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GENERAL STATEMENT

Already 15 years have passed since our Institute had been established. The 15th anniversary was held on September 19, 1964, in our roof garden. Officials from the Government and many colleagues were present.

My address delivered on that occasion contained the statistics of our activities of the past 15 years. I wish to repeat them here.

Until now, two thirds of our main building (3,200 m^2) have been constructed. We expect the remaining third part will be added in the coming years to make the whole building complete. As additional facilities, we have one radio-isotope laboratory, one insectarium, one special silkworm laboratory, two mousaries, one rice laboratory with a short day installation, one rabbit house, one glasshouse, one air-conditioned greenhouse, one isolation greenhouse, one gamma-greenhouse, and 33 living quarters for research workers and administrators.

Our library is not rich but we are proud of having the collection of genetic reprints and books of the late Dr. GOLDSCHMIDT.

At first we have started with three departments of Morphological Genetics, Cytogenetics and Physiological Genetics. However we have succeeded to add until now six more departments of Biochemical Genetics, Applied Genetics, Induced Mutation, Human Genetics, Microbial Genetics and Population Genetics. As to our research staff, we have 40 research workers, 38 full time technical assistants and about 50 part time workers in addition to 18 administrative officials.

The number of our published papers reached to 539 until the end of August, 1964. Most of them were read and corrected by Dr. F. A. LILIENFELD, to whom we are most grateful.

In addition to research activities, we have organized several international symposia and a genetic course for students of Southeastern Asia.

Our institute is governmental and is given every year by our government a budget. However we have been receiving grants from various sources for special projects. For instance The Rockefeller Foundation gave us a grant for the study of rice genetics and also funds for collecting the rice species of the world. Also the National Science Foundation and National Institutes of Health have contributed to the study of human as well as animal genetics. The Toyo Rayon Foundation for Promotion of Science and Technology has also given us a grant for radiation work. To all these Foundations we want to express our hearty thanks.

Hitoshi Kihara

ABSTRACT OF DIARY FOR 1964

Jan.	24	The 123rd Meeting of Misima Geneticists' Club.
Feb.	18	The 56th Biological Symposia.
Feb.	21	The 124th Meeting of Misima Geneticists' Club.
Mar.	25	The 57th Biological Symposia.
Mar.	27	The 125th Meeting of Misima Geneticists' Club.
Mar.	30	The 58th Biological Symposia.
May	1	The 126th Meeting of Misima Geneticists' Club.
May	22	The 127th Meeting of Misima Geneticists' Club.
May	27	The 59th Biological Symposia.
June	5	The 60th Biological Symposia.
July	9	The 1st Seminar of the Institute.
July	11	
July	24	The 128th Meeting of Misima Geneticists' Club.
Sep.	19	Ceremony for the 15th Anniversary of the Institute.
Oct.	2	The 129th Meeting of Misima Geneticists' Club.
Oct.	30	The 61st Biological Symposia.
Nov.	2	The 62nd Biological Symposia.
Nov.	6	The 130th Meeting of Misima Geneticists' Club.
Nov.	13	The 63rd Biological Symposia.
Nov.	20	The 131st Meeting of Misima Geneticists' Club.
Nov.	28	Public Lectures Memorializing the 15th Anniversary of the Institute.
Dec.	11	The 132nd Meeting of Misima Geneticists' Club.

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Clerks (6), Librarians (2), Typist (1), Chauffeur (1), Janitors (4)

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TACHI, Minoru, Director of Institute of Population Problems
TSUKAMOTO, Kempo, Director of National Institute of Radiological Sciences
WADA, Bungo, Professor of Sizuoka University

PROJECTS OF RESEARCH FOR 1964

Department of Morphological Genetics

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

Department of Cytogenetics

Study on chromosomal polymorphism of Muridae (YOSIDA, NAKAMURA and FUKAYA)
Cytogenetical and biochemical studies on tumor cells (YOSIDA, MORIWAKI and KURITA)
Mechanism of chromosomal abnormalities by treatment with chemicals (YOSIDA, KURITA and MORIWAKI)
Corelation between taxonomy and karyology of ants (IMAI and YOSIDA)
Experimental breeding and genetics of mice and rats (YOSIDA, MORIWAKI, KURITA and NAKAMURA)
Determination and differentiation of sex in higher plants (TAKENAKA and YONEDA)
Induction of abnormal mitosis and inhibition of growth by substances extracted from certain plants (TAKENAKA)

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 and YONEDA)

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 isozyme 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 study of *Oryza* (KIHARA and KATAYAMA)
Investigation of photoperiodic responses of *Oryza* species (KATAYAMA)

Department of Biochemical Genetics

Studies on transformation in higher organisms (NAWA and TSUJITA)
Genetical and biochemical studies of pteridine metabolisms in insects (NAWA and TSUJITA)
Studies on balanced lethality 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)
Studies on emanation of alkaloids from the leaves of tobacco plant (TSUJITA)

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)
Biochemical research on the tissue specificity in plant (ENDO)
Studies on biosynthesis of protein and nucleic acid in plant seed (ENDO)
Comparative studies on seed proteins of rice plant by electrophoretic analysis (SAKURAI)

Department of Applied Genetics

Studies on developmental instability in poultry (SAKAI, KAWAHARA and FUJISHIMA)
Quantitative genetic studies in poultry (KAWAHARA)
Competition studies in poultry (FUJISHIMA)
Theoretical studies on breeding techniques (SAKAI and IYAMA)
Studies on competition in plants (SAKAI and IYAMA)
Estimation of genetic parameters in forest trees (SAKAI and MUKAIDE)
Developmental genetics of quantitative characters in plants (SAKAI, SHIMAMOTO, EL-BALAL and TAKEDA)
Genetic studies on developmental instability in plants (SAKAI and SHIMAMOTO)
Studies on the effects of linkage disequilibrium in *Drosophila* population (IYAMA)
Genetic studies of *Oryza sativa*×*glaberrima* hybrids (OKA and MORISHIMA)
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)

Department of Induced Mutation

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

Department of Human Genetics

Selection in ABO blood groups (MATSUNAGA and HIRAIZUMI)

Down's syndrome in Japan (MATSUNAGA and TONOMURA)
Cytogenetics in man (TONOMURA and OISHI, H.)
Sexual dimorphism in resting nuclei (TONOMURA)
Biochemical studies on plasma proteins, haemoglobins and G-6P-D (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)
Effects of radiation-induced mutations on fitness (HIRAIZUMI)
Populational implications of meiotic drive with special reference to the *SD* locus in *D. melanogaster* (HIRAIZUMI)

FOREIGN VISITORS IN 1964

Feb. 18~26 NACE, G. W., Dept. of Zoology, Univ. of Michigan, Ann Arbor, Mich., U.S.A.
Feb. 27 OETJEN, R. A., National Science Foundation, Tokyo
Feb. 27~28 EMERSON, A. E., Dept. of Zoology, Univ. of Chicago, Chicago, Ill., U.S.A.
Mar. 24~25 SÁNCHEZ-MONGE, E., Director, Centro de Mejora del Maiz, Instituto Nacional de Investigaciones Agronómicas, Avenida Puerta de Hierro, Madrid, Spain
Mar. 30 GRAY, L. H., British Empire Cancer Campaign, Research Unit in Radiobiology, London, England
Apr. 6 NEUREITER, N. P., National Science Foundation, Washington, D. C., U.S.A.
Apr. 6 O'CONNELL, J. E., National Science Foundation, Tokyo
Apr. 11 VERLEUR, J. D., Dept. of Botany, Free Univ., Amsterdam, The Netherlands
Apr. 20 FORREST, H. S., Genetics Foundation, Univ. of Texas, Austin, Texas, U.S.A.
Apr. 30 KAMEMOTO, H., Univ. of Hawaii, Honolulu, Hawaii, U.S.A.

