs, namely first, second and third upper leaves, panicle and first, second, third and fourth culm internodes. The materials used were 24 lines taken at random from each of the two populations, control and X-irradiated, originated from the same variety Norin No. 8 and propagated according to the "one plant-one offspring" scheme for five generations. The length of the upper three leaves, panicle and four culm internodes was measured on an individual culm basis and the developmental instability was determined for each organ in terms of intraplant variability.

Table 1. Mean and genetic variance of developmental instability in length of leaves, panicle and culm internodes of rice.

Population	Leaves			Panicle	Culm internodes			
	first	second	third		first	second	third	fourth
	Mean							
Control (C_6)	6.70	8.53	7.64	1.72	3.54	1.74	1.91	2.24
X-irradiated (X_6)	6.79	8.52	7.85	1.74	3.57	1.72	1.89	2.19
	Genetic variance							
Control (C_6)	0.1245	.4690	.4236	.0717	.0038†	.0137	.0038†	.0320
X-irradiated (X_6)	.2386	.1356	.7213	.0516	.1116	.0403	.0392	.0675

† Between lines variance is not statistically significant.

Mean values of developmental instability are given in Table 1. It is seen from the table that leaves in general have higher developmental instability than culm internodes and panicle. There is, however, a definite difference with regard to developmental instability among homologous organs, e.g. developmental instability in the first of the three leaves is the lowest, while that in the first of the four culm internodes is the highest. No remarkable difference was observed between the values of the control (C) and the X-irradiated (X) populations. Genetic variance is also shown in Table 1, which demonstrates that, with the exception of panicle and second leaf, it was higher in the X-irradiated than in the control population. Genetic correlations among developmental instabilities of panicle and four culm internodes reveal that neighboring organs are

highly correlated while distant ones are not, and the closer the location the higher the values.

Conclusions drawn from the present study are:

1. Degree of developmental instability is not the same among different organs and even among homologous parts in rice.
2. X-ray treatment seems to have induced genetic variation in the magnitude of developmental instability of different organs.
3. Genetic correlation among developmental instability of neighboring internodes is higher than that among distant ones.

59. Genetical studies on clustered spikelets in rice

Katuyoshi MORIMURA and Kan-Ichi SASAKI

The dominant gene *Cl* for clustered spikelets belongs to the first linkage group. This gene is characterized by producing clustered spikelets in the top part of rachises. There might, however, be polygenes which could modify the effect of *Cl* gene in plus or minus direction. In the present study the relation between the gene *Cl* and the presumed polygenes was investigated. The material for this study were two normal strains, seven F_4 lines possessing the *Cl* gene derived from the same cross, six F_1 hybrids and two F_2 populations between the two normal and some derived lines. In the latter, intraline variation in rachis length, grain number per plant, grain density in a panicle and the degree of clustering was observed, indicating that they are still segregating. In addition to this intraline variations, seven lines were found to be significantly different with respect to the degree of clustering. On the other hand, normal lines lacking the *Cl* gene were not always free from the occurrence of clustering. Two normal lines in our material differed in this respect, and they also behaved differently in crosses with the same *Cl*-line. This may suggest the presence of polygenes besides the major gene. In the F_2 population, normal and clustered segregate in 3 : 1 ratio. A comparison between the normal plants with those segregating ones, revealed that the first rachis as well as second rachises in the clustered plants were apparently shorter than in normal plants, although there was little difference with regard to grain number per plant. Thus, grain density on clustered plants tends to be higher than in normal plants, but the correlation between the degree of clustering and grain density was not as high as was expected from the above observation. There is a high positive correlation between the two following measurements of clustering; *a*, number of grains in clusters divided by total number of grains, and *b*, number of nodes from which the grains develop in clusters divided

by total node number.

It is found from this study that the *Cl*-gene or the polygenes concerned produce shortening of rachises and clustering of grain near the top of rachises, though the number of grains per panicle remains constant. Thus, it is concluded that the effect of *Cl*-gene as well as of polygenes concerned might be a growth inhibition of the top parts of rachises which should otherwise continue growing.

60. Biometric-genetic study on rachis deficiency in rice

Kikuo WASANO and Kan-Ichi SAKAI

A mutant character in rice, rachis deficiency, is controlled by an allele, *sp*, a recessive major gene effecting partial failure of rachis formation on the panicles. The *sp* gene was introduced into ten strains differing from each other with regard to their genetic backgrounds. From examination of those strains and their hybrids with normals it was found that:

1) The rachis deficiency tended to occur mostly in the lower half of a panicle.

2) In the normal strain, there was a tendency in the primary branches to be longest in the middle part of a panicle, whereas in the *sp* strain, the primary branches were longest near the top and the length decreased downward.

3) The expressivity of *sp* gene was variable among the strains : the expressivity in the lowest strain was 37.5, whereas it was in the highest strain 76.5%.

4) The expressivity was found to be genetically negatively correlated with the length of the nearest internode of the culm, length of culm and panicle, and also number of panicles, but it was positively correlated with the number of non-bearing tillers; the gene seems to prohibit normal development and growth of panicles.

5) In the F_1 hybrids between normal and *sp* strains, the dominance effect of the normal gene was not complete; *i.e.*, the F_1 appeared more or less affected by the *sp* gene. In the F_2 population, it was found that in addition to the 3 : 1 segregation of normal and the deficient character, quantitative variation in expressivity took place. This may mean that besides the major gene, there must be polygenes responsible for the expression of the mutant character.

61. Studies on genetic relationships among leaves, culm internodes and the panicle in rice

M. S. BALAL and Kan-Ichi SAKAI

The rice variety, Norin No. 8, revealed, though naturally self-fertilizing, genetic variability for quantitative characters. A number of lines selected at random from the variety proved to differ significantly with regard to leaf size, internode length and panicle size. The present paper reports the results of an investigation on genetic correlations among those characters in two groups of lines selected at random. The results and conclusions drawn from this study are as follows:

(1) It was found that lines from the same variety, Norin No. 8, were different with respect to the characters investigated.

(2) Genetic correlation between first and second leaf was as high as approximating 0.9, while that between first and third leaves as low as approximating zero. The genetic correlation between second and third leaves was about 0.4.

(3) With regard to internode length, it was found that genetic correlation between two adjacent parts, that is, between panicle and first internode, or between first and second internode and so on, were generally very high, the average being 0.765, while those between parts adjacent separated by one, two or three organs became increasingly lower, the corresponding values being 0.370, −0.010 and −0.415, respectively.

(4) Genetic correlations between panicle or first internode and first leaf, between third or fourth internode and third leaf were very high, whereas genetic correlations among distant organs tended to be low or negative.

(5) It is thus concluded that adjacent organs of the rice plant are under stronger control of common genes than distant ones irrespective of whether they are leaves, internodes or panicles.

62. Cytogenetic studies in the genus Nicotiana

YÔ TAKENAKA

The reduction division in PMC's was studied in F_1 hybrids of *Nicotiana* species. Taxonomically, some of them were crosses within sections *Alatae*, *Aluminatae*, *Paniculatae* and *Suaveolentes* while others were crosses between different sections. The haploid chromosome number of parental strains and the number of bivalent chromosomes found in the F_1 hybrids are given in Table 1. The F_1 hybrids within section *Alatae* and *Acuminatae*

showed normal pairing, but other crosses had a few bivalents or none, most chromosomes remaining as univalents.

Table 1. *Nicotiana* F_1 hybrids and the number of bivalent chrosomes in meiosis of the hybrids (haploid number of parental species in brackets)

Cross combination		Bivalent No.
Within section *Alatae*,		
plumbaginifolia (10)	×*forgetiana* (9)	9 II
〃	×*langsdorffii* (9)	9 II
Within section *Acuminatae*,		
pauciflora (12)	×*acuminata* (12)	12 II
Within section *Paniculatae*,		
paniculata (12)	×*glauca* (12)	0—1 II
Within section *Suaveolens*,		
suaveolens (16)	×*excelsior* (19)	0 II
goodspeedii (20)	×*suaveolens* (16)	0—1 II
benthamiana (19)	×*gossei* (18)	1—4 II
Between sections,		
suaveolens (16, *Suaveolens*)	×*glutinosa* (12, *Tomentosae*)	0 II
rustica (24, *Paniculatae*)	×*suaveolens* (16)	0 II
〃	×*megalosiphon Suaveolens*)	0—4 II
〃	×*gossei* (18, *Suaveolens*)	0—1 II
excelsior (19, *Suaveolens*)	×*longiflora* (10, *Alatae*)	0—1 II
undulata (12, *Paniculatae*)	×*occidentalis* (21)	0—1 II
debneyi (24, *Suaveolens*)	×*tomentosiformis* (12, *Tomentosae*)	1—3 II
knightiana (12, *Attenuatae*)	×*alata* (9, *Alatae*)	2—5 II
paniculata (12, *Paniculatae*)	×*glutinosa* (12, *Tomentosae*)	0—1 II
glauca (12, *Paniculatae*)	×*sylvestris* (12, *Tomentosae*)	0—1 II

63. Karyotypes in the genus Nicotiana

Yaw-En CHU and Yô TAKENAKA

TAKENAKA, one of authors, had devoted himself to studies in cytogenetics of *Nicotiana*. His main lines of work were observations of chromosome pairing and tumor formation in species hybrids (TAKENAKA 1950 in Cytologia; 1953, 1956, 1962 in Bot. Mag., Tokyo; 1953 in Chromosoma; 1962a, 1962b in Jap. J. Breed. 1955, 1962c, 1962d, 1963 in Jap. Jour. Genet.). In addition to these, the karyotypes of somatic chromosome have been observed. Strains belonging to four diploid species were used as the materials. The root tips of adult plants were fixed in Carnoy fluid, and acetocarmine smear technique was employed. The results so far obtained are as follows:

Species	*Median*	*Sub-median*	*Sub-terminal*	*Total*
undulata	8	4		12
corymbosa	6	5	1	12
trigonophylla	9	2	1	12
paniculata	2	3	7	12

Hereby it was confirmed that the above diploid species of *Nicotiana* have different karyotypes.

64. Genetical studies on vein formation in Nicotiana tabacum

Kan-Ichi SAKAI and Kikuo WASANO

Two lots of seeds from the same variety of tobacco, one for control and the other X-rayed, were propagated up to the third filial generation by the "one plant-one offspring" method. In the C_3 (control) and X_3 (X-rayed) populations, 44 lines selected at random from each population were grown and the vein number per leaf was counted in two leaves from each of 20 plants in each line. The within-line variance or the environmental variance estimated in C_3 and X_3 lines was 1.71 and 1.86, while the genetic variances were 1.62 and 2.33, respectively. The variation range of line means was greater in the X population than in the control, especially toward a lower vein number.

Selection experiment was conducted in the X-line population for higher as well as lower vein number. In the next generation, X_4, the selection was found to be ineffective for higher, but effective for lower vein number. Genetic and environmental correlation between vein number and other leaf characters were investigated. It was found that vein number per leaf was not correlated with leaf number per plant neither genetically nor environmentally. Vein number, however, was found to be positively correlated genetically with leaf length but negatively with leaf width, while it was environmentally positively correlated with either leaf length or leaf width. Thus, the vein number was found to be not correlated genetically with leaf area but positively correlated environmentally.

65. *Inheritance of developmental instability in leaves of tobacco*

Yoshiya SHIMAMOTO and Kan-Ichi SAKAI

Inheritance of vein distance variability and of bilateral asymmetry of leaves was investigated in hybrids between a developmentally stable tobacco variety, Connecticut Broad Leaf, and an unstable variety, Ambalema. The F_1 hybrid was approximately intermediate between the two parents without any noticeable sign of dominance. The segregation was apparent in the F_2 population, its variance being significantly larger than in P_1, P_2 and F_1. The heritability was found to be 0.7279 for vein distance variability and 0.4900 for bilateral asymmetry. It was found in a variety-trial conducted before that those two values were closely associated among 11 varieties, whereas no such association was observed in the present F_2 population, the coefficient of the genetic correlation being 0.2325. The vein distance variability, however, was found to be positively highly correlated genetically with the vein number per leaf with $r_G=0.8230$. Investigation of F_4 lines revealed that lines with higher variability in vein distance had longer but narrower leaves than others. The correlation between F_3 and F_4 lines of vein distance variability was 0.6712.

66. *Competition in forests and an evidence that plants of the same genotype do not compete*

Kan-Ichi SAKAI, Hiromasa MUKAIDE and Koji TOMITA

Intertree competition in a standing forest can be investigated by comparing the growth of adjoining trees since environmental or soil similarity may tend to make their growth similar while competition produces differences. Accordingly, detection of intertree competition in forests may be possible by examining the correlation between adjacent two trees or between two differences among three adjoining trees; that is, correlation between y_i and y_{i+1} or that between (y_i-y_{i+1}) and $(y_{i+1}-y_{i+2})$, where y stands for stem diameter. (In our study hitherto conducted, it has been found that tree height does not tell much about competition). Theoretically speaking, $r_{y_iy_{i+1}}$ would be positive if there is no competition, but smaller or negative if intertree competition takes place. The corresponding values of $r_{(y_i-y_{i+1})(y_{i+1}-y_{i+2})}$ would be larger than -0.5 in the absence of competition, but would become smaller in case of competition.

Twelve forests of Cryptomeria japonica L., nine of which raised from

seed and the remaining three isogenic clones afforested by cuttings, were investigated with regard to the two correlations above mentioned. Of importance for this study was that the forest consists of trees of the same age. The results are presented in Table 1.

Table 1. Competition in forests of Cryptomeria japonica L.

	Strain	Number of trees	r_a[1]	r_b[2]	Competition[3] effect
Clone	Kyushu	144	0.57	−0.32	Nil
	Hyogo (1)	169	0.31	−0.51	Nil
	Hyogo (2)	146	0.43	−0.37	Nil
	Average		0.436	−0.466	
Seed-propagated forest	Hokkaido (1)	128	0.08**	−0.75**	+
	Hokkaido (2)	106	−0.28**	−0.90**	++
	Hokkaido (3)	147	0.05**	−0.58	+
	Akita	140	−0.21**	−0.65	++
	Miyagi	202	0.06**	−0.25	+
	Shizuoka (1)	170	−0.09**	−0.51	+
	Shizuoka (2)	167	−0.12**	−0.53	++
	Hyogo (1)	198	0.33	−	Nil
	Hyogo (2)	208	−0.02**	−	+

[1] $r_a = r_{y_i y_{i+1}}$ [2] $r_b = r_{(y-y_{i+1})(y_{i+1}-y_{i+2})}$ [3] Conclusion drawn from r_a.

It is found from Table 1 that:

(1) r_a proved to be a better criterion than r_b for the detection of competition effect.

(2) The r_a values are all highly positive in the three clone forests where trees of the same genotype are growing. The χ^2 test of the three correlations obtained from the clone forests allowed to regard them as estimates from the same $\rho(P=0.10-0.20)$.

(3) Forests raised from seed showed apparently lower values of the correlation except for one forest from Hyogo.

It is hereby demonstrated that trees of the same genotype do not compete against each other. Information from other studies on the estimation of genetic, environmental and competitional parameters in Cryptomeria forests has shown that clone forests are completely free from competition. It is thus concluded that competition is the result of interference between different genotypes, at least in plants.

67. Callus tissue culture in Japanese morning glory

Yô TAKENAKA, Yoshiaki YONEDA and Yaw-En CHU

A strain of Japanese morning glory which frequently produced tumors was reported in 1964 (Ann. Rep. 15: No. 73). This strain (MT), three strains derived from it (*B*, *fe* and *dl*, carrying the genes for Blown, feather and delicate, respectively) and a wild strain (Tks) were compared regarding the growth *in vitro* of callus tissues which have been preserved in culture for two years. Pieces of tissue weighing 50 mg each were placed in the following three media and were observed at 28°–30°*C* for 28 days.

a) White's basal medium
b) White's + Casein hydrolyzate (1000 ppm)
c) White's + Casein + Kinetin (0.1 ppm) + 2.4 D (0.1 ppm)

In White's basal medium, the callus tissue from MT grew slowly, but those from the other strains did not show any perceptible growth. When

Table 1. Growth of callus tissues from five morning glory strains

Culture medium	Fresh weight of tissue block in mg.			
	7 days	14 days	21 days	28 days
basic				
MT	90	212	376	410
dl	66	142	304	119
fe	61	65	81	102
B	56	70	160	144
Tks	77	86	113	104
basic+Casein				
MT	95	211	434	894
dl	76	74	130	384
fe	73	206	280	478
B	59	95	164	334
Tks	79	142	272	516
modified				
MT	72	157	514	1090
dl	69	244	570	1232
fe	85	133	394	680
B	82	301	478	605
Tks	84	230	562	860

casein hydrolyzate was added to the medium, MT showed a rapid growth and the others were gradually growing. When kinetin and 2.4 D were further added, the tissues from all the five strains grew rapidly. The fresh weights of the growing tissue blocks taken at one-week intervals are given in Table 1. It seems that the callus tissue of the tumor-producing strain was growing in a minimum medium in which the other four could not grow.