May 7	WHEELER, M. R., Genetics Foundation, Univ. of Texas, Austin, Texas, U.S.A.
May 7	THROCKMORTON, L. H., Dept. of Zoology, Univ. of Chicago, Chicago, Ill., U.S.A.
May 9	KWACK, B. H., Hyosung Women's College, Taegu, Korea
May 25	SOON, I. K., Nam-do, Korea
May 25	CHUNG, S. H., Nam-do, Korea
May 27	AUXIER, J. A., Health Physics Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
May 27	CHEKA, J. S., Health Physics Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
June 5	HOWES, J. R., Auburn Univ., Auburn, Ala., U.S.A.
June 9	MEHRA, P. W., Head of Botany Dept., Panjab Univ., Chandiarh, India
June 25	VENKATESWARLU, J., Head of Botany Dept., Andhra Univ., Waltair, India
June 25	RAO, C. B. J., Foreign Research Student, Faculty of Agriculture, Univ. of Tokyo, Tokyo
June 28	PARK, Y. S., Section of Agricultural Production, Dept. of Agricultural Production, Ministry of Agriculture, Seoul, Korea
June 29	LAMMERTS, W. G., Director of Research, Germain's Horticultural Research Division, Livermore, Calif., U.S.A.
July 16	BAE, S. H., Crop Experiment Station Office of Rural Development, Suwon, Korea
July 17	RAYCHAUDHURI, S. P., Indian Agricultural Research Inst., New Delhi 12, India
Aug. 18	TER-AVANERYAN, D. V., Dept. Director, All Union Inst. of Plant Industry, Leningrad, U.S.S.R.
Aug. 18	KORSAKOV, N. I., All Union Inst. of Plant Industry, Leningrad, U.S.S.R.
Sep. 7~8	DASGUPTA, K. P., Central Sericultural Research Sub-Station, Kalimpong (West Bengal), India
Sep. 14~15	TAN, W. Y., The Inst. of Botany, Academia Sinica, Nankang, Taipei, Taiwan
Sep. 27~28	CHANG, T. T., International Rice Research Inst., Los Baros, Laguna, Philippines
Oct. 5	DOBZHANSKY, Th., The Rockefeller Inst., New York, N. Y., U.S.A.
Oct. 13~14	DOBZHANSKY, Th., The Rockefeller Inst., New York, N. Y., U.S.A.

Oct. 29~30 WOLFF, S., Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Oct. 29~30 DOBZHANSKY, Th., The Rockefeller Inst., New York, N. Y., U.S.A.
Oct. 30 OAKBERG, E. F., Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Oct. 30 RUSSELL, W. L., Oak Ridge National Laboratory, Oak Ridge Tenn., U.S.A.
Nov. 2 OAKBERG, E. F., Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Nov. 2 RUSSELL, W. L., Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Nov. 2 CHU, E. H. Y., Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Nov. 6 HINDY, S. S., Rice Breeder, Cairo, Egypt, U.A.R.
Nov. 6 INTSIFOL, J. K., Crops Research Inst., Kumasi, Ghana
Nov. 7 CHU, E. H. Y., Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Nov. 7 WOLFF, S., Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Nov. 7 KIMBALL, R. F., Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Nov. 7 ELKIND, M. M., National Cancer Inst., Bethesda, Md., U.S.A.
Nov. 7 KALLMAN, R. F., Stanford Univ., Palo Alto, Calif., U.S.A.
Nov. 7 OSTER, I. I., The Inst. for Cancer Research, Philadelphia, Pa., U.S.A.
Nov. 7 SINCLAIR, W. K., Argonne National Laboratory, Argonne, Ill., U.S.A.
Nov. 7 SPARROW, A. H., Brookhaven National Laboratory, Upton, N. Y., U.S.A.
Nov. 7 HOWARD-FLANDERS, P., Yale Univ., New Haven, Conn., U.S.A.
Nov. 7 ARVEY, M. D., National Science Foundation, Tokyo
Nov. 9 KIMBALL, R. F., Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Nov. 9 SPARROW, A. H., Brookhaven National Laboratory, Upton, N. Y., U.S.A.
Nov. 12 DOBZHANSKY, Th., The Rockefeller Inst., New York, N. Y., U.S.A.
Nov. 12~13 GAUL, H., Max-Planck Inst. für Züchtungsforschung, Deutschland
Nov. 13 SONNEBORN, T. M., Dept. of Zoology, Indiana Univ., Bloomington, Ind., U.S.A.

Nov. 23~24 OSTER, I. I., The Inst. for Cancer Research, Philadelphia, Pa., U.S.A.
Nov. 23~24 DOBZHANSKY, Th., The Rockefeller Inst., New York, N. Y., U.S.A.
Dec. 1 OSTER, I. I., The Inst. for Cancer Research, Philadelphia, Pa., U.S.A.
Dec. 10 DAVE, M. J., Indian Cancer Research Center, Bombay, India
Dec. 10 RAO, C. B. J., Foreign Research Student, Faculty of Agriculture, Univ. of Tokyo, Tokyo
Dec. 12 SUBBADAH, K. C., Biology Division, Atomic Energy Establishment, Torombay, Bombay, India
Dec. 12 CHEN, C. L., The Inst. of Botany, Academia Sinica, Nankang, Taipei, Taiwan
Dec. 28 OMATA, R. R., N. I. H. Pacific Office, American Embassy, Tokyo

RESEARCHES CARRIED OUT IN 1964

A. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF ANIMALS

1. Genetic variability of a large natural population of Drosophila melanogaster[1)]

Chozo OSHIMA and Takao WATANABE

In late October, 1964, many male flies were collected from large natural populations in five different graperies of Kofu and Katsunuma. The second chromosome isolated from each male fly was duplicated, in order to estimate the effect of genes on viability. Seven hundred and forty 2nd chromosomes were divided into four classes; lethal, semi-lethal, subvital and normal. Their respective frequencies were 12.30, 22.84, 7.57 and 57.30 per cent. The frequency of lethal and semi-lethal chromosomes was found to be a similar high level as that of samples in 1963. On the other hand, the mean viability of homozygous flies for 480 quasinormal chromosomes was 90.5, when the viability of *Curly* and quasinormal heterozygous flies was standardized as 100. This mean value was much higher than that of samples of 1963, because the observed frequency of subvital chromosomes was very low.

The two graperies in Kofu and the three graperies in Katsunuma, where the flies were collected, are about one or two kilometers apart from each other, but the distance between both localities is about 13 kilometers. The distribution frequencies for the four classes of about 150 chromosomes isolated from each of the natural populations of those graperies, were found to be very similar. From the results, their genetic structure, in spite of their locations over a wide area, is assumed to be fairly uniform.

2. Allelic rate between lethal genes concealed in natural populations of Kofu and Katsunuma[1)]

Chozo OSHIMA and Takao WATANABE

One hundred and fourteen lethal chromosomes were isolated from different male flies collected at the two localities in the middle of

[1)] This work was supported by a grant from the Japan Society for the Promotion of Science as part of the Japan-U.S. Cooperative Science Program and also partly supported by the United States Public Health Service Grant RG 7836.

October, 1963. A total of 6,441 crosses were performed between the lethal and the *Curly* balanced strains. The crosses were divided into three groups; two of them represented crosses within lethal strains extracted from the same population and the third was a cross between

Table 1. The allelic rate between lethal genes isolated from natural populations in two localities, Kofu and Katsunuma.

Year	Population	No. of lethal chromosome	No. of crosses	No. of allelic crosses	Allelic rate (%)
1963	Within lethals of Kofu	61	1830	60	3.28
	Within lethals of Katsunuma	53	1378	30	2.18
	Between lethals of Kf. and Kn.	114	3233	80	2.47
	Total	114	6441	170	2.64

Table 2. Allelic groups of single, double and multi-loci lethal genes.

Symbol of single lethal gene	No. of allelic strain	Symbol of double, multi-loci and synthetic lethal genes	No. of allelic strains
l(2)63j 201	12	11 63j 273	1
202	8		
203	5	11 63j 203	1
204	6		
205	4		
206	4		
207	2	11 63j 269	1
208	2		
209	2		
210	2	11 63j 272	1
211	2	11 63j 207	1
212	2		
213	2		
214	1		
215	2	11 63j 225	1
217	1	11 63j 227	1
218	1	11 63j 233	1
(219 - 265)	(40)	11 63j 259	1
266	1		
253	1	11 63j 274	1
267	0	11 63j 271	1
268	0		
Total strains	100		11

lethal strains extracted from different populations. The results are given in Table 1.

The lethal genes were classified into sixty-one non-allelic groups and the frequencies of their appearances could be determined by the results of diallel crosses mentioned above. The results are given in Table 2.

The allelic rate between the populations was relatively high as compared with those within the populations, although the two populations are about thirteen kilometers apart. This could be due to frequent migration of flies between the localities or to persistence of some common lethal genes in both natural populations.

Eleven chromosomes seemed to have multi-loci lethal genes. Five chromosomes among them were ascertained to have two lethal genes which were allelic to two different lethal genes respectively. However, the other six chromosomes were assumed to have multi-loci lethal genes, whose loci could not be exactly determined.

3. *Location of lethal genes on the second chromosome*[1]

Takao WATANABE and
Chozo OSHIMA

In the previous annual report (No. 14, 1963), loci of fifteen lethal genes were determined by the crossing over value between them and the dominant marker genes such as *Sp*, *Bl* and *L*. Their locations on the genetic map seemed to be non-random, but their number was too small for evaluating our results and comparing them with the data of other investigators. However, recently loci of 55 single lethal genes isolated from the natural populations in Kofu and Katsunuma were determined by the same method.

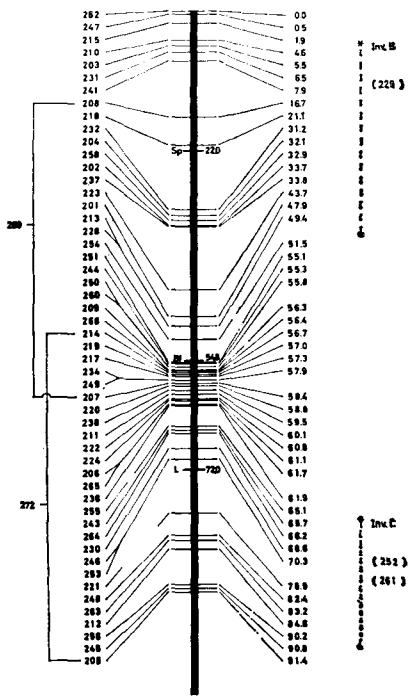

Fig. 1. Loci of lethal genes extracted from Kofu and Katsunuma populations.

[1] This work was supported by a grant from the Japan Society for the Promotion of Science as part of the Japan-U.S. Cooperative Science Program and also partly supported by the United States Public Health Service Grant RG 7836.

The results are shown in Fig. 1. About half of them (51.8%) were located in the central region (40~70) of the genetic map and twelve lethal genes were strikingly clustered within only five units, between 55 and 60.

The distribution of these lethal genes on the genetic map was compared with that of recessive visible mutant loci ($N \div 100$) described in the book "The Mutants of *Drosophila melanogaster*" by BRIDGES and BREHME (1944), and no significant difference between them was detected. Their distribution was confirmed to be non-random. As it is well known that the occurrence of recombination is not random on both arms of the large V-shaped chromosome, it may be assumed that the lethal genes are located randomly on the chromosome as well as the visible mutant genes. Two kinds of double lethal chromosomes had one lethal gene in the central region and another in a terminal region. Three different lethal genes were found to be surely included in inversion B or C, but their loci could not be determined.

4. *Frequencies of inversions in the Katsunuma natural population*[1]

Taishu WATANABE, Takao WATANABE
and Chozo OSHIMA

Frequencies of inversions which were found in small and large natural populations were reported previously (WATANABE, T. and OSHIMA, Annual Report No. 14, 1963). In late October, 1964, many female flies were collected simultaneously from two natural populations at Katsunuma locality. These females were allowed to lay eggs individually in a vial and then the salivary gland chromosomes of 100 larvae sampled from different cultures were observed. The results were compared with the data obtained in 1963.

These common inversions might have been maintained in the same population, but an endemic inversion, *In(3L)* F, could not be found in 1964.

The viabilities of heterozygotes for B or C inversion and the normal chromosome and of transformed heterozygotes for B and C inversion chromosome were estimated by using *Cy-Pm* technique. The materials used in the experiment were chromosomes extracted in 1963 from the Kofu and Katsunuma populations. The results are shown in Table 2.