68. Genetic analysis of a tumorous strain in Japanese morning glory[1)]

Yoshiaki YONEDA

Tumors in Japanese morning glory occur in association with certain combinations of several genes (Takenaka and Yoneda, 1963, 1965). Among these, a tumorous strain was further analysed in respect to the *Blown* (*B*), *feathered* (*fe*) and *delicate* (*dl*) genes.

Seeds of a plant showing characteristics of gene *B* were sowed and its offsprings were analysed. Among them, there were found four phenotypes, namely 1) *B fe dl*, 2) *B fe*, 3) *B dl* and 4) *B*. Each phenotype could be clearly distinguished by the shape of the cotyledons, leaves and flowers. The exception was type *B fe dl* which produced small flower buds which failed to bloom. Numbers of segregants of *B fe dl*, *B fe*, *B dl* and *B* were 5, 17, 14, 59, respectively, which almost matched the expected ratio of 1:3:3:9, if the original individual analysed had the genotype *BB+fe+dl*. Phenotypes *B fe dl* and *B fe* were completely sterile because of the presence of gene *fe*. Only a few seeds were obtained from *B dl* type and its offsprings segregated either 1) *B dl* or 2) *B dl* and *B fe dl*. This indicates that the genotypes of *B dl* are *BB++ dldl* or *BB+fe dldl*. 58 of the 59 type *B* plants obtained (one plant died before seed setting) were analysed further for their segregation. 25 plants produced all four phenotypes, while 13 plants yielded *B* and *B fe*, 14 plants yielded *B* and *B dl*, and 6 plants produed only phenotype *B*. This result is consistent with the expected ratio of 4:2:2:1, if assumed as above.

These data can be interpreted if we assume the original plant used had the genotype *BB+fe+dl*. It is impossible to determine the genotypes of *B fe dl* and *B fe* because of their sterility, but the segregation data coupled with the morphological features suggests that *B fe dl* had the genotype *BB fefe dldl*, and *B fe* had either *BB fefe++* or *BB fefe+dl*.

1) This work was supported by a fellowship from the Damon Runyon Fund (DRF-378).

All the *B fe dl* types showed tumorous growth, and some of the *B fe* also showed slightly tumorous growth. From these results, tumor formation in this strain seems to be predominantly high in *BB fefe dldl* and to be slightly in *BB fefe*++ or *BB fefe*+*dl*.

In conclusion, tumrous growth in this strain seems to be dependent on the combination of the *B* and *fe* genes, and to be enhanced by the *dl* gene.

69. *Auxin destruction and growth in Japanese morning glory stems*[1)]

T. STONIER and Y. YONEDA

A wild-type strain of the Japanese morning glory (*Pharbitis nil* Choisy) was investigated to ascertain the normal relationship between auxin and stem internode elongation. In young plants an age gradient was discovered in which the decreasing rate of elongation of older internodes correlated with an increasing ability of such tissue to destroy indoleacetic acid. Fragments of tissue from old internodes when incubated with indoleacetic acid, destroyed the hormone immediately and rapidly; in contrast, young rapidly elongating internode tissue destroyed IAA only after a lag of several hours. In older plants the gradient was more erratic towards the middle of the plant but old and young tissue behave as in young plants *i.e.*, old internodes destroyed IAA rapidly whereas young internodes did not. It appears reasonable to conclude that cessation of elongation in maturing internodes is brought about by developing an internal environment in which auxin is rapidly destroyed.

Further investigations to ascertain this relationship between stem internode maturation and increasing auxin destruction, established the following: There exists in young, elongating internode tissue, substances which prevent the destruction of indoleacetic acid by enzymes normally found in stem tissue. Most of the activity can be attributed to a substance whose molecular weight is in the 5,000 to 10,000 gm/mole range. A second inhibitor of auxin destruction has a molecular weight in the 1,500 to 5,000 gm/mole range. These substances are water soluble, and appear to be readily destroyed by heating, at least *in vitro*. It is further suggested that in the wild type, associated with stem maturation, is the loss of auxin-protecting substances, and as a consequence of this loss the loss of further endogenous auxin-induced elongation.

1) This work was supported by U.S. Public Health Service grant #CA-06957 to the senior author, and Damon Runyon Fellowship DRF-378 to the second author.

70. Induction of maize seedling catechol oxidase by maleic hydrazide

Toru ENDO and Drew SCHWARTZ[1)]

Investigations on the control of catechol oxidase in plumules of maize seedlings have indicated the existence of two distinct classes. One class shows high enzyme activity while in the second class activity is almost nil. Enzyme activity was measured photometrically by the oxidation of catechol. The determinations were made on homogenates of 10 plumules from 4 day-old seedlings. As is evident in Table 1 striking differences

Table 1. Catechol oxidase activity per plumule at 4 day-old in control and MH pretreatment

Active line	Control	MH pretreatment
F 536	213±171	539±45
K 201	316±92	664±131
K 4	512±201	823±29
Inactive line		
N 541	10±8	10±10
S 535	13±8	15±10
F_1 hybrid (Active×Inactive)		
F×S and S×F	163±35	194±43

in enzyme levels exist even between those lines which are active in producing the enzyme. These differences reflect variability in the proportion of seedlings in each line which are actively producing enzyme as well as differences in enzyme levels in the individual seedlings. In strain K 4 about 73 % of the seedlings are active while in strain F 536 only 22 % are active. This was determined by testing individual seedlings zymographically with starch gel electrophoresis. The enzyme appears as a sharp band which migrates to the cathode in pH 8.5 borate buffer system.

Pretreatment of seeds with 0.01 M maleic hydrazide (MH) for 24 hours resulted in a significant increase in catechol oxidase activity in the active lines (Table 1). MH had no effect on enzyme level in the inactive lines. Almost all of the seedlings in the active lines showed catechol oxidase activity with MH pretreatment. Thus in the active lines seedlings which do not normally produce the enzyme have the capacity to do so if treated with MH. The seedlings in the inactive lines can not be similarly induced. No increase in enzyme activity was noted when seeds were presoaked in

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solutions containing catechol oxidase substrates such as catechol or dihydroxyphenylalnine.

Preliminary analyses on F_1 hybrids between active and inactive lines are interesting and may shed some light on the control mechanism. Whereas enzyme activity is observed in the untreated controls characteristic of the active parent, the hybrids resemble the inactive parent in that no induction occurs with MH pretreatment. Increased enzyme activity in the active lines with MH pretreatment colud reflect either gene induction with increased enzyme synthesis or activation of latent enzymes by MH. The former is a more likely explanation since no increase in activity occurs in pretreated hybrids.

71. Tulip breaking virus and synthesis of water soluble proteins in tulip petals

Yoshito OGAWA

In order to examine the relation between virus infection and injection of RNA, we applied pure RNA isolated from virus infected petals of William Pitt to normal tulip leaves using the carborandam method. The influence of RNA on the host plant was examined at the level of protein synthesis in the petals. Besides the experimental group, three control groups were arranged, 1) non-treated normal group, 2) virus inoculated group and 3) group treated by RNA, extracted from normal tulip petals.

Fresh tulip petals were mashed with the same volume of distilled water in a glass homogenizer at 0°C. Immediately after centrifuging the homogenate at 1,500 g for 10 minutes, electrophoretic analysis of the obtained supernatant was carried out at room temperature on cellulose acetate strips (Separax) of 2.5×9.0 cm. size, using veronal buffer (pH 8.6,

Table 1. Influence of RNA isolated from virus inoculated tulip petals on the synthesis of soluble proteins in the petals of William Pitt

(%)

		A	B	C	D	E	F	G	H
Control groups	Normal group	4.2	4.2	12.6	19.4	30.4	20.8	5.6	2.8
	Virus infected group	1.6	3.2	12.8	0.0	47.2	22.4	9.6	3.2
	Treated with RNA isolated from normal petals groups	2.1	2.1	27.3	16.8	32.8	14.7	2.1	2.1
Experimental group	Treated with RNA isolated from virus inoculated petals	3.6	3.6	25.2	0.0	29.9	21.6	12.5	3.6

μ=0.06) at 0.6 mA/cm for two hours. Nigrosin staining was used for the estimation of the analyzed proteins.

The analytical results are given in Table 1. The soluble proteins extracted from normal tulip petals were separated into eight fractions (A, B, C, D, E, F, G, and H). In the virus inoculated group fraction D disappeared and only seven fractions were recognized, though no remarkable change was found in the group treated with RNA extracted from normal tulip petals. In the experimental group, treated with RNA isolated from virus infected petals, fraction D also disappeared just like in the virus inoculated group.

The assumption that tulip breaking virus could modify the genic action on the synthesis of water soluble proteins of tulip petals seems to be acceptable.

C. MATHEMATICAL GENETICS

72. A stochastic model concerning the maintenance of genetic variability in quantitative characters

Motoo KIMURA

A new model is proposed to explain the maintenance of genetic variability in quantitative characters. The basic assumptions of the model are: (1) At every locus involved with the quantitative character, mutation can produce an infinite sequence of alleles. (2) The effect of a new allele on the quantitative character is only slightly different from the parent allele from which it was derived by a single mutational step. (3) The genes are additive with respect to their effect on the quantitative character. (4) The optimum phenotype is fixed, and fitness decreases in proportion to the squared deviation from the optimum.

Based on those assumptions, the properties of the model were worked out, enabling one to make predictions about the relation between mutation rates, genotypic variance and mutational load.

The present model is in sharp contrast with the conventional models in which mutation is assumed to occur only between a pair of alleles, say A or a. For details, see Kimura (1965).

73. Quasi linkage equilibrium

Motoo KIMURA

Recently, a number of papers on population genetics have been published treating the effect of linkage and epistasis on selection.

The purpose of this note is to report a remarkable property inherent in the linked gene system, which may be summarized as follows:

In a large random mating population, if gene frequencies are changing by natural selection, under loose linkage and relatively weak epistatic interactions, a state is quickly realized in which chromosome frequencies change in such a way that

$$R=\frac{x_1x_4}{x_2x_3}=\text{constant},$$

where x_1, x_2, x_3 and x_4 are respectively the frequencies of 4 types of chromosomes, A_1B_1, A_2B_1, A_1B_2 and A_2B_2, in which A_1 and A_2 are a pair of alleles in the first locus and B_1 and B_2 are a pair in the second locus. In other words, R is the ratio between the frequencies of coupling and repulsion phases.

Such a state was termed *quasi linkage equilibrium* and it was shown that several important conclusions follow from its formulation, namely: (1) The rate of change in the mean fitness of a population is equal to the additive genetic variance in fitness. So, Fisher's fundamental theorem of natural selection holds. (2) The direction of change in gene frequencies by natural selection is such that the mean fitness is increased. (3) The stable equilibrium of gene frequencies corresponds to the local maximum of the mean fitness and Wright's classical picture of "adaptive topography" continues to be useful if the concept of quasi linkage equilibrium is taken into account.

In order to corroborate the above points, an extensive numerical study was carried out with the help of computers. For details, see KIMURA (1965).

74. Simulation studies on the number of self-sterility alleles maintained in a small population

Motoo KIMURA

The number of self-sterility alleles (S alleles) maintained in a plant population of finite size was first studied mathematically by Wright (1939) and later by Fisher (1958). Their treatments were based on approximations and the scientific validity of their results were recently questioned by

Ewens (1964), who claims that the alleles are lost at much slower rate than indicated by the previous treatments.

The purpose of the present work is to check these points by carrying out simulation experiments with the help of a high speed computer (IBM 7090). In each generation, the process of mutation, random sampling of gametes, testing of compatibility between pollen and stigma, fertilization and the sampling of zygotes for the next generation was simulated by generating pseudo-random numbers, until a given number of offspring were produced, and the experiments were carried out for many generations.

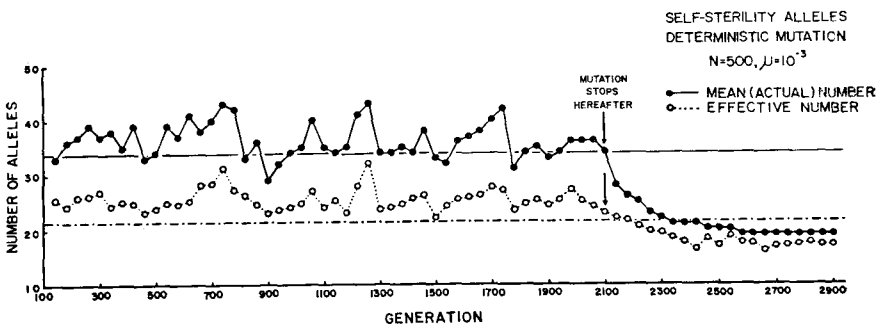


Fig. 1.

Fig. 1 shows the result of an experiment with a population of 500 individuals in which exactly one new mutation is produced in each generation until generation 2,100 after which mutation is stopped but simulation was carried out for another 800 generations. The initial population was assumed to contain 1,000 different S alleles. Equilibrium between mutation and random extinction appears to be reached by generation 100.

In the figure, both the mean and the effective numbers of alleles are given at intervals of 40 generations starting from generation 140. The mean number (n_a) is the one which is obtained by the reciprocal of the mean frequency ($\bar{x}$) of the alleles contained in the population. It is the same as the actual number of alleles in the population. The effective number (n_e) is computed by the reciprocal of the sum of squares of the allelic frequencies and it is usually smaller than the actual number. The average value of n_a computed from 50 outputs (generations 140~2,100) is

$$n_a = 36.2 ,$$

and this should be compared with the theoretical value obtained by Wright, *i. e.* $n_a = 33.8$. Similarly, the average value of n_e computed from 50 outputs is

$$n_e = 25.3 ,$$

and this should be compared with the theoretical value predicted by Fisher's theory, *i.e.* $n_e = 21.4$.

Thus, the agreement between the computer outputs and the theoretical predictions is sufficiently good for practical purposes. The figure also shows the results of stopped mutation and it may be seen that the mean number of alleles drops rather sharply during at least the first couple of hundreds of generations, while the effective number drops less markedly. This is due to the fact that, after the cessation of mutations the rare alleles are lost rather rapidly but this has little effect on reducing the effective number.

The same type of simulation experiments were also performed by Dr. T. Maruyama at the University of Wisconsin, assuming stochastic mutations, namely, assuming that the actual number of mutations follows Poisson distribution. The results obtained were essentially the same as those obtained by assuming deterministic mutations.

Hereby the validity of the results obtained by Wright and Fisher on the self-sterility alleles is confirmed and the arguments put forward by Ewens (1964), based on the extrapolation from his study of the 3 allelic system are disproved.

D. GENETICS AND BIOCHEMISTRY OF MICROORGANISMS

75. Leaky mutants of flagellation genes in Salmonella

Tetsuo IINO and Michiko MITANI

Strains SJ82, SJ122 and SJ376, originated from *Salmonella typhimurium* TM2, were found to be leaky non-flagellate mutants. By transduction analysis their mutant sites were located in *flaA*, *flaB*, and *flaC* cistrons respectively. On semisolid medium, these mutant clones formed satellite colonies; that is, a large colony was surrounded by many minute colonies, each formed by non-motile descendants of a motile multi-flagellate cell. Flagella of these mutants were observed under electron microscope. In all of these strains, flagellated cells were present at the frequencies of 5 to 10% of the populations in broth culture. Their average number of flagella per flagellated cell was one, while there were 7 flagella in the wild strain, TM2. It is worth noting that flagella of the leaky mutant cells were much less numerous but were as long as those of the wild strain.

From these observations the events occurring in the culture of the leaky mutants are described as follows. In the cell population of the mutants, cells which have recovered the ability to synthesize flagella less efficiently than the wild strain appeared at the frequency of one per 10^2 to 10^3 cells. They carried multi-flagella as long as the normal strain. Each minute colony of the satellites on semisolid media is inferred to be originated from such a cell. The ability to produce flagella lasted only one or at most a few cell generations, and the number of flagella per bacterium was decreasing until finally the offspring turned out to be single-flagellated or non-flagellated due to dilution during successive cell divisions.

76. Heteromorphism of flagella in a Salmonella *mutant*

Tetsuo IINO and Michiko MITANI

The heteromorphous mutant of *Salmonella abortus-equi* differs from the wild strain in the structural gene of phase-2 flagellin. It predominantly produces normal flagella, but at the frequency of 2%, cells with both normal and curly flagella appear in the broth culture. Often a flagellum has mixed normal and curly waves. No cells with curly flagella only have been ever detected. Each subclone started from a single cell of the mutant clones showed the same character. Therefore the heteromorphism is not explained by frequent mutation from normal to curly in the mutant culture. The strain was stable in flagellar phase-2. Consequently, the heteromorphism cannot be attributed to flagellar phase-variation. The remaining possibilities are that (1) both normal and curly flagellins are produced by coding degeneracy in the mutant, or (2) flagellin produced by the mutant is of the same kind regarding amino acid composition but has the potentiality to take either of the two alternative conformations depending on the condition of its polymerization.