[1] This work was supported by a grant from the Japan Society for the Promotion of Science as part of the Japan-U.S. Cooperative Science Program and also partly supported by the United States Public Health Service Grant RG 7836.

The inversion heterozygotes did not show heterosis in viability, but the viability of double inversion heterozygotes was proved to be significantly higher than that of normal heterozygotes.

Table 1. Frequencies of inversions in natural populations of Katsunuma.

Year	1963	1964
Standard and inversion	Frequency (%)	
2L (standard)	68.0	73.0
In (*2L*) B	32.0	27.0
2R (standard)	79.0	74.5
In (*2R*) C	21.0	25.5
3L (standard)	89.5	89.5
In (*3L*) E	8.5	10.5
In (*3L*) F	2.0	—
3R (standard)	61.0	71.0
In (*3R*) G	18.0	12.0
In (*3R*) H	10.0	8.5
In (*3R*) I	11.0	8.5

Table 2. Relative viability of inversion heterozygotes.

Chromosomal type	Pooled basis		Line basis	
	No. of counted flies	Relative viability	No. of lines	Relative viability
+/+	98,631	1.0583±0.00941	78	1.0699±0.01683
+/B	53,978	1.0587±0.01277	46	1.0727±0.01640
+/C	51,707	1.0512±0.01295	40	1.0539±0.01808
B/C	55,086	1.1253±0.01361**	49	1.1490±0.02678*

Viability of *Cy*/*Pm* flies = 1.0000

* Significant at the 5% level

** Significant at the 1% level

5. *Mechanism of maintenance of chromosomal polymorphism in natural populations*[1)]

Taishu WATANABE and Chozo OSHIMA

One of causes of maintained chromosomal polymorphism could be assumed to be the situation described in the preceding report. This assumption was doubly confirmed by the following experimental results. One hundred male flies, collected from natural populations at Kofu and Katsunuma in late October, 1964, were used in this experiment. Each male fly was crossed with several virgin female flies of Samarkand strain, and the salivary gland chromosomes of 8 or 10 F_1 larvae in each culture were observed. The chromosomal type of each male fly could be detected and the frequencies of various inversions in second and third chromosomes were also determined. The expected frequencies of all possible combinations of the second and the third chromosomes were calculated on the basis of Hardy-Weinberg's law and were compared with the observed

Table 1. Comparison of observed and expected frequencies of various inversion second chromosomes.

Chromosomal type		Observed frequency	Total	Expected frequency	Total	Difference
Standard	+/+	27		28.09		
Inversion homozygote	B/B	4		6.0025		
	C/C	2	33	1.96	36.695	−3.695
	BC/BC	0		0.64		
	L*/L*	0		0.0025		
Inversion heterozygote	+/B	27		25.97		
	+/C	13		14.84		
	+/L*	0	44	0.53	47.50	−3.50
	B/BC	4		3.92		
	C/BC	0		2.24		
Double inversion heterozygote	B/C	10		6.86		
	B/L*	0		0.245		
	C/L*	1	23	0.14	15.805	+7.195
	BC/+	12		8.48		
	BC/L*	0		0.08		
		100		100		

* *In* (*2R*) L: A new endemic inversion, which was found in 1964, is located on the right arm (43F−49F) of the second chromosome.

1) This work was supported by a grant from the Japan Society for the Promotion of Science as part of the Japan-U.S. Cooperative Science Program and also partly supported by the United States Public Health Service Grant RG 7836.

Table 2. Comparison of observed and expected frequencies of various inversion third chromosomes.

Chromosomal type		Observed frequency	Total	Expected frequency	Total	Difference
Standard	+/+	46		46.24		
Inversion homozygote	E/E	0	48	0.2025	48.425	−0.425
	G/G	1		0.7225		
	H/H	0		0.7225		
	I/I	1		0.4900		
	EG/EG	0		0.0025		
	EH/EH	0		0.0225		
	EI/EI	0		0.0225		
Inversion heterozygote	+/E	7	38	6.12	39.625	−1.625
	+/G	9		11.56		
	+/H	13		11.56		
	+/I	9		9.52		
	E/EG	0		0.045		
	E/EH	0		0.135		
	E/EI	0		0.135		
	G/EG	0		0.085		
	H/EH	0		0.255		
	I/EI	0		0.210		
Double inversion heterozygote	E/G	1	14	0.765	11.950	+2.050
	E/H	1		0.765		
	E/I	0		0.630		
	G/H	3		1.445		
	G/I	2		1.190		
	H/I	0		1.190		
	EG/EH	0		0.015		
	EG/EI	0		0.015		
	EH/EI	0		0.045		
	+/EG	1		0.680		
	+/EH	2		2.040		
	+/EI	3		2.040		
	G/EH	0		0.255		
	G/EI	0		0.255		
	H/EI	0		0.255		
	H/EG	0		0.085		
	I/EG	0		0.070		
	I/EH	1		0.210		
		100		100		

frequencies in Tables 1 and 2.

Total observed frequency of double inversion heterozygotes of both chromosomes was higher than the expected one regardless of their chromosomal types in any other chromosome. From the results, heterosis of double inversion heterozygotes could be a strong mechanism for maintaining chromosomal polymorphism in natural populations.

6. *The effect of insecticide selection on changing the frequencies of four chromosomal types in experimental populations of* Drosophila pseudoobscura[1]

Chozo OSHIMA and Taishu WATANABE

About forty homozygous strains for 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 (The Rockefeller Institute) in 1963. Heterozygous female flies for ST and AR were crossed with heterozygous male flies for CH and PP. Two initial populations were made with 250 female and 250 male offspring and, after two generations (one generation period lasts about 20 days), the salivary gland chromosomes of 150 larvae hatched from sampled eggs were observed. In the F_2 generation of both populations, the frequencies of the four kinds of chromosomes should be theoretically equal, *i.e.* 25 per cent of each. However, the observed frequencies were ST:33.3, AR:26.3, CH:22.7, PP:17.7 per cent in Population I and ST:26.3, AR:28.0, CH:26.3, PP:19.3 per cent in Population II. Further, each population was divided into two populations A and B. Flies in Population A were exposed to insecticide test paper (DDT 1%, 2% or Dieldrin 0.1%) for one hour and transferred into a new cage in each new generation. Flies in Population B were transferred into a new cage without exposure. The test papers and test kits were prepared by WHO in Geneva and sent to us. These populations are being maintained, but the varying frequencies of chromosomes from F_2 to F_7 in each population were observed as shown in the following table.

The frequency of ST chromosomes has increased in both selected and non-selected populations, but after F_5 the increase in the former (IA, IIA) was more remarkable than in the latter (IB, IIB). On the other hand, AR chromosomes showed changes which were opposite to those of ST. The frequencies of both CH and PP chromosomes have decreased. However, their relative frequencies in selected populations were different

[1] This work was supported by a grant from the Japan Society for the Promotion of Science as part of the Japan-U.S. Cooperative Science Program.

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

	F_2			Frequency (%)				
					F_3	F_4	F_5	F_7
Pop. I	ST	33.3	IA (DDT selected)	ST	40.3	55.0	55.3	73.3
	AR	26.3		AR	18.3	23.0	24.3	15.7
	CH	22.7		CH	22.0	11.7	8.3	4.0
	PP	17.7		PP	19.3	10.3	12.0	7.0
			IB (non-selected)	ST	42.0	45.7	53.7	65.3
				AR	24.7	25.7	26.3	22.0
				CH	18.3	17.3	10.0	7.0
				PP	15.0	11.3	10.0	5.7
Pop. II	ST	26.3	IIA (DL selected)	ST	43.7	41.0	43.0	71.0
	AR	28.0		AR	19.7	30.0	29.7	16.7
	CH	26.3		CH	15.3	9.7	10.3	4.3
	PP	19.3		PP	21.3	19.3	17.0	8.0
			IIB (non-selected)	ST	36.3	45.7	49.3	52.3
				AR	21.7	29.7	27.3	30.0
				CH	23.3	14.7	13.3	12.0
				PP	18.7	10.0	10.0	5.7

from those in non-selected populations; PP became more frequent than CH after several selections, and, on the other hand, CH was found more frequent than PP in non-selected populations. These results seem to be an interesting illustrations of the genetic changes which DOBZHANSKY observed for about twenty years from 1940 in natural populations of California locality.

7. *Distribution pattern of tryptophan and pteridine metabolites in* Drosophila *larval fatbody*

Toshifumi TAIRA and Tahir M. RIZKI[1]

Larval fatbodies are morphologically symmetrical and those of both sides are connected by a narrow band of single cells at the top of the anterior region. The fatbody of each side is a lobed monolayered cell mass, which consists of about 900 cells at the 96 hour stage. Its cells are distinguishable under the UV-microscope in the anterior and the posterior

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region by cytoplasmic autofluorescent inclusions. Kynurenine granules are characteristically found in the anterior region cells constituting the tryptophan metabolic zone, and pteridine granules are observed in the posterior region which is the pteridine metabolic zone. Such distribution pattern is highly specific for eye color mutants. The pattern of both metabolic zones of the fatbody, however, can be changed by feeding the larvae with L-tryptophan and 2-amino-4-hydroxypteridine (AHP). Due to tryptophan feeding, the distribution pattern of kynurenine cells in the anterior region extends to the posterior pteridine zone along the ventral line of the fatbody, and kynurenine granules can be often observed in the pteridine cells of the posterior region. Such an extension of the kynurenine zone can not be suppressed by pre-treatment with chloramphenicol feeding. In AHP feeding experiments, kynurenine granules in the anterior region gradually disappear, changing their shape, in the direction from the rear to the front, along the dorsal line of the fatbody. Eventually, the region is completely converted to cells containing pteridine granules within one hour. Such a conversion is considerably suppressed by pre-treatment with chloramphenicol feeding, but not all by the same treatment given simultaneously. The pre-treatment with AHP feeding given even at the 93 hour stage causes kynurenine cells to convert to pteridine cells in the anterior region. After 7 hours, the converted pteridine cells revert only in the anterior region to kynurenine ones, even if tryptophan is not fed to the same larvae.

These observations lead to following conclusions: (1) Distribution pattern of tryptophan and pteridine metabolic zones found in the larval fatbody is determined by the relative amount of both metabolites produced *in vivo*. (2) The activity of tryptophan pyrrolase by which tryptophan is converted to kynurenine, is induced by the additional feeding without a new synthesis of the enzyme protein. However, the enzyme catalyzing pteridine is probably synthesized *de novo*. (3) These phenomena are assumed to depend on the physiological potential at cellular level, such as permeability and so on.

8. *Mapping of acid soluble nucleotides in insects*

Toshifumi TAIRA and Sakaru SUZUKI[1]

A standard mapping of acid soluble nucleotides in insects has been attempted using fertilized eggs, larvae, pupae and adults of *Drosophila*, *Bombyx* and *Ephestia*, by means of the following procedures:

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A) *Extraction and separation*: Materials were homogenized in a Waring Blender in cold 50 % ethanol, at the ratio of 1 g per 5 ml. The homogenate was boiled for 2 min in a water bath and, after cooling, was quickly filtrated. This extraction was carried out at least twice. The combined filtrate was concentrated to one third in volume with a rotary evaporator. The concentrated solution was mixed with a given weight of charcoal (Nolite extra) in an ice bath under acidic condition (pH 5), and left standing for 15 min under occasional stirring. The cold mixture was again filtrated and the charcoal adsorbed the nucleotides on the filter paper was quickly washed with cold 0.001 *N* HCl. The washed charcoal was placed in a given volume of 50 % ethanol containing 0.1 % of ammonium water and left standing for 30 min at room temperature, under occasional stirring. After two elutions, the solution was concentrated again to a small volume for paper chromatographical fractionation of the nucleotides. In order to remove other compounds, a descending paper chromatography was used with filter paper (Toyo, #527, 60×60 cm), by a solvent of *n*-butanol, ethanol and water (52:32:16), for 6 to 8 hours at room temperature.

B) *Indentification and mapping*: Two-dimensional chromatography was

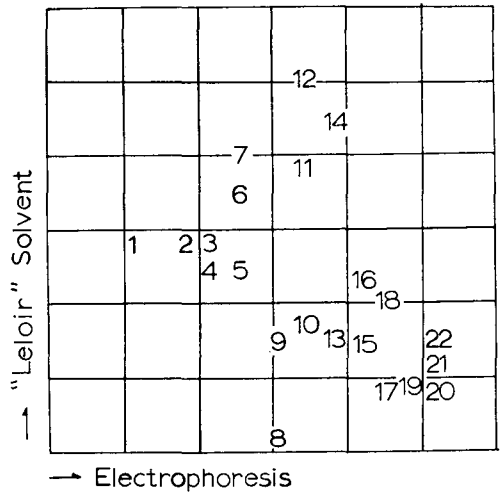

Fig. 1. Map of acid soluble nucleotides.
1. β-NAD 2. CMP 3. AMP 4. GMP 5. IMP 6. UMP 7. TMP 8. NADP 9. CDP 10. ADP 11. ADP-ribose 12. UDP-N-acetyl-D-glucosamine 13. GDP 14. UDP-D-glucosamine 15. CoA 16. TDP 17. CTP 18. UDP 19. ATP 20. GTP 21. UTP 22. TTP

applied to identify and to map the nucleotides, *i.e.* first by paper electrophoresis and then by paper chromatography. Paper electrophoresis was conducted on filter paper (Toyo, #51, 20×60 cm) using 0.05 *M* ammonium acetate and acetic acid buffer (pH 5), a cooling system of carbon tetrachloride and an electric run with 30 volts per cm for 90 min. The separated electrograms were eluted from each fraction with water after localization by UV-photograph, and every fraction was simultaneously applied to second paper chromatography. A filter paper (Toyo, #50, 60×60 cm) was used for carrying out descending chromatography, and the solvent "Leloir", *i.e.* mixed solution of 95% ethanol and 1 *M* ammonium acetate (15:6), pH 7, containing 2% of EDTA, was applied for 48 to 80 hours at room temperature. The chromatograms were located on the filter paper by UV-photograph. In each separated nucleotide, furthermore, the base component, sugar content and the amount of phosphorus were determined. Thus, the results allowed to draw a map of nucleotides, as shown in Fig. 1. Detailed analyses are now in progress.