77. Dimorphic nature of bundled flagella in a small-amplitude mutant of Salmonella abortus-equi

Michiko MITANI and Tetsuo IINO

By both darkfield microscopy and electronmicroscopy, morphological conversion of bundled flagella was found to appear in a small-amplitude mutant of *Salmonella abortus-equi* when the cells were suspended in 0.25% methylcellulose solution. The change in the shape of bundled flagella from small-amplitude to curly was observed about 40 minutes after the

cells had been suspended in the solution, and thereafter the fraction of curly cells increased in the course of time up to 80% of the cell population. Dispersed small-amplitude flagella did not undergo morphological change in the solution. Tight association of the component flagella in each bundle caused by viscous solution of methylcellulose may produce a stress among them resulting in the alteration of their shape. The process of the transition in the flagellar shape was observed on a cell under darkfield microscope: the twirl of the bundle accompanied by a sudden change from small-amplitude to curly started to occur from the tip of the bundled flagella and proceeded to the base. As the small-amplitude mutant differs from the normal in the structural gene of flagellin, the dimorphism is primarily attributed to the elastic bipotential conformation of the flagellin produced by the mutant.

78. An assay for newly-synthesized flagellin of Salmonella

Hideho SUZUKI and Tetsuo IINO

An attempt was made to apply the reconstitution of flagella for separating flagellin from a cell-free extract which might include a newly-synthesized fraction of flagellin.

Flagellin monomers produced by treatment with dilute HCl at pH 2 were reconstituted into flagellar filaments with efficiency of more than 80% in the following mixture: flagellin 4–10 mg/ml flagella fragments as seeds 1/5–1/4 amount of flagellin, phosphate buffer (pH 6.8) 20 mM, KCl 150 mM, $MgCl_2$ 5 mM.

Cell-free extract from ^{14}C-labelled culture of *Salmonella abortus-equi* strain SL23 (*enx*) was subjected to heat treatment in order to dissociate all flagellar fragments into monomers and to denature the bulk of cellular protein, then the extract was added to a reconstituting mixture which consisted of flagellin and seeds originated from *Salmonella* strain SJ25 (*1, 2*). The reconstituted flagella were analyzed by starch gel zone electrophoresis. Flagellin *enx* and *1, 2* were separable electrophoretically at pH 8.4 (buffered with 10 mM tris); i.e. flagella reconstituted incorporated labelled material and gave a peak of radioactivity at the position corresponding to *enx*. When an aliquot of the sample was mixed with a γ-globulin fraction containing antibody for *enx*, prior to electrophoresis, this peak practically disappeared and almost all of the radioactivity remained unmoved at the original point, indicating that the radioactive material was reactive specifically with anti-*enx* globulin.

A similar method was applied to detect intracellular flagellin. The

labelled cell-free suspension prepared from cells of SJ28, the non-flagellate mutant strain which was known to produce *enx* flagellin CRM (IINO and HARUNA: Annual Report No. 11, 1960), was acidified to pH 2 with HCl and the resulting denatured protein was removed. The acid-treated extract was brought into the reconstituting system with *1*, *2* flagellin and seed, after the volume of the extract had decreased to less than 0.5 ml by freeze-drying. An electrophoretic analysis again displayed the presence of the radioactive material at the position in which flagellin *enx* should reside. This radioactive material was also reactive with γ-globulin fraction of anti-*enx* serum.

Flagellin produced by the CRM-type mutant, SJ28, is incorporated into flagella in the reconstituting system, so that it is inferred that lack of a mechanism to organize its flagellin into a flagellum, rather than an abnormality of flagellin, is responsible for the loss of flagella in this mutant.

79. *Composition of chromosome fragments participating in P22 mediated transduction of* fla·H1·mot *loci in* Salmonella typhimurium

Masatoshi ENOMOTO

Based on the hypothesis proposed by PEARCE and STOCKER (1965) that exogenotic *H1* allele in transductional heterogenote involving cotransducible *fla·H1* or *mot·H1* loci could be expressed when it was carried with mot^+ or fla^+ locus, the composition of the transducing fragments participating in transduction of *fla*, *H1*, and *mot* loci was estimated. Three *H1*-linked nonmotile mutants, *mot C-244*, *fla-48*, and *mot C-244·fla-48* derived from *Salmonella typhimurium* TM2 (*i*: *1.2*), were used as recipients. The order of the genes in this chromosomal region is *fla-48·H1·mot C-244*. Phage P22 was propagated on line SJ697 of TM2 strain whose phase-1 flagellar antigen was replaced by *gp* by transduction of *H1* from *Salmonella dublin* SJ11 (*gp*:-). Overnight broth cultures of the recipients in phase-1 (*mot C-244*) or in latent phase-1 (*fla-48* and double mutant) were mixed with phage at moi 10^{-2} (single mutant) or at 5 (double mutant). The mixtures were plated, if necessary after dilution, on semisolid medium containing anti-*1.2* or anti-*1.2* and -*gp* sera. After incubation for 9–10 hr at 37°, the number of trails was counted under binocular microscope. Frequency of cotransduction of *fla* and / or *mot* with *H1* was determined by slide agglutination test of swarms obtained from another transduction test. The results are shown in Table 1.

It is necessary for trail production in the transduction to the double mutant (fla^-·*H1*·mot^-) that the donor allele *H1gp* is carried together with

Table 1. Transduction from SJ697 (*gp*: *1.2*) to *H1*-linked nonmotile mutants.

Recipient	Trail (abortive transductant)						Swarm (complete transductant)	
	Medium containing	No. of transductants per 10^5PFU	Transductant (per 10^5 PFU) expressing *i* (recipient) No.	%	*gp* (donor) No.	%	Percent of *H1*-cotransduction	No. of transductants per 10^5 PFU
fla-48	anti-*1.2*	5.27	4.97	67.3	3.00×10^{-1}	4.1	2.1	4.77×10^{-1}
	anti-*1.2, gp*	4.97						
mot C-244	anti-*1.2*	2.10	1.40×10^{-1}	1.9	1.96	26.5	36.0	1.36×10^{-1}
	anti-*1.2, gp*	1.40×10^{-1}						
fla-48	anti-*1.2*	1.56×10^{-2}	—	—	1.56×10^{-2}	0.2	74.7	7.20×10^{-4}
mot C-244	anti-*1.2, gp*	2.60×10^{-4}						

both *fla*$^+$ and *mot*$^+$ genes. When the transducing fragments participating in transduction of *fla* and / or *mot* were taken as 100, those carrying *fla*, *fla·H1*, *fla·H1·mot*, *H1·mot*, and *mot* loci were 67.3, 4.1, 0.2, 26.5, and 1.9% respectively. The transducing fragments ending between *fla* and *H1* were 93.8% and those between *H1* and *mot* were 6.0%. This indicates that most of the transducing fragments are cut off at a predetermined position.

The frequency of wild type recombinants in the transduction to the double mutant was less than 2×10^{-2} as compared with that of single mutants. The frequency of *H1*-cotransduction with *fla-48* was very low. These low frequencies may be explained by the above estimation that chromosome fragments carrying either *fla·H1·mot* or *fla·H1* are few.

80. *Dependency of* arg-s *mutant of* Salmonella typhimurium *not only on uracil but also on arginine during arginine inhibition*

Jun-ichi ISHIDSU

Among various physiological characteristics of *arg-s-1*, an arginine sensitive mutant of *S. typhimurium*, it has been reported that transfer of cells from a medium containing both arginine and uracil to the same medium which contains only arginine at any stage of growth brings about immediate and absolute growth inhibition (ISHIDSU, Annual Report No. 15, 1964). This suggests that, during the normal growth in the first medium, formation of uracil synthesizing enzymes, or of one of them at least, is strongly repressed and cannot be recovered even after removal of uracil from the medium.

Effect of similar transfer to other media was examined. Cells were grown in a medium supplemented with both arginine and uracil as far as middle log phase. They were washed twice by saline and transferred to fresh media supplemented with (1) both arginine and uracil (control), (2) only uracil and (3) neither (minimal medium). Care was taken not to change markedly the titer of cells upon transfer. When medium (1) was used, no interruption of logarithmical growth was observed. But transfer to medium (2), as well as to (3), affected the cells and a 1 to 2 hour lag period was brought about before the cells could recover logarithmical growth. Additionally, it was found that this lag disappears if medium (2) is supplemented with citrulline, one of the biosynthetic precursors of arginine. Another precursor, ornithine, could not save the cells from the lag following the transfer to the medium (2).

These findings suggest the following considerations: (1) The mutant requires arginine as well as uracil during arginine inhibition. (2) This dependency on arginine is due to the inhibition of the conversion of ornithine to citrulline by arginine. (3) The inhibition at this specific step is the result of the inhibition of carbamyl phosphate formation which acts as a common substrate of ornithine- and aspartate-transcarbamylase in this organism.

Enzymatical studies of carbamyl phosphate synthetase of this mutant are now under way.

81. *Subunit analysis of* g*-complex antigens of* Salmonella

Shigeru YAMAGUCHI

It is known that the flagellar antigens of *Salmonella* are composed of several antigenic subunits. Among them, the so-called *g*-complex antigens, all of which belonging to phase-1, have been studied most accurately in respect of their subunit composition. The already known subunits of *g*-complex antigens are *f*, *g*, *m*, *s*, *t*, *p*, *q*, *u*, and z_{51}. However, it has often been recognized in the process of serum absorption when preparing single factor antisera that subunit *g* may be further subdivided.

Table 1. Subunit compositions of *g*-complex antigens.

Strains		Antigen type	Subunit composition of phase-1
Salmonella abortus-equi	Tr6	*4, 12; gt : (enx)*	$g_1\ g_2\ g_3\ t$
〃	Tr11	*4, 12; gp : (enx)*	$g_1\ g_4\ g_5\ p$
〃	Tr16	*4, 12; fg : (enx)*	$g_3\ g_4\ f$
〃	Tr17	*4, 12; gm: (enx)*	$g_1\ g_2\ g_4\ g_5\ m$

A subunit analysis of *g*-complex antigens was carried out by means of reciprocal absorption tests of rabbit antisera and slide agglutination tests among four strains of phase-1 stable *Salmonella abortus-equi*, each having *H1* transduced from one of the four serotypes of *g*-group *Salmonella*, that is, *Salmonella* sp. (*gt*:-), *S. dublin* (*gp*:-), *S. derby* (*fg*:-), and *S. enteritidis* (*gm*:-). As shown in Table 1, it was found that subunit *g* is not single but can be further subdivided into at least five.

82. Mutation of g-complex antigen in Salmonella

Shigeru YAMAGUCHI and Tetsuo IINO

Flagellin, flagellar protein of *Salmonella*, has complex antigenicities. Its antigenic specifficity is determined by a single gene, *H1* in phase-1 and *H2* in phase-2. It is well known that mutants slightly altered in the serological character can be selected for by incubating the cells on a semi-solid medium containing homologous or cross-reacting antiserum. Serological and genetical investigations of such serologically altered mutants of *Salmonella* having the *g*-complex antigen were carried out.

Tr16 (*4,12*; g_3g_4f: (*enx*)) is a phase-1 stable strain of *S. abortus-equi*. *H1* gene of the strain was transduced from *S. derby* (*fg*:-). This strain was cultivated on semi-solid medium containing rabbit anti-*f* serum at the minimum concentration in order to immobilize the Tr16 cells. After 48 hr incubation five mutants (Tr16-fM1 to -fM5) were obtained as swarms spreading faster in the medium than the parental type cells. Tr16 was also cultivated on semi-solid medium containing anti-g_3t serum or anti-g_4g_5p serum and each of five mutants (Tr16-g_3M1 to -g_3M5, and Tr16-g_4M1 to g_4M5) were isolated from the respective medium.

Agglutinabilities of these fifteen mutants with anti-*f*, anti-g_3m, and anti-g_4g_5p sera were compared with those of the wild type cells by tube agglutination test. In nine mutants (Tr16-fM1 to -fM5, -g_3M1, -g_4M2, -g_4M4, and -g_4M5), only agglutinabilities of the antigenic subunits corresponding to the agglutinins used for the selection have decreased to one fourth to one tenth of those of the wild type cells in agglutination titer, and those of the other antigenic subunits have remained unchanged. In the other six mutants, agglutinabilities not only of the subunits corresponding to the agglutinins used for selection but also of all the other subunits decreased to one tenth of those of the wild type cells. Since the rabbit antisera against mutants Tr16-fM2, -g_3M1, -g_3M2, -g_4M1, and -g_4M2 were completely absorbed by the wild type cells, it is thought that the antigenic specificities of these mutants are not qualitatively different from those of the parental strain.

Transduction of *H1* genes from Tr16-fM2, -g_3M1, -g_3M2, -g_4M1 and -g_4M2 to *S. abortus-equi* strain SJ241 (*4,12*; *a*: (*enx*)) was carried out by using phage P22 as mediator and a semi-solid medium containing anti-*a* serum as selecting medium. All the transductants obtained had the antigenicity exactly the same as the donor mutants, indicating that the mutation sites of these serological mutants are within *H1*. Tryptic peptide maps of flagellins of Tr16-g_3M1 was compared with that of the wild type flagellin. In the peptide map of Tr16-g_3M1 flagellin, a neutral spot of wild type flagellin was substituted by a somewhat basic spot. The other 33-34 spots were identical between these maps.

83. Lack of host cell reactivation of bacteriophage T1 inactivated by ^{32}P decay

Mituo IKENAGA and Sohei KONDO

In ultraviolet (UV) inactivated phage T1, a fraction of the UV lesions can be reactivated by the host cell (GAREN and ZINDER 1955). The reactivation, later called "host cell reactivation" (HCR), is believed to result from elimination of UV damage of the phage's DNA, due to some repair enzyme of the host cell. According to a recent report by SAUERBIER (1964), phage T1 damaged by ionizing radiation also undergoes repair by host cell reactivation. Thus, HCR might be a versatile repair mechanism capable of eliminating various kinds of damage sustained by irradiated phage DNA.

In order to gain more insight into the mechanism of HCR, the survival of ^{32}P decay inactivated T1 was determined under HCR conditions and in absence of HCR. Heavily ^{32}P labeled T1 was prepared as reported by STENT and FUERST (1960), and stored in air or in nitrogen saturated glycerol casaminoacid medium at 4°C. The survivors were counted day by day by parallel plating on the host bacterium *E. coli* H/r, capable of performing HCR, and *E. coli* B_{s-1}, a mutant lacking the reactivation enzyme. Fig. 1 shows the survival curve of ^{32}P decay inactivated T1 in air or nitrogen saturated medium when plated on *E. coli* H/r and B_{s-1}, respectively. Apparently, survivals of ^{32}P labeled T1 are the same in both host bacteria, irrespective of having or not the HCR enzyme. HCR of T1 inactivated by ^{32}P decay was completely inhibited even in nitrogen saturated medium in which HCR efficiency of γ-rayed T1 is found to be about two times larger than in air. The lack of HCR in the present study indicates that ^{32}P decay induced lesions in phage DNA are not host cell reactivable. The lesions responsible for T1 inactivation may be double strand scissions of T1 DNA molecule most probably due to ^{32}P transmutation.

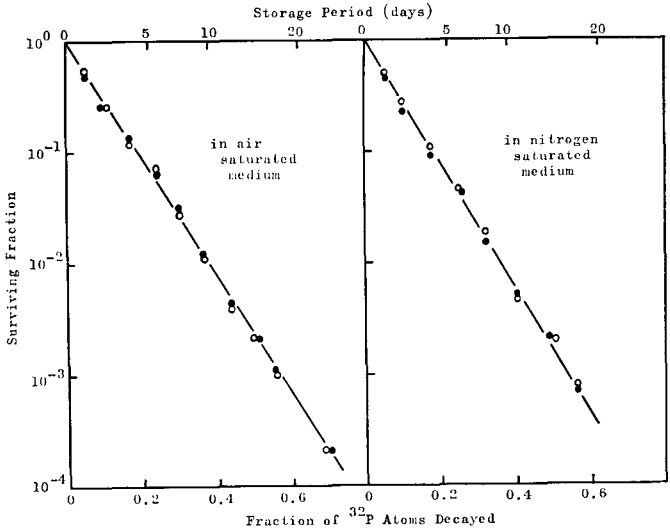


Fig. 1. Survival curve of phage T1 inactivated by ^{32}P decay (300 mCi/mg-P) plated either on *E. coli* H/r (open circle) or B_{s-1} (solid circle).

E. RADIATION GENETICS IN ANIMALS

84. *Mechanisms underlying the enhancing effect of induced-mutation frequency after fractionated γ-irradiation of silkworm gonia*

Yataro TAZIMA and Kimiharu ONIMARU

As reported previously, it was found that fractionated exposure to γ-rays enhances the induced mutation frequency in silkworm gonia. As a possible interpretation of this phenomenon some kind of modification* in the cellular metabolism was assumed presumably being caused by a disturbance, which hindered the repair of premutational lesions until after a certain while the condition disappeared. Hence, experiments were carried out to seek supporting evidences for this assumption. In an experiment, in which irradiated insects had been kept at high or low temperatures during the period between two exposures, it was found that the duration

*) In a previous report the term "metastasis" was used for representing a "modified state" of the irradiated cell. Since in medical science "metastasis" is used for representing "transfer of a disease-producing agency", the term "modified state" will be used hereafter instead of "metastasis".

of the persistenc of the modified state was temperature dependent. Post-treatment of irradiated cells with agents that are known to affect cellular metabolism proved to be effective in modifying mutation frequencies. Those results were consistent with the expectation that the post-irradiation modification may be a change in the metabolic activity of the cell.

However, a finding that the enhancing effect did not give a decay curve, but instead exhibited a peak at 18-24 hours after the initial exposure, did not support the view of a biochemical disturbance, but seemed to suggest cell synchronization. Nevertheless, this latter view contradicted our previous finding that mutation frequencies never fell below a single acute exposure level for 48 hours. In other words, no effect in opposite direction was observed during this period. Questions arose, therefore, if the tested interval was not short enough or had we not tested a period long enough to reveal an opposite effect due to cell synchronization. In order to answer those questions, the following experiments have been carried out.

a) Fractionation experiment with different initial doses.

Total 1000 r were given in two fractions, 250 r and 750 r, to two experimental series in eversed order at various time intervals. In one series 250 r were given first followed by the second exposure of 750 r at 6 hour intervals. Whereas, in the other group the initial dose was 750 r and then

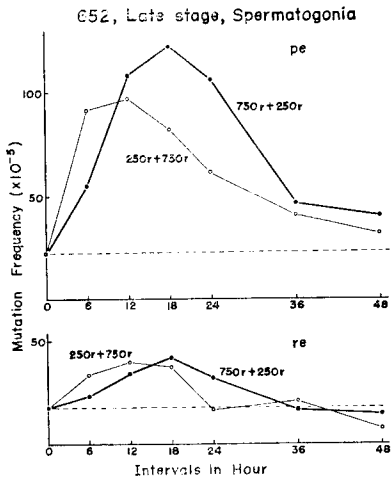


Fig. 1. Comparison of induced mutaiton frequencies between two fractionation experiments of total 1000 r with eversed first and second doses.

250 r were given at the same intervals. The results are given in Fig. 1.

It may be seen from the figure that the peak appeared earlier for the initial 250 r series than for the initial 750 r series, although it was lower. Those results can better be interpreted by assuming cell-synchronization than by postulating a biochemical disturbance.

b) Fractionation experiment with protracted intervals.

If cell synchronization had actually occurred after the initial exposure, the opposite effect of fractionated irradiation could be revealed when longer intervals were examined. Furthermore, such experiment might reveal even a second peak of the next synchronized cell cycle. Since one cell cycle has been estimated to last 34 hours for non-irradiated primary spermatogonia (Sado, this Report), our previous observation for a 48 hour period seemed to be insufficient for such purpose.

An experiment was, therefore, undertaken by extending the intervals between two exposures up to 72 hours. The initial and the second doses were the same, 500 r. The results are given in Fig. 2.

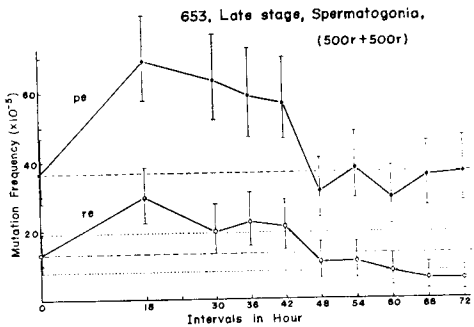


Fig. 2. Results of a fractionation experiment of 500 r+500 r with extended intervals up to 72 hours.

As seen from the figure, the enhancing effect of fractionated exposure disappeared after 48 hours and thereafter the mutation frequency was maintained almost at the single exposure level. The apparent decline of the curve after 48 hours, seems, instead of representing an effect in opposite direction, to show a general trend of frequency decrease in accordance with the progress of the development of the larvae. Why, then, was the opposite effect not observed? Since cell synchronization became plausible from experiment (a), how could we interpret the result of experiment (b)? The contradiction seems to be resolved by assuming that synchronization is destroyed immediately in the subsequent cell cycle.

In this regard the phenomenon is not cell synchronization in true sence, but a temporary accumulation of cells that are blocked at a certain phase of their cycle. Presumably, the blockade is gradually released after a certain while.

If the above view is correct, the nature of the "modified state" of the irradiated cell can be interpreted as a shift of cell compartment in regard to the DNA synthetic cycle among germ cell population. Thus the enhancing effect of fractionated irradiation may be explained by assuming an accumulation of cells at a certain phase in which a repair of premutational lesions can hardly occur.

85. *Time of reversal of radiation does-rate effect on mutation induction in silkworm gonia*

Yataro TAZIMA and Yosoji FUKASE

With the purpose of studying in more detail at what stage of development the dose-rate effect on mutation induction becomes reversed, an experiment has been carried out with silkworm gonia within the period from 3 days before to 5 days after hatching. Total 1000 r γ-rays were given either acutely or chronically. Dose rates used were 100 r/min for acute and 0.3 r/min for chronic treatment. In the acute series irradiation was given in succession every 24 hours to seven groups, whereas the exposure of the chronic series lasted 3 days and was started every 24 hours in order to examine the variation in mutation response during the 8 day period indicated above. The results are shown in Fig. 1. In this figure the response curve for the chronic treatment was obtained by connecting the observed mutation frequencies plotted at the start of each exposure, since the cytological observation revealed that the progression of the cell cycle is arrested shortly after the onset of chronic exposure at a dose-rate of 0.16 r/min (SUGAI, in preparation and SADO, this Report).

It can be seen from the figure, that for the male germ cells the mutation frequency at chronic exposure rushed ahead of the acute as early as one day afrer hatching. In female germ cells mutation response to different dose rates was somewhat different from that of male germ cells. Yet, a similar overshoot was observed two days after hatching. These findings suggest that the critical time of development at which the dose-rate effect becomes reversed is closely correlated with the metabolic state of the germ cells. Presumably, the cells become active after hatching.

It was found in our previous experiments (TAZIMA, 1965) that the enhancing effect of fractionated irradiation is stronger for advanced stage gonia (7 day old larva) than in their younger stages (hatching day larva).

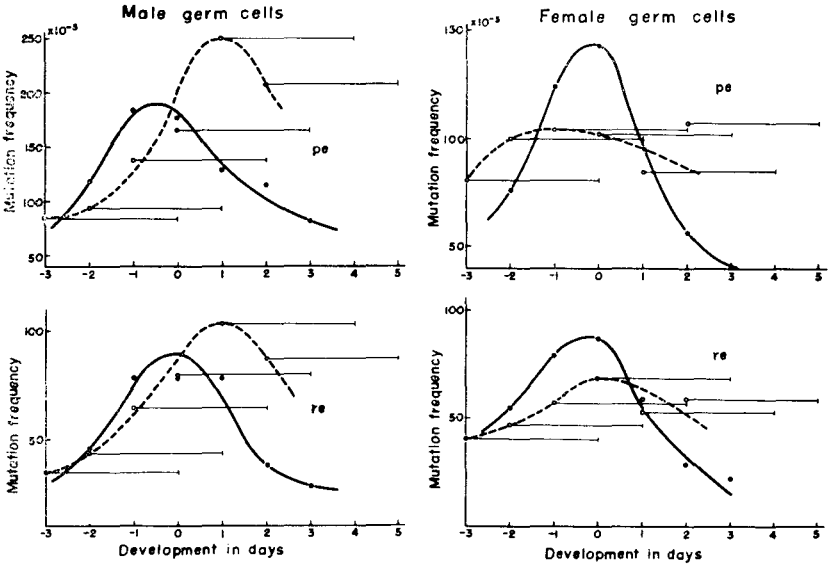


Fig. 1. Changes in mutation frequency after acute and chronic exposures of silkworm gonia with their development.
Solid line: acute, Broken line: chronic

Since a chronic exposure can be regarded as an integrated form of fractionated exposures, it may give rise, in analogy to fractionated irradiation (preceding article in this Report), to higher mutation frequencies in advanced stage gonia than the acute treatment.

86. *Further data on post-irradiation treatment of silkworm spermatogonia with various metabolic inhibitors*

Yotaro TAZIMA and Kimiharu ONIMARU

Experiments have been continued with various metabolic inhibitors and a radiation-protecting chemical in order to confirm the effect of post-treatment on modifying mutation frequency (*cf.* Annual Report No. 15). Agents tested in this experiment were nitrogen gas, 8-azaguanine, mitomycin C, chloramphenicol and a known radiation protecting chemical, AET. Except for nitrogen gas, chemicals were administered to the larvae by feeding them with fresh mulberry leaves, which had been coated with an aqueous solution of the chemicals. Wild type larvae of C108 strain were irradiated with 1,000 r γ-rays at 7 day age. Two hours after irradiation, the insects were allowed to feed on the prepared leaves for 9-21

hours. For gas treatment, the insects were placed immediately after irradiation in a vial filled with nitrogen gas. The condition for each treatment was as follows:

Cont. =1,000 r γ-rays only.
Gas 1=Raised 24 hours in a current of nitrogen gas mixed with a minimum quantity of air in order to avoid asphyxia.
Gas 2=Same gas as above, but in tightly shut vials. Opened twice to introduce air when asphyxia observed.
AG 1=8-azaguanine ("Azan", Tanabe Seiyaku Co., Ltd.), 105 mg per 1,000 larvae.
AG 2=8-azaguanine, 210 mg per 1,000 larvae. Given twice in two half doses.
MMC =Mitomycin C (Kyowa Hakko Co., Ltd.), 6 mg per 1,000 larvae.
CAP =Chloramphenicol ("Chloromycetin", Sankyo Seiyaku Co., Ltd.), 100 mg per 1,000 larvae. Given twice in two half doses.
AET =2-Aminoethyl isothiouronium bromide (Koch-Light Laboratories, Ltd.), 150 mg per 1,000 larvae. Given twice in half doses.

The results, given in Table 1, show that treatment with those agents,

Table 1. Results of post-irradiation treatment of spermatogonia of 7 day old larvae (653).

Treatment	Observed number	Number of mutants				Mutation frequency ($\times 10^{-5}$)			
		pe	*pe*,+*mos*	*re*	*re*,+*mos*	*pe*	*pe*,+*mos*	*re*	*re*,+*mos*
Cont.	177,599	69	0	18	2	38.85	0.00	10.14	1.13
Gas 1	173,255	93	1	41	2	53.68	0.58	23.66	1.15
Gas 2	173,258	99	2	36	3	57.14	1.15	20.78	1.73
AG 1	173,998	107	0	38	3	61.49	0.00	21.84	1.72
AG 2	172,507	96	0	40	3	55.65	0.00	23.19	1.74
MMC	163,445	152	2	56	5	93.00	1.22	34.26	3.06
CAP	172,515	101	3	25	0	58.55	1.74	14.49	0.00
AET	164,803	80	1	22	0	48.54	0.61	13.35	0.00

except for AET, increased mutation frequency to a considerable extent. Considering that those agents either disturb respiration or inhibit nucleic acid synthesis or protein synthesis, it can be surmised that they had suppressed the repair of radiation induced premutational lesions resulting in increased mutation frequencies. An alternative interpretation is also possible, by assuming that those agents affected the progression of the

cell cycle by prolonging the duration of radiation-caused blockade (*cf.* an article by SADO in this Report).

In conclusion, the present results are consistent with both hypotheses of metabolic disturbance and progression-blockade, but they do not furnish a conclusive clue which would allow us to determine which of the two is more reasonable.

87. Further studies on proliferation kinetics of silkworm spermatogonia during chronic γ-irradiations

Toshihiko SADO

In a previous report (SADO 1965), the kinetics of proliferation and killing of spermatogonia of young silkworm larvae (2.5–7.5 day old) during chronic exposure was studied. This stage was selected because the number of cells at the beginning of exposure was not very large and they were more homogeneous than at more advanced stages. It was thought that this was advantageous for the kinetic studies of primary spermatogonial populations during chronic irradiation. In the present experiment, however, the irradiation of the larvae was performed between 5 and 10 days after hatching. This is the interval where Type II dose-rate effect on mutation induction is observed. The following kinetic indices were determined at various intervals after the onset of chronic exposure (Cs-137 γ-rays, 0.130 r/min for 5 days): (a) total viable cell (or gonocyst) counts, (b) incidence of pycnosis, (c) mitotic index, and (d) S index, or the percentage of cells that take up H^3-thymidine in 1 hour after isotope administration. It was hoped that this will make possible the assessment of dynamic changes in a spermatogonial population with regard to the distribution of irradiated cell stages during chronic exposure.

The results show that there was a considerable difference in various indices from those obtained in the previous experiment. Roughly speaking, the cells were more sensitive to radiation killing and mitotic inhibition in this experiment (5–10 day old larvae) than in the previous study (2.5–7.5 day old larvae). 1) The majority of secondary spermatogonia present at the beginning of the exposure were killed by the end of exposure. Thus, only a few secondary spermatogonia (12 cysts per testis) survived the exposure (total dose: 900 r). 2) The maximum depletion of primary spermatogonia was observed 3 days after the onset of exposure. 3) Incidence of pycnosis of primary spermatogonia reached the maximum values of 33.3 and 34.0% at 48 and 72 hours, respectively, after the onset of exposure, or after accumulated doses of 360 r and 540 r, respectively. At the termination of the exposure, only 4.4% was found to be pycnotic.

In contrast, 10.4% of secondary spermatogonia (cyst) was still pycnotic at this time. 4) Mitotic activity of primary spermatogonia was considerably suppressed during the treatment. Maximum suppression was observed on the third day in one experiment (17% of the control), whereas it was found on the fourth day (12% of the control) in another experiment. It is obvious that the maximum depletion period corresponds to the time when mitotic activity is extremely low and incidence of pycnosis is very high. These results suggest that there was a significant, but not complete, block in cells at premitotic stage (G_2) entering into mitosis (M) during chronic irradiations. 5) S index of primary spermatogonia also decreased to 10% in contrast to 30% of the control as early as 12 hours after the onset of the exposure (accumulated dose: 90r) and was constant for 36 hours. This indicates that a fraction of cells in S phase decreased to 1/3 of the control soon after onset of chronic exposure. This may have been due to a partial block of cells in G_1 entering into S phase.

These results show that there was a considerable accumulation of cells in (late?) G_1 as well as in G_2 period during chronic irradiation. It is obvious that the distribution of cells at various phases of the cell cycle during irradiation is significantly different from that of unirradiated cell population to which a single acute dose is generally given to compare the dose rate effect on mutation induction. As we know that radiosensitivity of cells is not constant throughout the cell cycle, the change in distribution of primary spermatogonia at various phases of the cell cycle should no doubt affect the mutation frequency. We cannot determine at this moment, however, which of the two *i.e.*, accumulation of cells in G_1 or in G_2, is more important in enhancing the mutation rate by chronic irradiation.

88. Change in distribution of primary spermatogonia of the silkworm at various phases of the cell cycle after irradiation

Toshihiko SADO and Kugao OISHI

It has been held by many workers that the effect of dose fractionation on cell survival or on mutation frequency should be similar to the effect of reducing the dose-rate of chronic irradiation. TAZIMA's (1965) recent finding that acute doses given to silkworm larvae in two or more fractions give a much higher mutation frequency than that given in a single acute exposure raised the question if synchronization of cells by the first dose might be responsible for the result. Because of the great significance in the interpretation of the mechanisms involved in Type II dose-rate

effect on mutation induction in silkworm gonia, experiments were undertaken to examine how the distribution of spermatogonia throughout various phases of the cell cycle would be affected by radiation.

First, the distribution of primary spermatogonia of unirradiated larvae at various phases of the cell cycle was studied. This was done by estimating the relative time occupied by each phase of the cell cycle, on the assumption that the cells are distributed randomly throughout the cell cycle. As will be reported elsewhere, the generation time of these cells is approximately 34 hours, the distribution of cells in various phases being roughly as follows: G_1, 40%; S, 40%; G_2, 18%; and M, 2%.