9. *Heterotic effect of linkage disequilibrium in* Drosophila *populations*

Shin-ya IYAMA

The segregating population of *Drosophila melanogaster* which was derived from the cross between a natural population collected in St. Paul, Minn., U.S.A., and the *ebony* mutant (*e*) stock, have been kept for more than 70 generations under random mating so as to reach nearly linkage equilibrium between the ebony locus and other loci concerning fitness. Both kinds of the third chromosome, one carrying the wild and the other the mutant allele for ebony locus, were sampled from this population. The third chromosomes of the isogenic line derived from one genome of the Samarkand inbred stock were replaced by the sampled chromosomes in heterozygous condition, *i.e.*, $+/e$.

Experimental populations were initiated by mating the above mentioned flies with eight replications as follows:

Population I: one pair of identical flies.
Population II: one pair of unrelated flies.
Population III: six pairs of unrelated flies and one pair of identical flies.

Each replicate was kept in a 200 ml milk bottle containing 250 to 300 flies. Soon after the emergence, in every generation wild and ebony fly frequencies were counted and ebony flies were discarded before mating making the ebony gene artificially lethal. The change in average ebony

fly frequencies in each population is shown in Table 1 up to the eleventh generation together with the expected frequencies calculated on the assumption of equal fitness for +/+ and +/*e*.

Table 1. Ebony fly frequencies in per cent in three populations I~III in which ebony flies were made artificially lethal.

Population	G_0	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	G_9	G_{10}	G_{11}
I	23.42	18.30	18.02	17.31	13.64	14.68	10.08	11.01	15.45	12.83	10.46	10.09
II	23.08	14.82	13.37	11.26	9.52	8.62	7.58	8.21	8.51	8.93	8.44	8.39
III	21.02	13.18	10.81	6.54	4.66	3.58	3.60	2.59	3.78	4.19	3.72	3.36
Expected	25.00	11.11	6.25	4.00	2.78	2.04	1.56	1.23	1.00	0.83	0.69	0.59

The results reveal the fact that ebony fly frequencies were higher than expected in all three populations, indicating a heterotic effect in +/*e* heterozygotes; its magnitude was largest in population I and smallest in population III, depending on the number of pairs used for the initial mating, that is, on the degree of linkage disequlibrium involved in the population with regard to linkage between ebony and other loci concerned with fitness.

10. *Position effect of spontaneous mutant polygenes controlling viability in* Drosophila melanogaster

Terumi MUKAI and Tsuneyuki YAMAZAKI

As described in previous reports (MUKAI *et al.*, Annual Report No. 12, 13 and 14, 1961~1963), spontaneous polygenic mutations affecting viability had been accumulated under the minimum pressure of natural selection in 104 chromosomes derived from a single chromosome. In Generations 25, 32, 52 and 60, homozygous viabilities of these chromosome lines were tested. In addition, the viabilities of flies carrying random combinations of these chromosomes were estimated in Generations 25, 32 and 52 and the correlation coefficient between the sum of homozygote viabilities and the corresponding heterozygote viability was calculated for each generation. The estimated values were positive and highly significant.

On the other hand, in Generations 32 and 60, the viabilities of heterozygotes between these experimental chromosomes and a chromosome supposed to be identical to the original chromosome were estimated and the correlation coefficient between homozygote and heterozygote viabilities was calculated for each generation. The results showed significantly negative values.

From these experimental results, it can be said that newly arising

mutant polygenes clearly show overdominance in homozygous genetic background when the chromosomes carrying them are combined with the original normal chromosome, but are heterozygously deleterious in trans-phase heterozygotes. These phenomena are significant in clarifying the mechanism by which natural populations carry genetic variation.

11. The viabilities of heterozygotes for newly arising spontaneous lethal genes in Drosophila melanogaster

Isao YOSHIKAWA and Terumi MUKAI

In the experiment described above (This Report, **10**), forty-one second chromosomes carrying newly arising spontaneous recessive lethal genes were accumulated by Generation 60. These chromosomes were derived from a single normal second chromosome. Heterozygous effects of these lethal genes on viability were tested in three genetic backgrounds (almost homozygous, intra-population hybrid and inter-population hybrid). The control experiment was conducted by employing 41 chromosomes which were randomly picked up from lethal-free chromosomes. These chromosomes are of the same origin as the lethal-carrying chromosomes and had been maintained simultaneously in the same way. Therefore, the genetic backgrounds of lethal heterozygotes and their controls were, on the average, the same.

The following findings were obtained by statistical analysis: (1) All these lethal genes were detrimental in heterozygous condition, *i.e.*, any lethal gene did not show overdominance. (2) Interaction between lethal genes and genetic backgrounds could not be observed, namely, that lethal genes that showed more deleterious effect in one genetic background were more detrimental in any other genetic background. (3) Overall average degree of dominance ($\bar{h}$) was 0.0143.

12. Average degree of dominance of spontaneous mutant polygenes controlling viability

Terumi MUKAI and Tsuneyuki YAMAZAKI

After the sums of the viabilities of homozygotes corresponding to random heterozygotes (This Report, **10**) were divided into four groups in Generations 32 and 52 according to the order of their magnitudes, the relationship between the averages of the sums of homozygote viabilities and those of corresponding heterozygote viabilities were examined, taking all the data together into consideration. A clear linear relationship was

observed between them. Thus, the average degree of mutant polygenes ($\bar{h}$) in the classical model, in which the viabilities of AA, Aa and aa are assumed to be 1, $1-hs$ and $1-s$ (A = wild-type gene, a = mutant gene and s = selection coefficient against the mutant homozygote), was estimated. In the present analysis, only chromosome lines which had new mutant polygenes and their heterozygotes were employed.

The average degree of dominance ($\bar{h}$) was estimated by the following two formulae, under the assumption of no correlation between selection coefficient against mutant homozygotes and the h in the range of polygenes:

$$\bar{h} = \frac{\widehat{\mathrm{cov}}(v_i+v_j \text{ and } v_{ij})}{2\hat{\sigma}_G^2} \quad \cdots\cdots(1)$$

and

$$\bar{h} = \frac{v_0-\bar{v}'}{2(v_0-\bar{v})} \quad \cdots\cdots(2)$$

where v_0 = viability index of the original homozygotes (ca. 33.3); $\bar{v}$ = average viability of homozygote lines; $\bar{v}'$ = average viability of random heterozygotes; σ_G^2 = genotypic variance among homozygote lines; $\mathrm{cov}(v_i+v_j \text{ and } v_{ij})$ = covariance between the sum of the homozygote viabilities of Line i and Line j and viability of their hybrid.