In the following experiment, 6 day old male larvae were exposed to a single acute dose of 1,000 r (100 r/min) and various kinetic indices such as (a) total surviving cells, (b) incidence of pycnosis, (c) mitotic activity, and (d) S index, as a function of time after irradiation were studied. 1) It was found that primary spermatogonia were not homogeneous in respect to radiosensitivity, possibly due to difference in celll generations or in phases of the cell cycle. (These cells are indistinguishable morphologically from each other but there is no doubt that they represent a mixed population comprising stem cells, or *predefinitive* spermatogonia, and *indefinitive* spermatogonia, *i.e.*, intermediate generations between the stem cells and the first generation of secondary, or *definitive* spermatogonia (*cf.* HANNAH-ALAVA 1965)). 2) Mitotic activity dropped abruptly following irradiation and no mitoses were observed between 3 and 12 hours. Recovery of mitoses proceeded very slowly. Thus after 24 and 36 hours the frequency of mitoses was still 5 and 25% of the control, respectively. These observations indicate that there was a complete block of cells in G_2 entering into M phase (G_2 block) immediately following irradiation and that recovery from this block went on a very slow rate. 3) S index also decreased linearly during the first 12 hours, from 40% at time 0 to 9% after 12 hours. This slope can be extrapolated to 0% at 15 hours on the time axis. After 24 hours S index was 3.6% and after 36 hours it was 14%. Extrapolation of these two values goes back to 0% at 20 hours on the time axis.

These results suggest a nearly complete block of cells in G_1 entering into S phase immediately following irradiation on the one hand and a normal transit of cells from S into G_2 phase on the other. Since mitosis was nearly completely blocked during the first 24 hours, there was a decrease of the cell fraction in S phase and concomitant increase of G_2 population during this period. G_1 block seems to have been lost after about 20 hours when S index began to increase. Distribution of cells in various phases of the cell cycle at different intervals after irradiation can, therefore, be

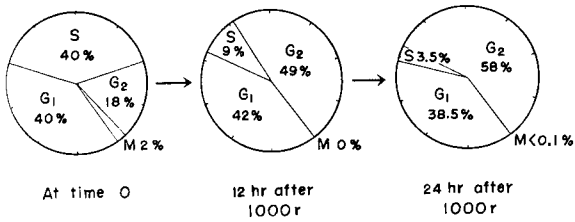


Fig. 1.

represented schematically as in Fig. 1. It is clear from this figure that there is a high correlation between the size of G_2 population and the enhancement of mutation frequency by fractionated doses given at intervals after the first dose (*cf.* TAZIMA 1965). However, we can not rule out the possibility that accumulation of cells in late G_1 might also be responsible, because G_1 block was nearly complete immediately following irradiation and continued for as long as 20 hours.

89. Radiosensitivity of silkworm spermatogonia during the cell cycle
I. Fate of primary spermatogonia irradiated in S phase

Toshihiko SADO

The foregoing experiment showed that as many as 58% of primary spermatogonia in contrast to 18% of the control was occupied by G_2 population 24 hours after irradiation with 1,000 r, whereas the proportion of G_1 population was not significantly different from that in the unirradiated group, *i.e.*, approximately 40%. This is because radiation caused an immediate block of cells in G_1 entering into S (G_1 block) and of cells in G_2 going into M phase (G_2 block), without affecting the transit of cells from S into G_2. For the evaluation of these results in relation to the enhancement of mutation frequency by dose fractionation, it is essential to ascertain the radiosensitivity of cells during various phases of the cell cycle. This includes sensitivity to mutation induction, induction of chromosome aberrations and radiation killing.

In the following experiment, the fate of primary spermatogonia irradiated in S phase was studied with H^3-thymidine (H^3-Tdr) as cell marker. 6 day old male larvae were injected with H^3-Tdr (0.2 μc/larva). One hour later the injected larvae were exposed to a single acute dose of 1,000 r. At intervals, frequencies of label among survivors, pycnotic cells and mitotic cells were studied. The results are presented in Table 1.

1) Frequency of label among survivors is relatively constant during the first 60 hours. Thereafter, the frequency begins to fall and after 120 hours

Table 1.

Time after irradiation	Survivals	Pycnosis	Mitosis
	Labeled/total (%)	Labeled/total (%)	Labeled/total (%)
30 min	271/ 849 (30.3)	0/0	0/0
24 hrs	475/1069 (44.4)	0/0	0/0
36 〃	166/ 404 (41.1)	0/1 (0.0)	1/4 (25.0)
48 〃	251/ 399 (38.0)	26/78 (33.3)	1/1 (100.0)
60 〃	74/ 157 (47.1)	42/102 (41.2)	0/0
72 〃	80/ 392 (20.4)	12/40 (30.0)	0/2 (0.0)
120 〃	51/ 744 (6.9)	5/13 (38.5)	0/15 (0.0)

Dose: 1,000 r

(5th day) it was only 7%. At a first glance this result might be understood to mean that the majority of cells irradiated in S period were killed within 5 days and, therefore, did not contribute to the repopulation of spermatogonia in the irradiated testes. However, if we take into consideration the number of grains/cell as a function of time after irradiation, we will see that this is not the case. The number of grains/cell was as low as 8.8 ± 1.39 five days after irradiation in contrast to 30.9 ± 4.04 after 48 hours. This can be best explained by the fact that normal mitotic activity was restored between 48 and 60 hours after irradiation which resulted in the dilution of the label thereafter. 2) Using a half-time of the mean grain count/cell between 48 and 120 hours, the generation time of primary spermatogonia in irradiated testes was estimated to be approximately 35 hours which is not significantly different from that known for unirradiated cells. In other words, irradiation caused inhibition of mitosis for nearly 24 hours after irradiation but once the mitoses were restored the generation time was not affected to a noticeable degree. 3) Pycnosis of primary spermatogonia was almost absent during the first 36 hours after irradiation with 1,000 r. During the next 24 hours its incidence increased rapidly. Thus, after 48 and 60 hours it was nearly 20 and 40%, respectively. During the next 12 hours it fell down suddenly to 8%. Throughout the intervals studied in this experiment the frequency of labeled pycnosis was not much different from that of the surviving cells. This suggests that the cells irradiated in S phase have the same sensitivity to radiation killing as cells irradiated in other phases of the cell cycle. An experiment is now in progress to examine the sensitivity of cells irradiated in G_1 or in G_2 periods.

90. Variation in radiosensitivity during the early developmental stage of the silkworm egg

Akio MURAKAMI

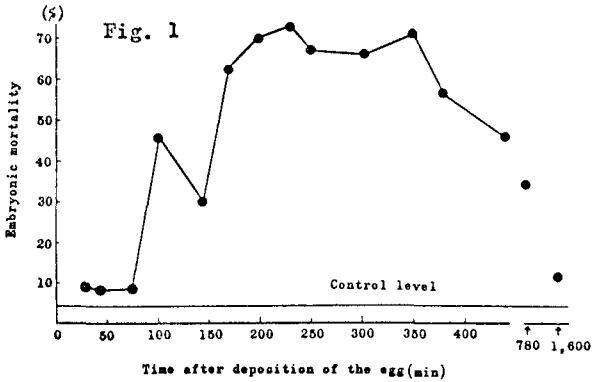
In order to elucidate the differences in radiosensitivity at various phases of DNA synthetic cycle of the cell, experiments have been carried out with newly deposited eggs of the silkworm using the hatchability as a criterion. The materials used were eggs from wild type C108 female moths, mated with males having the double recessive egg colour genes, *pe* and *re*. Moths were allowed to lay eggs which were collected at definite intervals at 25°C.

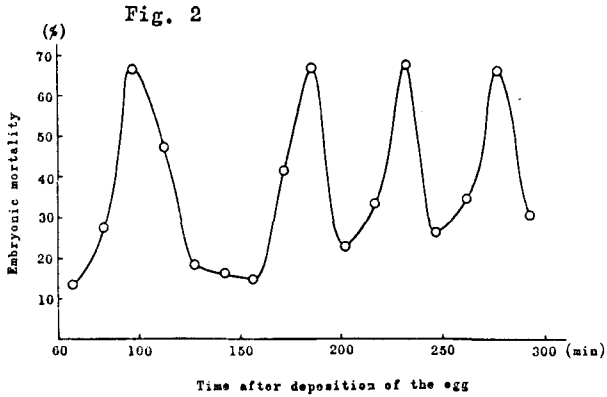
Expt. 1. The eggs were collected at 30 minute intervals. They were irradiated with 1,000 r of ^{137}Cs gamma-rays at a dose-rate of 100 r per minute at their various age. The results are given in Fig. 1. No cyclic changes were observed in hatchability among different age groups. Presumably this result might have been due to the heterogeneity of the material with regard to the division cycle of the eggs.

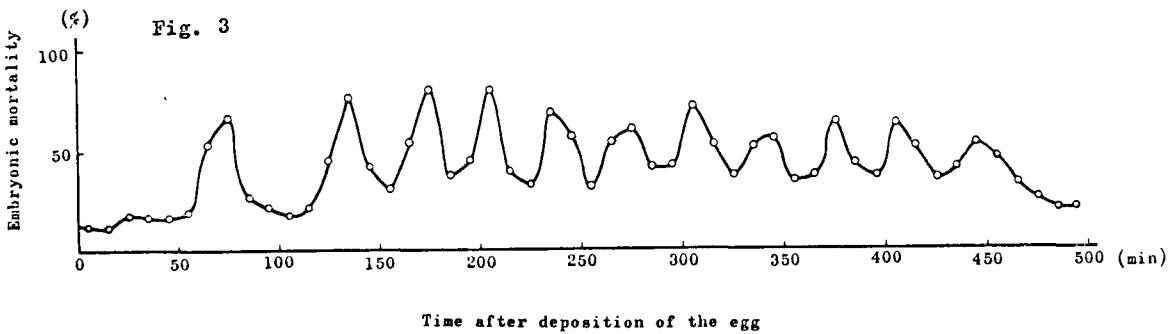
Expt. 2. Eggs were collected every 15 minutes and irradiation was applied at 15 minute intervals from 60 to 300 minutes after oviposition. 1,000 r of 173 kVp X-rays were administered at a dose-rate of 1,000 r per minute. As shown in Fig. 2, a rhythmical change in embryonic mortality was observed with a 45 to 50 minute cycle. It is known in this species that the 2nd maturation division of oöcyte begins approximately 60 minutes after oviposition and is completed about 20 minutes later, and that the fusion of female and male pronuclei occurs about 120 minutes after oviposition. The first peak of the curve may, therefore, correspond to the end of the 2nd maturation division, and the 2nd, 3rd and 4th peaks of the curve correspond to cleavage I, II, and III, respectively. It is also clear from the figure that the degree of radiosensitivity is essentially the same for every cycle regardless of the increase in the number of cleaved nuclei. The same sensitivity pattern has been reported for *Drosophila* (Würgler *et al.*, 1963).

Expt. 3. The eggs were collected at 10 minute intervals at 30–31°C and were exposed to 1,000 r of X-rays at a dose-rate of 1,000 r per minute during the period from 0 to 500 minutes after oviposition. As shown in Fig. 3, the cyclic change in radiosensitivity was again demonstrated clearly during the first 500 minutes of egg development. Each cycle was repeated after 30 minutes and its length was kept almost constant at least up to the 10th division.

Cytological observation by squash method showed that the time required







Figs. 1–3. Observed change in X-ray induced embryonic mortality during meiosis and early cleavage of the silkworm egg.

for one division cycle was 40–50 minutes at 25°C, and 30 minute at 30–31°C. The results were in good agreement with those obtained from hatchability data, indicating that the radiosensitivity pattern may well be correlated with a cyclic change in the cell division cycle. The cytological studies now in progress indicate that the peak of radiosensitivity appears around late prophase and metaphase.

91. Enhancing effect of fractionated irradiation with 14 MeV neutrons on the induction of visible recessive mutations in silkworm gonia

Akio MURAKAMI, Sohei KONDO and Yataro TAZIMA

Since 1962, the effect of fractionated doses on the induction of mutations has been investigated in silkworm gonia by one of the authors, Y. TAZIMA, using low LET radiations. The results showed that the induced mutation rates were approximately 2 to 2.5 times higher in the dose fractionation group than in the single exposure group, for both spermatogonia and oögonia, when irradiations were given around the stage of hatching. In the present experiment it was confirmed that the similar enhancing effect of fractionated irradiation was also observable even in 14 MeV neutron irradiation. Our materials were the same as described in the previous paper (TAZIMA, 1963). Larvae of the wild type C108 strain were exposed at one to three days after hatching to 14 MeV neutrons of T (d, n) He reaction at the Research Institute of Nuclear Medicine and Biology, Hiroshima University. Neutrons of total 1,000 rad were administered in two fractions (500+500) 10 to 12 hours apart at a dose-rate of 0.8 rad per minute. Owing to technical limitations the 1,000 rad single exposure group was not examined.

The results are shown in Figs. 1 and 2 for spermatogonia and oögonia, separately. As seen from these figures, an enhancing effect of dose-fractionation is more marked for spermatogonia than for oögonia. The phenomenon is quite similar to those reported previously by Y. Tazima for X- and gamma-rays. The mutation frequencies were about two times as high as those at a single exposure. The highest enhancing effect was observed in spermatogonia when the second dose was given 36 hours after the initial exposure, in contrast to the 18 hours for X- and gamma-rays (Tazima, 1963). It is noted, therefore, that a delay of 18 hours occurred in the manifestation of the peak effect in case of 14 MeV neutrons compared with γ-irradiation. A possible cause of this delay may be that the 14 MeV neutrons have higher efficiency to affect cell divisions than X- or gamma-rays.

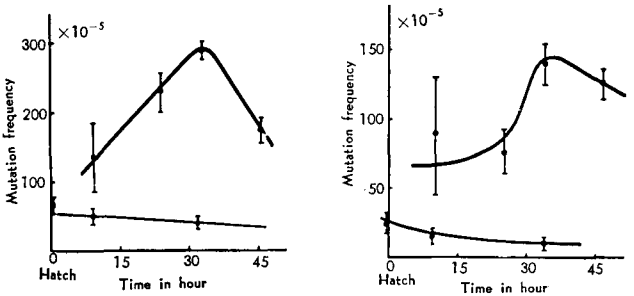


Fig. 1. Spermatogonia

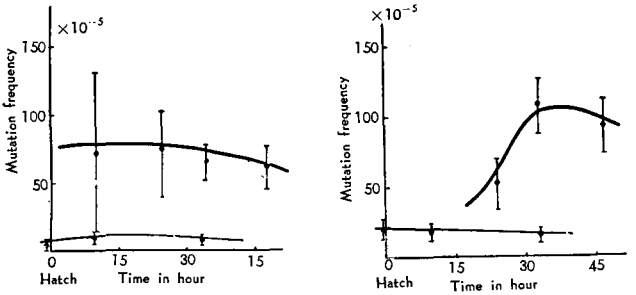


Fig. 2. Oögonia

Fig. 1-2. Variation in induced mutation frequency with time interval between two neutron exposures.

92. Chromosome aberrations induced in mouse bone marrow cells by gamma irradiation

Yoshinori KURITA, Reiko TSURUTA, Tosihide H. YOSIDA and Katsumi SAKAKIBARA

Many mouse leukemias induced by ionizing radiation are characterized by aberrant karyotypes (Ford, 1958). It is also known that irradiation causes a persistent damage to the chromosomes of living cells. To investigate the mechanism of the persistence of the damage, primary chromosome aberrations induced by ionizing radiations were examined in bone marrow cells of C57BL/6 male mice within 24 hours after a single 450 r gamma irradiation. Eight hours after irradiation, four different types of aberrations were observed; namely (1) chromatid breaks, (2) chromatid translocations, (3) isochromatid aberrations, and (4) chromosome type aberrations. Their frequencies were 0.42/cell, 0.08/cell, 00.5/cell, and 0.35/cell,

respectively. Minute chromosomes only were observed 8 hours after treatment. They seem to have resulted from breakage in the secondary constriction of certain chromosomes. The most striking feature of chromosome aberrations 24 hours after irradiation was the disappearance of chromatid translocations. The types of aberrations that appeared 24 hours after irradiation were; chromatid fragments (0.15/cell), ring chromosomes (0.04/cell), and minutes (0.37/cell). Nonrandom distribution of those chromosome aberrations was evident.

93. Persistent chromosome aberrations in mouse bone marrow cells after gamma irradiation[1]

Tosihide H. YOSIDA, Yoshinori KURITA, Reiko TSURUTA and Katsumi SAKAKIBARA

Although the significance of chromosomal abnormalities in relation to leukemia pathogenesis remains uncertain, it was of utmost importance to investigate the persisting type of chromosome abnormalities induced by irradiation. In order to supply the badly needed information, the types and incidence of long-time persisting chromosomal aberrations were studied in bone marrow cells of C57BL/6 and RF strain mice after three fractionated 450 r gamma treatments.

It was observed that the persisting aberrations were of chromosome type only. Four kinds were observed, namely (1) minute chromosomes, (2) D-type chromosomes, closely linked at the middle part, (3) subtelocentric chromosomes, arisen from secondary chromosome rearrangement, and (4) giant chromosomes. None of them were found in the control specimens. 12 out of 18 mice examined showed persisting chromosome abnormalities. Their incidence was slightly lower than that observed in LAf mice by Nowell and Cole (1963), but considerably higher than that obtained in C57BL/6J mice irradiated with four fractionated 600 r of X-ray by Nadler (1963). From these results, it seems highly probable that the cells with persisting abnormal karyotypes participate in the development of mouse leukemias after irradiation.

[1] This work was supported by a research grant from the National Cancer Institute (CA 07798-02), Public Health Service, U.S.A.

F. RADIATION GENETICS IN PLANTS

94. Photoreactivation of ultraviolet-induced mutation in maize pollen

Seiji MATSUMURA and Tomoo MABUCHI

From our preliminary experiment (cf. Ann. Rep. No. 15:137–138) it was assumed that photoreactivation of ultraviolet (UV)-induced mutation in higher plants really exists. In order to confirm this assumption, a similar experiment was repeated with the same material. Pollen grains of normal maize (*Su*) were irradiated in a single layer in a Petri dish with UV of 254 mμ at a dose rate of 90.3 ergs/mm^2/sec for 30, 60 and 90 seconds. Immediately after UV exposure, the irradiated pollen grains were divided into two batches, one was respread on a Petri dish and exposed to visible light (7,400 lux) from 440W high pressure mercury lamp for 30 minutes at the distance of 110 cm (VL treatment) and the other batch was held for 30 minutes is darkroom (dark treatment). After VL and dark treatment, the pollen grains were dusted on the silks of a recessive sugary line (*su*).

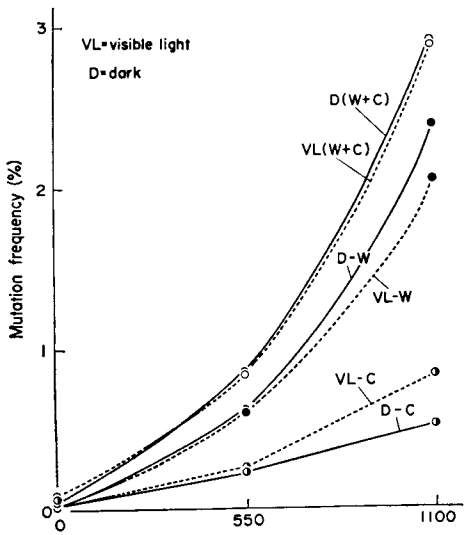


Fig. 1. Effect of visible light on γ-ray induced mutations in maize pollen.

in the field under sun light and in the darkroom under red light, respectively. For comparison they were irradiated with 550 and 1,100 R of γ-rays from ^{137}Cs and dusted on the silks in the same way.

The percentage of seeds set was likewise high in both doses of γ-rays, while it decreased with the increase of UV-dose and was about 50% of the control lot at the highest dose. Induced whole and chimeral endosperm mutants were scored separately, and the obtained mutation rates from γ-ray irradiation are given in Fig. 1. There were no marked differences in the frequency of whole and chimeral mutants between VL and dark treatment. As to the frequency of the two types, whole mutation was more frequent than partly mutated chimeras. The frequency of chimeras fits roughly a linear relation with increasing doses, while this relation is rather exponential than linear for whole mutation. It is assumed that the VL treatment was completely ineffective in γ-ray irradiation.

On the other hand, the frequency of whole and chimeral mutations in UV irradiation showed almost linear relation, as shown in Fig. 2. It was

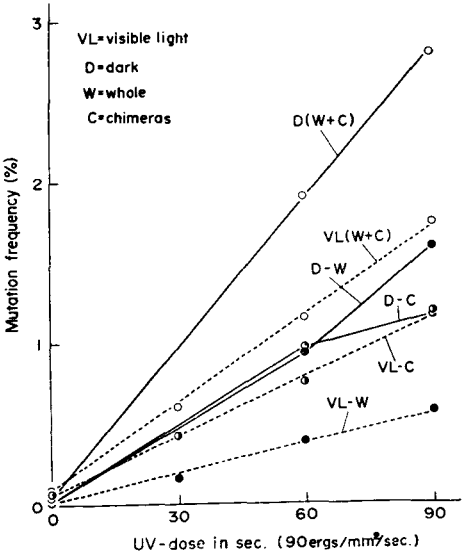


Fig. 2. Effect of visible light on UV-induced mutations in maize pollen.

considerably decreased by exposure to visible light, while there was no difference in the frequency of chimeras between VL and dark treatment. It may be assumed that chimeral mutation may be caused by UV-induced

alteration in one or a half chromatid at *Su* locus and whole mutation by alteration in two chromatids. The fact that whole mutation is reversible by visible light more frequently than the chimeras suggests that photoreactivation may proceed in some way from whole to chimeral mutation and from there to normal endosperm.

95. *Effects of dose fractionation of γ-rays on mutation in maize pollen*

Tomoo MABUCHI

In order to elucidate the radiological response of the sperm chromosomes in pollen grains, an experiment was carried out on the effects of dose fractionation of γ-rays on mutation rate. Mature pollen grains of a dominant line (*Su*) of maize were irradiated by ^{137}Cs γ-rays and dusted on the silks of a recessive line (*su*). A single dose of 550 or 1,100 R was applied in acute (917 R/min) irradiation. Fractionated doses (550 R) were given twice with intervals of 0.5, 1.0, 2.5, 5.0, 10, 30, 60 and 120 minutes.

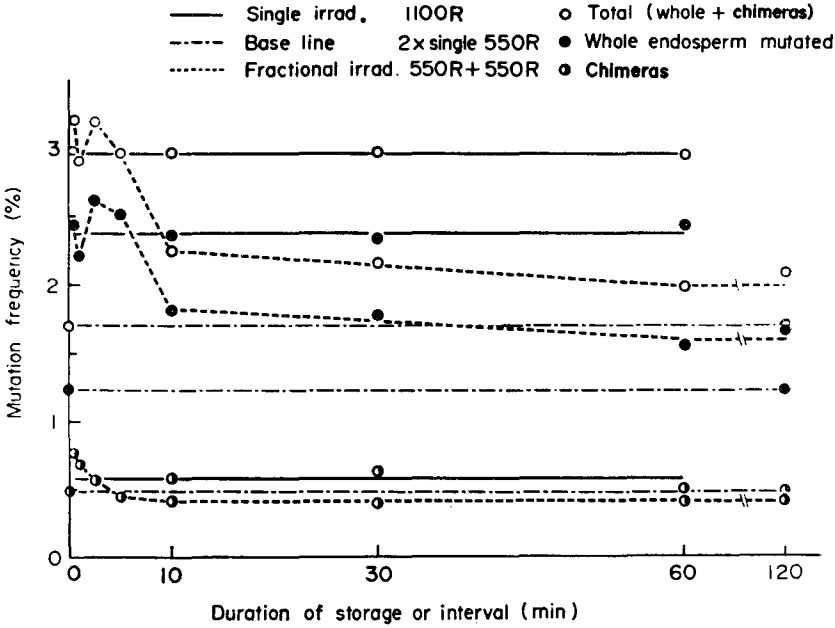


Fig. 1. The relation between storage or radiation fractionation and the frequency of mutation in maize.

The base line was obtained from single 550 R dose by multiplying the mutation frequency by two. Mutations (*Su→su*) were detected in the kernels developed from the fertilization by the treated pollen. The results are shown in Fig. 1.

There was no significant difference in the frequency of chimeral (partial) endosperm mutations between the base line and a single 1,100 R dose or fractionated doses with time intervals 0.5~120 min. But whole mutations in a single 1,100 R dose were produced twice as high as the base line, as did the fractionated doses with 0.5 to 5 min intervals. In the experiment with fractionated doses, whole mutations dropped rapidly at 5 to 10 min intervals and slightly decreased with increasing intervals (10~120 min).

The results of the dose fractionation experiment indicate that most of the breaks induced in the sperm chromosome of pollen grains remained open at least for 10 minutes.

96. Storage and oxygen effects on γ-ray induced mutation in einkorn wheat

Tomoo MABUCHI and Seiji MATSUMURA

Dry seeds of *Triticum monococcum flavescens* containing 13% water were sealed in ampules filled with oxygen, nitrogen and air, exposed to 10 and 15 kR γ-rays. For acute and chronic irradiation dose rates of 10,000 R/hr with ^{137}Cs and 19.6 R/hr with ^{60}Co were used, respectively.

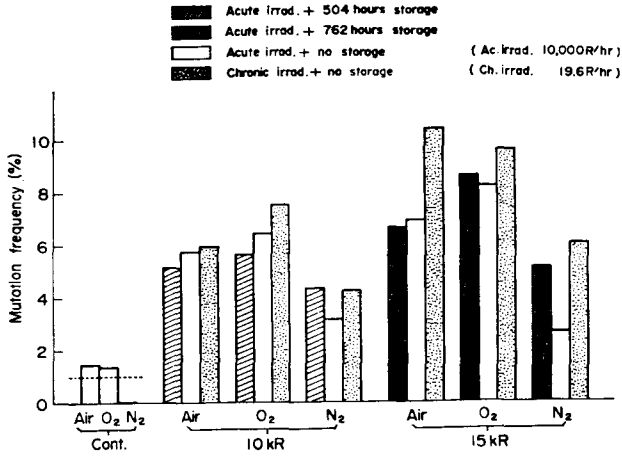


Fig. 1. Oxygen and storage effects on chlorophyll mutation in einkorn wheat (1965).

Two chronic irradiations (10 and 15 kR) were terminated simultaneously before sowing. Acute irradiations were given at the time coinciding with the beginning and termination of the chronic ones. The data for chlorophyll mutations in X_2 seedlings are shown in Fig. 1. In general, chronic irradiations were found to be more effective than the acute ones, especially at higher irradiation with 15 kR, as already found for the inhibition of seedling growth (cf. Ann. Rep. No. 15:139-141). There were no clear differences between air and oxygen treatment either in acute or chronic irradiation. On the other hand, nitrogen treatments clearly showed a protective effect on mutation frequencies. Also post-irradiation storage was effective on inducing mutation, especially in acute irradiations with nitrogen treatments.

These results might be explained by the relationship between production and decay of free radicals and radiation damage, as already mentioned. It is assumed that the storage effect is due to the decay of long-lived free radicals which combine with biologically important molecules, like water molecules, and the nitrogen treatment decreases the radical yield, and production and decay of the radicals are more gradual and slower in chronic than in acute irradiation.

97. Genetic effects of X-irradiated pollen in wheats

Tomoo MABUCHI and Seiji MATSUMURA

Anthers before dehiscence of *T. monococcum*, *T. durum* and *T. Spelta* were irradiated by X-rays at 1 kR (200 R/min). The pollen grains were dusted on emasculated florets of the same species. Reciprocal translocation (RT), plant height at maturity, seed fertility in X_1 and chlorophyll mutations in X_2 were investigated. The results are given in Table 1.

Plants with RT in *T. monococcum* and *T. durum* were observed in high frequency in irradiated lots, but not in the non-irradiated control. In diploid species, there was no significant difference in plant height between irradiated and non-irradiated lots. Plant height of polyploid species, however, was considerably inhibited by irradiation. No correlation between plant height and RT was found in *T. monococcum*, but it was found in *T. durum*. Seed fertility was reduced markedly by irradiation in all species tested and there was a positive correlation between seed sterility and RT. Chlorophyll mutations in X_2 were also found to be induced by pollen irradiation like from seed irradiation.

From these results, it is assumed that in polyploid wheats plants with RT and other chromosome aberrations which reduce plant height have

considerable viability, but not in diploid wheats.

Table 1. Effects of X-irradiated pollen on chromosome aberration at MI, plant height and fertility in X_1 and mutation rate in X_2 of wheats

Species		% of plants with RT at MI	Plant height (cm)	Seed fertility (%)	Chlorophyll mutation rate per spike (%)
T. monococcum (2n=14)	C	0.0 (0/24)	124.0± 9.8	74.1± 8.5	0.0 (0/32)
	X	40.0 (26/65)	127.5±10.5	42.2±24.6	3.9 (2/51)
T. durum (2n=28)	C	0.0 (0/11)	158.0± 6.1	84.8±12.4	0.0 (0/26)
	X	61.3 (27/44)	141.5±20.9	43.9±35.6	1.4 (1/70)
T. Spelta (2n=42)	C	—	134.6± 9.5	54.7±20.1	0.0 (0/33)
	X	—	107.8±18.5	14.8±21.1	0.0 (0/46)

C: Control, X: X-irradiation

98. Relation between polyploidy and radio-sensitivity under chronic condition

Seiji MATSUMURA and Tarô FUJII

The facility for chronic γ-ray irradiation of growing plants, the so-called gamma-greenhouse, was built in our institute in 1964. Effects of chronic irradiation on growing wheat were examined in the gamma-greenhouse.

Two diploid, 3 tetraploid and 2 hexaploid species were used in this experiment. The employed seven species constituted one block. Four blocks were set up for irradiation at various distances from the source or at various dose rates. Radiation intensity was 0.31~3.06 R/hr. Therefore, total dosages varied considerably from 870 R at the minimum to 8,700 R at the maximum.

For examination of seed fertility, spikes of the tallest, main tiller of each individual were collected from all surviving plants and the average seed set was calculated. Decrease of fertility was clear in each species with increasing dosage, and unexpectedly, there were no marked differences among the three ploids. But when the decreasing rate was compared between the cultivated and wild types at each ploidy level, in general the rate for the wild type was slightly lower than for the cultivated types.

From our experiments, we would say roughly that the difference in radio-sensitivity between ploids became smaller under chronic than under acute condition. The smaller radiation effect in chronic than in acute condition may be due to recovery phenomena during a long irradiation

period at low intensity. A protective effect in polyploids is also an important factor in radiation tolerance. Therefore, in polyploid plants radiation effects under chronic condition may be influenced by these factors, and differences in mitotic rate and genome complexity in allo-polyploids should be additional factors in the appearance of radiation damage.

99. Development of mutated cells in diploid wheat

Tarô FUJII

Normals and recessive *chlorina* mutants of diploid wheat were used as female and male parents, respectively. 500 R of γ-rays were given to young embryos 24, 28, 72 and 96 hrs after pollinations. The F_1 seeds were harvested and the mutated stripes were scored in the F_1 or X_1 generation. Germination rate in all irradiated lots were lower than in the control and that of the 24 hr lot was as low as 35%. TSUNEWAKI (1955) examined the number of cells several times after pollination and found that it was 24, 48, 72 and 96 hrs after pollination about 1.0, 8.2, 37.8 and 105.7, respectively. When the cell at one-cell stage were damaged by irradiation, all cells in the ripe embryo would have the same kind of aberration which could affect germinability.

Longitudinal *chlorina* and necrotic stripes caused by somatic mutations were detected in young seedling having one or two leaves. The widest *chlorina* stripes occupied about half the area of a leaf and the narrowest stripes were restricted to about 1/4 leaf area. One seedling in the 48 hr lot was *chlorina* without any trace of green. The number of mutated spikes in the present experiment was 10 times larger than after seed exposures. Of course, if mutations occurred in some cells among a small number of cells in the young embryo, mutated cells should be incorporated into a large part of tissues through many steps of development.

100. Effects of ultraviolet-rays on Arabidopsis thaliana

Tarô FUJII

F_2 seeds obtained from the F_1 of crosses between the hairless mutant and the wild hairy strain were used because it is somewhat difficult to obtain many F_1 seeds. Seeds were sown on wet filter paper in Petri dishes. About 5 mm long seedlings were exposed to UV-rays. Wave length of the UV-rays was 253.7 mμ; the absorbed energy was 90.3 ergs/mm^2/sec. About 1,500 seedlings were used in each lot and 0.5 and

1 min exposures were applied. Irradiated seedlings were kept in a dark room for 24 hours.

During the growth of seedlings, the segregation ratio of hairless homozygotes was examined. In the 0.5 min lot, hairless homozygotes segregated according to the ratio 3:1 as expected. Therefore, two thirds of hairy plants were expected to be heterozygotes. Hairless sectors appeared in two plants in the 0.5 min lot among 581 presumably heterozygotic plants. From the results, mutation rate was calculated as 0.34 per cent. Segregation ratio of hairless homozygotes in the 1 min lot did not fit the 3:1 ratio. The small number of hairless plants in the 1 min lot might be due to their low survival rate.

Only one mutated plant was observed in the 1 min lot among 552 hairy plants. The low mutation rate in this lot seems to be the result of a too small number of surviving plants. Photoreactivation effect and dose-versus-mutation curve are now under examination.