The estimated $\bar{h}$ value is 0.403 ± 0.017, which is remarkably large in comparison with that of lethal genes ($\bar{h}=0.04$, STERN *et al.*, 1952).

13. *Homozygous loads and genetic structure of natural populations of* Drosophila melanogaster

Terumi MUKAI

According to the results in Generation 60 reported in a preceding article (This Report, **10**), the viabilities of heterozygotes with the original chromosome increased with the number of mutant polygenes, but reached a plateau when about eleven mutant polygenes, on the average, have been accumulated. This is one restriction to the manifestation of overdominance in natural populations. In addition, these mutant polygenes cannot be overdominant in *trans-phase*.

GREENBERG and CROW (1960) have developed a method for the test of the existence of overdominance in natural populations, using homozygous loads. On the basis of our findings, homozygous loads were calculated assuming no overdominance. Using the relationship derived by WRIGHT (1931) for an equilibrium random mating population, $q \approx \frac{\mu}{hs}$, where μ

is the mutation rate per locus; h stands for the degree of dominance; s for selective disadvantage of mutant homozygote; and q for the frequency of the mutant gene, the homozygous loads were calculated to be $\sum \frac{\mu}{hs} s = \left(\frac{\bar{1}}{h}\right) \sum \mu$. In this derivation, it was assumed that $\frac{1}{h}$ and μ are uncorrelated.

The magnitude of homozygous load due to lethal genes in the second chromosomes (L) was approximately $\frac{0.0063}{0.0143} = 0.4406$ where $\left(\frac{\bar{1}}{h}\right) = \frac{1}{0.0143}$ was employed as an approximate value, because we could not estimate $\left(\frac{\bar{1}}{h}\right)$. $\bar{h}(=0.0143)$ and $\mu(=0.0063)$ were estimated in our experiment. The load due to detrimental genes (D) was approximately $\frac{0.1411}{0.403} = 0.3501$. $\mu(=0.1411)$ and $\bar{h}(=0.403)$ were also estimated by us (MUKAI, 1964; This Report, **10**). Therefore, $D:L = 0.79$. The estimated $D:L$ values from random mating populations ranged from 0.502 to 1.012 (GREENBERG and CROW, 1960).

On the other hand, $D:L$ ratio was calculated for newly arising mutations, by using the experimental result in Generation 32. The results are: $D:L = 0.98$ ($L = 0.1843$ and $D = 0.1797$ in Generation 32). Thus, the $D:L$ ratio of equilibrium populations to that of new mutants becomes 0.806. This ratio is very close to 1 which can be expected from hs = constant (GREENBERG and CROW, 1960). Actually, our data almost satisfy this relationship.

In conclusion, it might be said that the manifestation of overdominance in natural populations is rare. Accordingly, the classical hypothesis might be generally accepted.

14. *Synergistic interaction between spontaneous mutant polygenes controlling viability in* Drosophila melanogaster

Terumi MUAKI

As reported in a preceding article (This Report, **10**), homozygous viabilities of the chromosome lines derived from a single second chromosome were estimated in Generations 10, 15, 20, 25, 32, 52 and 60. Excluding lethal and semi-lethal lines, seventy-two lines were examined out of 104 lines. However, since the result in Generation 15 was abnormal, it was not employed in the present analysis. The probable reason for this abnormal phenomenon was described in my report (MUKAI, 1964). The average viability in each generation is given in Table 1.

Table 1. Relationship between average homozygote viabilities and generation number.

Generation	10	20	25	32	52	60
Average homozygote viability	31.57	30.85	28.53	28.14	21.32	16.42

The viability of the original homozygotes was ca. 33.0 (per cent of wild-type flies by Wallace's *Cy* method (1956)).

From Table 1, it can be said that a linearity does not exist between generation number and average viability, and especially, the decrement of viability per generation became large with increasing generation number after Generation 32. This result implies that there is a synergistic interaction among mutant polygenes. A quadratic equation was fitted to the experimental data by the least-squares method and the following equation was obtained:

$$\hat{y}=-0.004691x^2+0.03547x+31.57 \quad \cdots\cdots\cdots\cdots\cdots\cdots (1)$$

where x and y stand for generation number and the average viability, respectively. This formula shows a good accord with the experimental data.

From this formula, it can be expected that the average viability becomes zero in Generation 85.9 where the average number of polygenic mutations per second chromosome is expected to be: $0.1411\times 85.9\fallingdotseq 12.12$.

15. Heterosis and Hardy-Weinberg equilibrium

Terumi MUKAI

Fitness of *Drosophila* may be separated into several components. In this short report, the author will discuss the characteristic of equilibrium populations where a mutant gene has been maintained by the superiority of heterozygotes over either homozygote with respect to viability, female fecundity or male mating ability.

In order to set up mathematical models, the following parameters were defined: $f(m, v)$ = selection coefficient in female fecundity (male mating ability, zygotic viability) against recessive homozygotes (*aa*), that of wild-type homozygotes (*AA*) being 0. We assume that these parameters are positive and less than 1. h = degree of dominance of *a* gene over *A* gene in female fecundity, male mating ability or zygotic viability. Thus, for example, female fecundities of *AA*, *Aa* and *aa* individuals are 1, $1-hf$ and $1-f$, respectively. x = frequency of heterozygotes in an adult population; p = frequency of a wild-type

gene (A); and q = frequency of the mutant gene (a) allelic to A gene, and $p+q=1$.

Zygotic viability: When the fitnesses of wild-type and heterozygotic flies are different only in zygotic viability, the necessary and sufficient condition for the maintenance of the recessive gene in stable state is obtained to be $h<0$ by the ordinary procedure. The equilibrium frequency of the recessive gene becomes $\frac{h}{2h-1}$, and the Hardy-Weinberg equilibrium can not be formed in adult populations.

Female fecundity: The zygotic array in an arbitrary population can be represented as that in Table 1. In an equilibrium population, the

Table 1. Zygotic array and female fecundity of each genotype in an arbitrary population.