101. Mutagenic efficiency of heavy ionizing particles in Arabidopsis thaliana

Tarô FUJII, Mituo IKENAGA and John T. LYMAN[1)]

F_2 seeds obtained from F_1 hybrid between a hairless mutant and the wild hairy strain were subjected to 1~5 krad He^4 and C^{12}-ions, respectively, and 3 and 5 krad of Ar^{40}-ions, initial particle energy being 41.6, 124.8 and 416 MeV, respectively. For comparison of RBE values and establishment of a standard, 27 and 47 krad γ-ray exposures were given to the same material using 6 kCi of ^{137}Cs source. About 67% of hairy plants may be assumed to be heterozygotes for the hairy-hairless allele pair. When a leaf or a part of a leaf was hairless it was scored as one mutational event occurring in the dominant hairy allele.

The frequency of plants with hairless sectors increased with increasing dosage in γ-ray, C-ion and Ar-ion lots. Somatic mutation rates in C-ion lots were very high; those of Ar-ion lots were very much lower. On the other hand, only one mutated plant was observed in the He-ion 2 krad lot. Dose-versus-mutation curves for the C- and Ar-ion lots were fairly linear, while a non-linear relation taking the shape of an exponential curve was observed between mutation frequency and γ-ray dosage. Thus the shapes of dose-response curves are different, and therefore the RBE value of heavy ionizing particles cannot be expressed by a single parameter.

1) Donner Laboratory and Lawrence Radiation Laboratory, University of California, California, U.S.A.

The frequencies of somatic mutations of γ-rays and heavy ionizing particles were compared at 0.5% mutation rates from their dose-versus-frequency curves. About 33 krad of γ-rays was necessary for producing the 0.5% mutation rate, and about 0.9 and 6.7 krad of C-ions and Ar-ions could produce the same mutation frequency. From the results, RBE for somatic mutation at 0.5% frequency was roughly estimated as 35 for C-ions and 5 for Ar-ions. Neither could we decide about He-ions' RBE, but if the mutation rate at 2 krad lot was sustained their RBE for somatic mutation might be about 10.

G. HUMAN GENETICS

102. Genetic epidemiology of Down's syndrome in Japan[1)]

Ei MATSUNAGA

It is now known that the overwhelming majority of cases with Down's syndrome are due to primary non-disjunction of the 21st autosomes, either at gametogenesis or at an early cleavage of the fertilized egg. Theoretically, this event may be caused by factors either on paternal or maternal side, or on both sides, but it is generally believed, from the Caucasian data, that the maternal side is of relative importance, since there is a strong dependency of the occurrence of this abnormality upon the maternal, not the paternal age if the two are appropriately separated. Consequently, it is natural to inquire whether such agents as hormones acting upon ovarial function may affect the aging process or the tendency of the stocked ova to chromosomal non-disjunction. This question may best be answered by testing the effects of birth order and of pregnancy free intervals preceding the births of the patients.

Another question of genetic interest is concerned with whether recessive genes are involved in non-disjunction in man. In *Drosophila melanogaster* and in *Zea mays* the existence of such genes is well known. If recessive genes were involved in the etiology of Down's syndrome, a higher incidence of consanguinity among maternal grandparents would be expected.

Keeping the above considerations in mind, data obtained from 834 cases of Down's syndrome in institutions for mental defectives were compared

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

with various sets of matched controls including standardized vital statistics. The rise of relative incidence of this abnormality with maternal age showed a pattern quite similar to that found for the Caucasians; this pattern did not vary by the socio-economic conditions of the mothers. When the maternal age effect was eliminated, the paternal age had in practice no effect, while a significant decrease in the relative incidence was noted according to live-birth rank, irrespective of the socio-economic conditions. This is likely to be due, however, not to birth order effect as such, but to some selection, practiced probably by the parents in bringing their children to institutions, because the same tendency was found for other mental defectives than those with Down's syndrome. As to pregnancy free intervals preceding the births of the patients, there was no indication that either a too short or a too long interval is of etiological significance.

Consanguinity was investigated, by referring to "koseki" records, among grandparents of 104 patients born to younger mothers. There was no increase in frequency of consanguineous marriages among maternal grandparents as compared with that among the paternal ones, suggesting that recessive genes of the mothers, either autosomal or sex-linked, play no role in chromosomal aberrations leading to Down's syndrome.

Details of this work will be published elsewhere.

103. Parental age and sporadic retinoblastoma[1)]

Ei MATSUNAGA

It has been pointed out by some workers, notably Penrose, that for certain hereditary diseases such as chondrodystrophy and acrocephalosyndactyly the mean paternal age is characteristically increased above the population's average for sporadic cases attributable to fresh mutation, indicating that the site of the genic mutation is mainly in the paternal gonads. For another group of diseases, *e.g.*, retinoblastoma and neurofibromatosis, the mean paternal and maternal ages are both only slightly raised, and it had been impossible to make any inference as to which of the parental ages is of primary significance. Those observations were based on Caucasian data.

Using data from 65 cases of sporadic retinoblastoma previously ascertained in Hokkaido, the parental ages have been carefully checked against "koseki" records. Of the 65 cases 21 were bilateral and 45 unilateral. Although the year of birth of the patients range from 1930 to 1958, all

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

but four were born after 1940, for which, with the exception of 1944-46, the distributions of maternal ages in quinquenniums are available for all Japan from vital statistics data. Their percentage distributions for each year of the period were then weighted by the number of cases born in the respective year, and from them the mean maternal age for the control group was estimated. As to controls for paternal age, the vital statistics provide only data for 1952 and thereafter. Because the mean paternal age in the vital statistics has been gradually decreasing, the control was taken from the data for 1952.

Table 1. Mean parental ages of sporadic retinoblastoma cases compared with those of control group

Paternal ages							
Affected eyes	No. of cases	p_1	p_0	p_1-p_0	V_1	t	P (one tailed test)
Bilateral	21	34.4	32.0	+2.4	39.05	1.76	$P<0.05$
Unilateral	45	33.0	32.0	+1.0	34.77	1.14	$P>0.1$
Combined	66	33.4	32.0	+1.4	35.97	1.79	$P<0.02$
Maternal ages							
Affected eyes	No. of cases	m_1	m_0	m_1-m_0	V_1	t	P (one tailed test)
Bilateral	21	28.9	28.2	+0.7	31.19	0.61	$P>0.2$
Unilateral	45	28.9	28.5	+0.4	29.87	0.54	$P>0.2$
Combined	66	28.9	28.4	+0.5	29.82	0.79	$P>0.1$

Key:
p_1, m_1=mean parental ages for retinoblastoma.
p_0, m_0=mean parental ages for controls.
V_1=variance of parental age for retinobastoma.
Control for paternal age=vital statistics data for 1952.
Control for maternal age=weighted average from 1940-58 for all Japan.

As can be seen from Table 1, the mean paternal age shows a significant increase for the bilateral but not for the unilateral cases, while the mean maternal ages for both bilateral and unilateral cases remain almost the same as the mean ages of the mothers in control group.

A sensitive indication of the primary importance of paternal or maternal age is obtained by comparing the mean difference in ages, p-m, of the parents of the patients with that in the general population. If the disease were primarily associated with maternal age, the mean difference should

be considerably lower than the difference in the general population, while the reverse would be expected if the disease were primarily dependent upon paternal age. Table 2 shows the mean difference between parental

Table 2. Mean of the difference between parental ages, p-m for the retinoblastoma cases compared with a control population

Affected eyes	No. of cases	Mean of p-m for the retinoblastoma cases	Mean of p-m for controls	V_1	t	P (one tailed test)
Bilateral	21	+5.1	+3.9	8.45	1.75	$P<0.05$
Unilateral	45	+4.0	+3.9	10.77	0.12	$P>0.9$
Combined	66	+4.3	+3.9	10.13	1.00	$P>0.15$

Key:
V_1=variance of p-m for retinoblastoma.
Control for p-m was based on the vital statistics for 1952.

ages for our retinoblastoma cases compared with a control value calculated from the vital statistics data for 1952. The mean difference is significantly raised for the bilateral cases, while for the unilateral cases this figure scarcely differs from the control's.

In our previour report (1961), we presented some evidence, based on the followed-up studies of patients with retinoblastoma, that most cases of sporadic bilateral retinoblastoma appear to be due to new mutation, while the great majority of unilateral sporadic cases are to be regarded as phenocopies. The results given here are consistent with this view. Furthermore, they suggest that the primary site of the mutation is mainly in the paternal gonads, as is the case for chondrodystrophy.

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104. Genetically controlled variations of red cell acid phosphatase

Tomotaka SHINODA

Attempts have been made to estimate frequency of variations of the acid phosphatase types in human red cells. When hemolyzates are tested by means of starch gel electrophoresis, at least three zones of acid

phosphatase activity can be obtained. These patterns are detectable as colored zones by incubation of the gel with a substrate solution.

Blood samples were collected in Numazu City and in Tokyo during the summer of 1965. A total of 437 samples were tested. In all individuals, ABO blood types and enzyme activity were determined. Table 1 summarizes the data.

Table 1. Numbers of individuals of the different enzyme phenotypes

Phenotypes	No. of individual	Incidence
A	77	0.176
AB	202	0.462
B	146	0.334
AC	3	0.007
BC	8	0.018
C	1	0.003
Total	437	1.000

It has been suggested that these phenotypic differences are determined by three allelic autosomal genes, P^a, P^b, and P^c. If this hypothesis is correct, then the frequencies $p=0.410$, $q=0.574$, and $r=0.016$ of the genes P^a, P^b, and P^c may be obtained from the results given in Table 1.

By the appropriate calculations it will be seen that there is a good agreement between the observed numbers of the various phenotypes and the numbers expected.

Further analysis of the data is now in progress.

105. Alkaline phosphatase in human serum

Tomotaka SHINODA

It has been demonstrated that alkaline phosphatase in human serum fell into two groups, type 1 and type 2. One of these was found to be strongly associated with certain red-cell antigens. Type 1 had a single zone of activity, while type 2 had an additional slower migrating zone the intensity of which varied considerably from sample to sample.

Attempts were made to obtain some population data, by using part of the samples collected for classification of acid phosphatases in red cells. Total activity of serum alkaline phosphatase was determined at 410 mμ using *p*-nitrophenyl phosphate as a substrate. Some sera were found to

have a slow migrating band which was quite weak compared with that of type 2, especially in the case of undiluted sera. These were not, however, used for classification, because the resolution of zone on the gel was inferior to that obtained with diluted specimens. Other zones with enzyme activity are occasionally seen in normal serum. One of these migrates between the fast and the slow bands seen in the type 2. Another migrates very slowly and appears just ahead of the origin.

Among 438 sera tested in all, 92 were found to be type 2, giving a frequency of 21 per cent. The relation between the phosphatase groups and the ABO blood groups is summarized in Table 1.

Table 1. Relation between phosphatase groups and ABO blood groups

Phenotypes	Blood group				Total
	A	B	O	AB	
1	176	59	83	28	346
2	5	37	43	7	92

It is apparent that there is a much higher incidence of type 2 sera with groups B and O than with A. The AB individuals constitute a small sample (n=30) with an intermediate frequency.

There is considerable variation in level of enzyme activity from serum to serum within each of the two groups. The average level of enzyme activity in type 2 sera is about 27 per cent greater than in type 1.

Two different hypotheses have been advanced concerning the nature of the slower migrating component. The more likely of these suggests that the two enzyme zones in normal serum are qualitatively different.

106. Studies on haptoglobin

Tomotaka SHINODA

Haptoglobin, a serum protein with hemoglobin-combining ability, usually falls into three common types 1-1, 2-1, and 2-2, the relative frequencies of which vary among ethnic groups so far studied.

In order to obtain more population data, a search was carried out in 1965.

Blood samples were collected from unrelated persons in Izu area. Among 694 sera so far tested the frequencies of the types 1-1, 2-1, and

2-2 were respectively 38, 271, and 381. Accordingly, the frequency of the gene Hp^1 may be estimated to be 0.251. In this study three sera were found to be of type 0. One sample appeared to contain a variant of type 2-1. The second and third fastest haptoglobin zones of this variant were less intense than normal, and seemed to overlap. Some attempts were made to find out the difference between the variant and type 2-1. However, it could not be shown that the variant contained an altered polypeptide chain in the molecule. There was no significant difference in hemoglobin-combining capacity between type 2-1 and the variant so far examined. The overall frequency of the gene Hp^1 was 0.254, and no differences in distribution have so far been obtained among the samples collected from different areas in Japan. Further analyses are in progress.

107. Cytogenetic survey of 127 cases with Down's syndrome

Akira TONOMURA, Hidetsune OISHI and Yasumoto KIKUCHI

The incidence of translocation and mosaicism among patients with Down's syndrome is about 8% in Western Europe and North America, but comparable figures from other parts of the world have so far been very limited. The purpose of the present investigation was to estimate the incidence of translocation and mosaicism in a sample of Japanese patients with Down's syndrome. The karyotypes of 127 cases with this abnormality (76 males and 51 females) have been examined by the use of leucocyte culture of peripheral blood. Clinical examination and diagnosis of the patients have been made at the Pediatric Clinic of the Konodai National Hospital in Ichikawa and a few other University Hospitals in Tokyo. The patients' age at the time of chromosome examination varied from a few months after birth to 12 years, but about 85% of these were under five years. There was no case in the present series which contained a second affected child in the sibships.

In 127 cases with Down's syndrome, 118 (92.9%) were found to be of the standard 21-trisomy type. There were six cases with translocations, three of them showed 13-15/21 translocation and the other three had 21-22/21 translocation or an isochromosome for the long arm of chromosome no. 21. Analysis of cultured blood cells from their parents showed apparently normal karyotypes. Mosaicism was observed in three patients. One case was a chromosomal mosaic with 46 (normal)/47 (trisomic 21)/48 (tetrasomic 21) chromosomes. This patient has been described before. The other two cases consisted of two different cell types, one with 46 (normal)/47 (trisomic 21) and the other with 47 (trisomic 21)/48 (tetrasomic 21) chromosomes.

The results obtained are summarized in Table 1. In the present series the frequency of Down's syndrome with chromosome constitutions other than the standard 21-trisomy type is about 7.2%. This figure may be comparable to those obtained in Western Europe and North America, and there is no indication of any difference between Japanese and Caucasians in the relative rate of Down's syndrome with translocation and mosaicism.

Table 1. Results of chromosome survey of 127 cases of Down's syndrome

Karyotypes	21 trisomy	13-15:21 transl.	21-22:21 transl.	mosaics	Total
Number of patients (%)	118 (92.9%)	3 (2.4%)	3 (2.4%)	3* (2.4%)	127 (100.1%)

* 46/47, 46/47/48, 47/48

108. Identification of human somatic chromosomes from measurements of their areas

Hidetsune OISHI and Akira TONOMURA

In general, the Denver classification proposed in 1960 by the Denver study group is now widely accepted as the standard system of nomenclature of human somatic chromosomes. However, there are some important differences of views on specific points, particularly on the morphological identification of the X chromosome. In that study group, some participants considered that the X was larger than no. 6 autosome, while the others thought that it was smaller. In this system, classification of individual chromosomes was based on the unidimensional measurement, which was concerned with length and arm ratio of the chromosomes. This method is not reliable, since the larger chromosomes tend to contract more in later stages of the metaphase than the smaller ones. In the present study, an attempt has been made to give to distinguish the chromosomes more accurately by measuring their area rather than length only.

Chromosome preparations from human peripheral leucocytes were made by standard culture technique. The cells were spread by air-drying method and stained with Giemsa. A suitable metaphase figure was obtained from each of ten normal males; the distinction of the X from the autosomes, especially nos. 6 and 7, is usually very difficult in female cells, while it is less difficult in male cells where pairs of nos. 6 and 7 are clearly ascertained so that an unpaired chromosome can be identified as

the X. All chromosomes were magnified about 18,000 times from photomicrographs by a photographic enlarger. The projected image was traced on a sheet of white typing paper, and the figures were then measured by a planimeter. The lengths of the chromosomes were also recorded in the same cells. The measurement for each chromosome relative to the total of a normal X-containing haploid set was expressed per thousand.

The results are summarized in Table 1. There is good agreement between the two numberings of the chromosomes obtained by area and those by length measurements. However, values obtained by area

Table 1. Relative lengths and areas in normal human chromosomes

No.	Relative area	Arm ratio by area	Relative length	Arm ratio by length
1	84.7±3.72	1.1	81.8±5.47	1.1
2	82.2±3.28	1.6	78.3±6.42	1.6
3	67.9±3.49	1.2	67.1±3.25	1.1
4	63.4±2.16	2.8	60.5±3.09	2.5
5	59.2±3.13	2.8	56.5±1.68	2.5
6	57.5±2.59	1.7	57.3±3.00	1.5
X	56.7±2.49	1.7	51.9±1.55	1.6
7	51.0±3.68	1.6	49.8±1.81	1.5
8	46.9±2.37	1.8	46.7±1.11	1.7
9	46.2±1.21	2.5	45.4±1.50	2.2
10	44.2±2.13	2.4	43.8±0.98	2.1
11	42.4±1.88	1.5	42.6±0.93	1.5
12	42.0±1.25	2.4	41.6±1.71	2.2
13	36.4±2.36	10.9	37.5±1.54	5.5
14	34.1±2.14	11.5	35.0±2.11	5.5
15	31.3±1.14	9.0	33.4±1.70	4.6
16	29.3±1.71	1.4	31.4±1.60	1.3
17	27.4±2.14	2.6	29.1±1.24	2.1
18	24.3±1.89	3.5	26.1±1.48	2.6
19	21.9±1.58	1.3	24.4±1.28	1.2
20	19.5±1.44	1.2	22.7±0.86	1.2
21	16.7±2.03	5.9	19.2±2.45	3.4
22	14.7±1.08	4.6	18.0±1.27	2.6
Y	19.6±3.02	8.3	22.6±3.59	4.9

measurements tended to be somewhat larger for the larger chromosomes than the values from length measurements, while the relation was reversed for the smaller chromosomes. Further, the estimates of the arm ratios in terms of relative areas were found to be larger for the submetacentrics, such as nos. 6–12 and 16–18, than the corresponding estimates based on length measurement.

In comparison with the data of length measurements by the Denver study group, the presents results are very similar to the data of LEVAN and HSU, and BUCKTON, JACOBS and HARNDEN, who suggested that the X was smaller than no. 6. In the present study, the value of the X was 56.7 ± 2.49 by area measurement and 51.9 ± 1.55 by length measurement.

An independent test for the accuracy of our identification of the X chromosome may be made by referring to the so-called drumstick appendage of the polymorphonuclear leucocytes, which is generally considered, on the basis of recent cytogenetic discoveries, to be an expression of an X in somatic cells of the female. Therefore, we can estimate, from the observed ratio of the size of the drumstick to the total nuclear size of the polymorphs, the expected ratio of an X chromosome to the total of an X-containing haploid set in terms of area measurement. From the observation of 60 polymorphonuclear cells with drumsticks, the ratio of the drumstick to the total nucleus had been found to be about 0.03, and this is equivalent to 0.058 for the ratio of an X to the haploid set. Thus, the latter is rather close to the value of 0.057 obtained by the direct measurement of the area.

Briefly the area measurement may sometimes be useful for the identification of individual chromosomes, especially, for the measurement of quantitative changes involved in the X chromosome material.

109. Clinical conditions of patients with apparently normal chromosomes, II

Hidetsune OISHI, Yasumoto KIKUCHI and Akira TONOMURA

Based on leucocyte cultures of peripheral blood, patients with the following clinical conditions were found to have 46 chromosomes of apparently normal karyotype.

	Name	Age	Legal sex	Clinical conditions
26	S. M.	2 years	M	Congenital cataracta
27	Y. T.	3 years	F	Sjögren's syndrome
28	T. Y.	5 years	M	Male pseudohermaphroditism
29	N. I.	10 years	M	Retentio testis abdominalis

30	T. S.	1 year	M	True hermaphroditism
31	H. A.	19 years	F	Testicular feminization
32	N. A.	17 years	F	,,
33	M. O.	3 months	M	Hirschsprung's disease
34	A. S.	7 years	M	Turner's syndrome in the male
35	K. T.	6 years	M	Female intersex
36	Y. Y.	14 years	M	Eunuchoidism
37	—. M.	1 day	M	Anencephaly
38	M. Y.	6 months	F	Pterygium coli
39	M. Y.	10 years	F	Gonadal disorder
40	T. O.	3 years	M	Chondro-osteo dystrophy
41	A. S.	46 years	M	Eunuchoidism
42	A. H.	12 years	F	Pterygium coli
43	T. K.	2 years	M	Mental retardation
44	K. K.	3 years	M	Klippel-Feil's disease
45	K. K.	7 years	M	de Lange's syndrome
46	T. H.	3 years	F	Female pseudohermaphroditism

110. DNA replication pattern of the human Y chromosome

Yasumoto KIKUCHI

In human karyotype, certain structural characteristics make it easy to distinguish the Y chromosame from the acrocentric chromosomes of G group (nos. 21–22), namely absence of satellites on the Y chromosome, its longer size, lower arm ratio, and condensed or pycnotic condition. In a considerable number of metaphases, however, it may not be possible to distinguish the Y chromosome from the acrocentrics of G group. Hence, we made a special effort to ascertain the autoradiographic pattern of the Y chromosome as compared to that of the small acrocentric chromosomes, hoping to find out if the DNA replication pattern can be used for identification.

Leucocytes obtained from venous blood secured from male subjects with XXY, XYY, X-long Y, and normal XY were cultured acording to a modified method of MOORHEAD *et al.* (1960). DNA replication was analysed by means of autoradiography, tritiated thymidine, and pulse labeling technique (KIKUCHI and SANDBERG 1964, 1965).

To demonstrate possible differences in grain content of the Y and the G chromosomes, 20 metaphase plates of each subject were examined. The results are shown in Table 1. In normal male cells, the average grain number of Y, 4 G's, and the most heavily labeled G chromosome were 10.0, 2.3, and 4.2, respectively. Grain distribution over the Y

Table 1. Summary of the result of grain counts for the Y and the chromosomes of G group from 5 male subjects. The data are based on counts obtained from 20 metaphases in each case.

	Normal (XY)	XXY	X-long Y	XYY	XYY
Average no. of grains on 1 Y chromosome	10.0	11.7	12.4	8.2	8.4
Average no. of grains on 1 G chromosome	2.3	2.2	2.8	2.4	2.6
Average no. of grains on the most heavily labeled G chromosome	4.2	5.0	5.6	4.5	4.7

chromosome was about four times heavier than over the G chromosomes. Further, the Y chromosome was about two times heavier labeled as the most heavily labeled G chromosome. Similar results were obtained in cells of XXY, X-long Y, and XYY subjects.

In general, it was noted that the human Y chromosome has a markedly late DNA replicating pattern which differs from that of G group. The results obtained from detail analysis of grain content may indicate that the DNA replication of the Y chromosome is later than that of any chromosome of G group, and that the grain count by autoradiography is a useful tool for distinguishing the Y chromosome from the chromosomes of G group.

H. TECHNICAL NOTE

111. Separax, a new Japanese cellulose acetate strip for electrophoresis

Yoshito OGAWA

Cellulose scetate strips for zone electrophoresis manufactured by Fuji Photo Film Company in Japan under the guidance of the present writer and sold giving the name of "Separax" have several advantages over the presently used kinds, namely Oxoid (England), Membranfolien (Germany), Millipore, Sepraphore III (U.S.A.) and Cellogel (Italy).

The benefits of Separax strips are as follows:

1. Separax is not fragile and does not roll.
2. It does not take color, *e.g.* Ponceau 3R or Nigrosin, and remains

completely colorless.

3. Its ability of separation is excellent (Fig. 1).

4. Rapid separation and drying, with a great saving of time and labor.

On Separax strips, electro-osmotic phenomena are clearly observed. The material must be applied near the anode (Fig. 1).

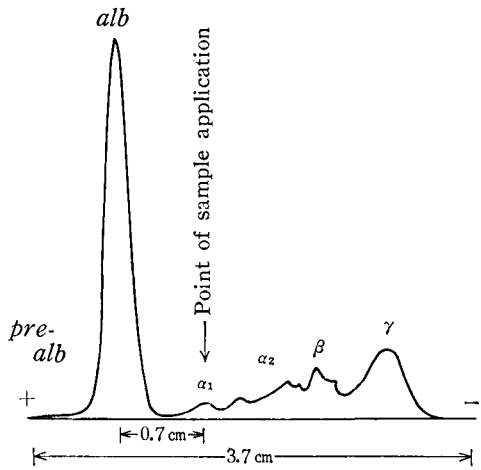

Fig. 1. Curve obtained by scanning a Ponceau 3R stained Separax (1×6cm.) cleared with paraffin oil. α_2 and β-fractions were separated into three and two fractions respectively.

Results:	Total protein	7.0 gm%
	Albumin	59.5%
	α_1 globulin	3.2%
	α_2 globulin	10.1%
	β globulin	8.2%
	γ globulin	19.0%
	A/G	1.47

Separax is opaque-white and is usually sold in 6×22 cm. strips, but othr sizes can also be obtained*.

The physical and chemical characteristics are as follows:

Thickness: approximately 130—150 μ.

Wet to dry ratio, after blotting: 2.6 : 1.

Refractive index of strips immersed in paraffin oil: Approximately 1.477 (N_D^{20}).

* Jō-Kō Sangyō CO. LTD.: Tamagawa Okuzawa 3-164, Setagaya, Tokyo. Japan.

Table 1. Technical errors in the analysis of human sera on Separax (%)

Kind of strips	Method of quantitative determination	Size of strips (cm)	Number of experiments	Albumin	Globulin			
					α_1	α_2	β	γ
Separax	Scanning	1×6	5	61.02±1.149	2.33±0.149	9.40±0.584	7.37±0.343	19.98±0.355
Separax	Elution	2×6	5	61.09±0.894	4.91±0.609	9.18±0.308	7.81±0.264	17.01±0.397
Separax	Elution	4×6	5	62.89±0.429	3.22±0.009	9.26±0.285	6.95±0.012	17.68±0.230
Oxoid (Control)	Scanning	1×6	5	63.40±1.151	4.45±0.645	8.95±0.388	7.94±0.613	15.26±0.641

Examination was carried out using the same sample under optimal experimental condition of each strip.
Separax: Electric run, 0.8 mA/cm, 33 minutes at 28°C., Staining 90 sec. with 0.8% ponceau 3R solution.
Oxoid: Electric run, 0.6 mA/cm, 50 minutes at 28°C., Staining 60 sec. with 0.4% ponceau 3R solution.

Table 2. Normal values of Japanese serum analysed on Separax (%)

Method of quantitative determination	Blood donors		Albumin	Globulin			
				α_1	α_2	β	γ
Scanning	Male	10 persons	68.3±2.2	3.3±0.9	8.0±1.3	7.6±0.9	12.8±2.4
	Female	10 persons	67.8±4.4	3.7±0.3	7.7±1.1	8.2±1.5	12.6±2.8
Elution	Male	10 persons	70.3±0.9	3.4±0.3	6.8±0.7	7.0±0.8	12.5±0.6
	Female	10 persons	69.3±0.7	3.6±0.3	6.8±0.2	7.7±0.5	12.6±0.6

Electrical resistance of strips immersed in barbitone buffer pH 8.6, ionic strength 0.06: 323,000 ohms/cm. length/cm. width.

Chemical composition and solubility are generally similar to those of the other commercial kinds.

The optimal experimental conditions for analysing human serum for clinical examination are as follows:

1. 1×6 cm. strips for scanning and 3×6 cm. strips for elution are convenient. When many samples have to be analysed on the same strip wider strips may be convenient.
2. Buffer solution: Barbitone buffer pH 8.6, ionic strength 0,06 is recommended. (Barbitone 1.62 g., Sodium diethyl-barbitone 12.38 g., Distilled water to 1 liter).
3. Application of sample: Samples at normal protein concentration should be applied on aproximately 0.8 μl/cm. strips.
4. Electrophoretic run: 0.8 mA/cm. strips should be applied when using current constant power stabilizer until an adequate separation of 3.7 cm. length is produced (ca. 33 minutes at 28°C.).
5. Staining: The strips are treated with 0.8 per cent Ponceau 3R solution of 6 per cent aqueous trichloracetic acid for abuot 60 seconds.
6. Washing: Continue washing with 1 per cent aqueous acetic acid until the background is quite white. This takes only 5–10 minutes.
7. Densitometry: Cleared strips by paraffine oil are measured with densitometer using 0.5×8 mm. slit adapter with 500 mμ wavelength.
8. Elution method: Immediately after blotting off the washing solution, the wet strip is cut in separate portions. Each part is treated with N/100 NaOH solution separately for 5 minutes under constant stirring and the extracted Ponceau 3R is estimated in the usual manner by colorimeter with 500 mμ wavelength. White part of the strip of 1 cm. length, usually on the cathode side of γ-globulin fraction, is utilized for control.

Standard error of this technique and normal values of Japanese sera on Separax are given in Tables 1 and 2.

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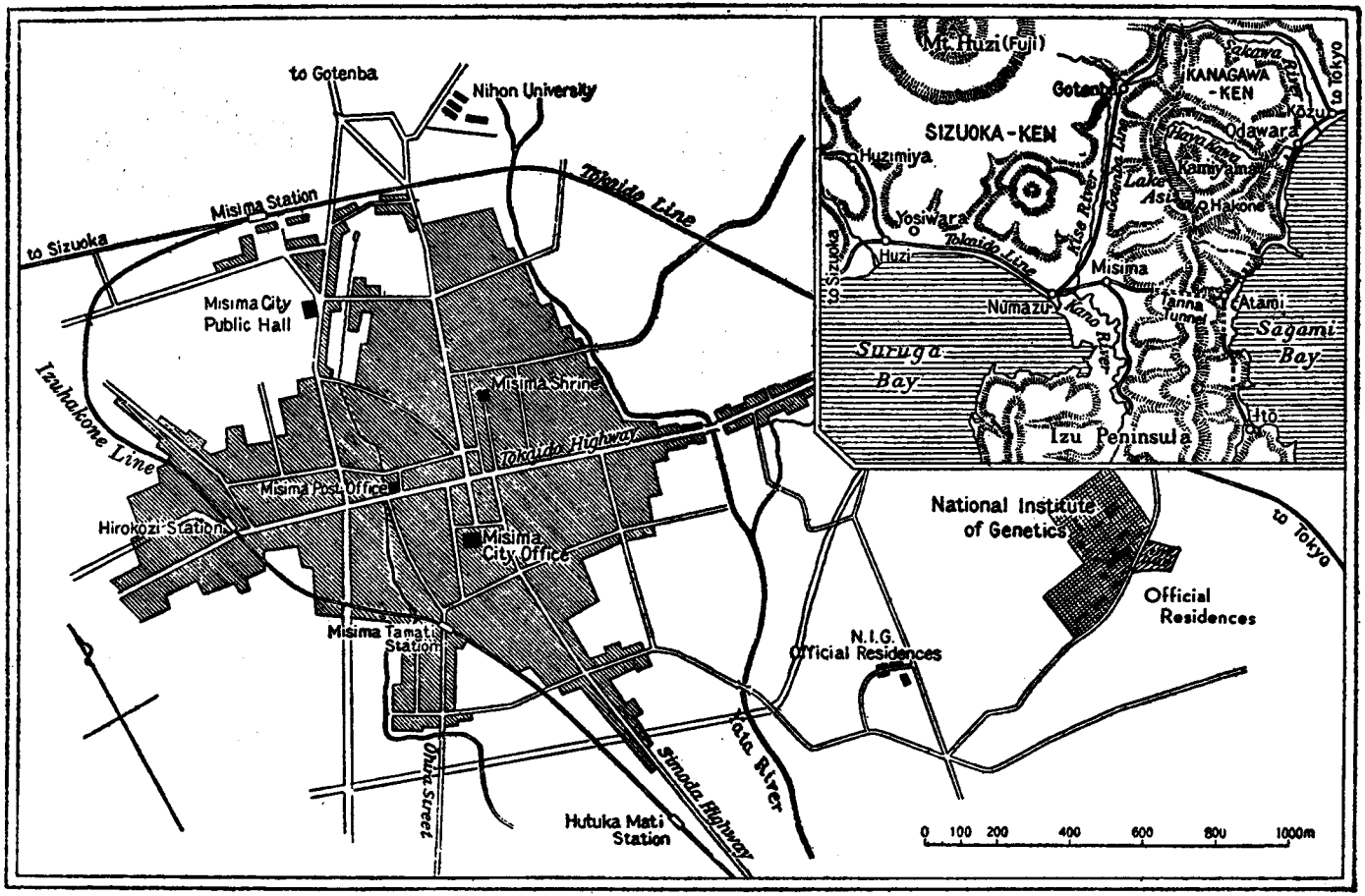
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Official Residences
to Tokyo
0 100 200 400 600 800 1000m
Mt. Huzi (Fuji)
Gotenba
KANAGAWA - KEN
Sakawa River
Kozu
SIZUOKA - KEN
Odawara
Huzimiya
Hayakawa
Kamiyama
Lake Asi
Hakone
Yosiwara
Tokaido Line
Kise River
Izu Mountains
Sizuoka
Huzi
Misima
Numazu
Kano River
Tanna Tunnel
Atami
Sagami Bay
Suruga Bay
Itō
Izu Peninsula