Genotype	AA	Aa	aa
Frequency	$p-\frac{x}{2}$	x	$q-\frac{x}{2}$
Female fecundity	1	$1-hf$	$1-f$
Male mating ability	1	1	1

magnitudes of change in gene frequency and heterozygote frequency should be zero. From these conditions, the following simultaneous equations (1) can be obtained:

$$\left.\begin{aligned} x\{p(1-h)+h(1-p)\}-2p(1-p)&=0 \\ \left\{2f\left(h-\frac{1}{2}\right)\right\}x^2-\{f(h-2+p)+2\}x+2p(1-p)(2-f)&=0 \end{aligned}\right\} \cdots\cdots\cdots(1)$$

Under the condition of $0<p<1$, $\hat{x}$ and $\hat{p}$ can be obtained as follows:

$$\left.\begin{aligned} \hat{p}&=\frac{h-1}{2h-1} \qquad \left(\hat{q}=\frac{h}{2h-1}\right) \\ \hat{x}&=\frac{2(h-1)h}{(2h-1)^2} \end{aligned}\right\} \cdots\cdots\cdots\cdots\cdots(2)$$

The necessary and sufficient condition for these $\hat{p}$ and $\hat{x}$ being stable is $h<0$.

Exactly the same relationship as the above can be obtained when only male mating abilities are different among the three genotypes.

The following important finding should be pointed out: When only female fecundity or only male mating ability is concerned with the maintenance of mutant genes in populations, the Hardy-Weinberg equilibrium can be formed, in contrast with the case of zygotic viability.

16. A condition for pseudo-overdominance

Terumi MUKAI

It has been said that single mutant genes can be maintained in populations with the help of adaptive genes linked with them, even though they are detrimental to their carriers both in homozygous and heterozygous conditions. In this article, the condition for such pseudo-overdominance is derived.

Following parameters are defined for setting up a mathematical model: A (or a) = wild-type (or mutant) gene in Locus 1; p = gene frequency of A; $1-p$ = gene frequency of a; B (or b) = wild-type (or mutant) gene in Locus 2, and Loci 1 and 2 are localized in the same chromosome. q = gene frequency of B; $1-q$ = gene frequency of b; D = magnitude of linkage disequilibrium (LEWONTIN and KOJIMA, 1960), $PS-QR$ in Table 1.

Table 1. Frequencies of gametes and zygotes and adaptive values of zygotes in an arbitrary generation.

Female gamete \ Male gamete	$P(AB)$	$Q(Ab)$	$R(aB)$	$S(ab)$
$P(AB)$	P^2 $1\left(\frac{AB}{AB}\right)$	PQ $1-hs\left(\frac{Ab}{AB}\right)$	PR $1-hs\left(\frac{aB}{AB}\right)$	PS $1-2hs\left(\frac{ab}{AB}\right)$
$Q(Ab)$	QP $1-hs\left(\frac{AB}{Ab}\right)$	Q^2 $1-s\left(\frac{Ab}{Ab}\right)$	QR $1-2hs\left(\frac{aB}{Ab}\right)$	QS $1-hs-s\left(\frac{ab}{Ab}\right)$
$R(aB)$	RP $1-hs\left(\frac{AB}{aB}\right)$	RQ $1-2hs\left(\frac{Ab}{aB}\right)$	R^2 $1-s\left(\frac{aB}{aB}\right)$	RS $1-hs-s\left(\frac{ab}{aB}\right)$
$S(ab)$	SP $1-2hs\left(\frac{AB}{ab}\right)$	SQ $1-hs-s\left(\frac{Ab}{ab}\right)$	SR $1-hs-s\left(\frac{aB}{ab}\right)$	S^2 $1-2s\left(\frac{ab}{ab}\right)$

With these parameters, the frequencies of four kinds of gametes can be expressed as follows:

$$\begin{aligned} AB&: \quad pq+D=P \\ Ab&: \quad p(1-q)-D=Q \\ aB&: \quad (1-p)q-D=R \\ ab&: \quad (1-p)(1-q)+D=S \end{aligned}$$

The adaptive value of each genotype is given in Table 1, where h denotes the degree of dominance of a or b gene over A or B gene, respectively, and s indicates the selection coefficient of aa or bb homozygotes in comparison with AA or BB homozygotes (h and s are positive).

If we calculate the average adaptive values of AA homozygotes ($\bar{W}_{AA}$) and Aa heterozygotes ($\bar{W}_{Aa}$) and the difference of these values, the

following formula can be obtained:

$$\bar{W}_{Aa}-\bar{W}_{AA}=\{D^2-p(1-q)D\}s-\{2D^2+p(2q-1)D+p^2(1-p)\}hs\cdots\cdots(1)$$

If this value becomes positive, it implies that, on the average, *Aa* heterozygotes show adaptive superiority to the homozygotes (pseudo-overdominance) even if *a* gene itself does not show overdominance.

The necessary and sufficient conditions for the above pseudo-overdominance can be obtained on the assumption that h is very small in comparison with 1. The results are:

1) When $p+q>1$,

$$\frac{1}{2}p(1-q)\left\{1-\sqrt{1+\frac{4h(1-p)}{(1-q)^2}}\right\}<D \quad\cdots\cdots(2\text{-}1)$$

$$(1-q)^2>hp^2$$

2) When $p+q<1$,

$$\frac{1}{2}p(1-q)\left\{1-\sqrt{1+\frac{4h(1-p)}{(1-q)^2}}\right\}>D \quad\cdots\cdots(2\text{-}2)$$

$$q>h(1-p)$$

Accordingly, it can be concluded that some magnitude of linkage disequilibrium could cause pseudo-overdominance of a certain gene in a population and that, if this D is not changed permanently by epistatic effect of these genes, heterozygote superiority and the mutant gene *a* will be maintained permanently in the population.

17. Examples of permanent linkage disequilibrium

Terumi MUKAI and Tsuneyuki YAMAZAKI

CHIGUSA and MUKAI (1964) have established eight random mating populations using *sepia*-carrying and wild-type third chromosomes of *Drosophila melanogaster*. The former were originated from ten chromosomes and the latter from a single chromosome. Initial gene frequencies of *sepia* were 0.5 in all populations. Thus, the expectation of *sepia* homozygote frequencies was 25 per cent. The frequencies of *sepia* homozygotes decreased to less than three per cent in six of the eight populations by Generation 55. However, after the frequencies of *sepia* homozygotes decreased to about seven per cent, they began to increase in Populations 6 and 7, and reached equilibria approximately in Generation 50, where their frequencies were 15~20 per cent. Since then, the *sepia* genes have been maintained at high frequencies. The results are shown in Table 1.

These results can be best explained by the appearance of permanent

linkage disequilibrium owing to the development of some gene complex around the *sepia* genes.

Table 1. Frequencies of *sepia* homozygotes in experimental populations of *D. melanogaster*.

Population	Generation						
	(Average) 51~55	67	81	82	83	84	(Average) 81~84
#6	0.15	0.20	0.24	0.24	0.23	0.22	0.23
#7	0.21	0.26	0.13	0.15	0.14	0.13	0.14

18. *Persistence of the recessive gene* ew *in experimental populations in* Drosophila melanogaster

Tsuneyuki YAMAZAKI and Terumi MUKAI

This experiment was designed to obtain some information as to whether or not the third chromosome recessive mutant gene *ew* (*expanded wing*) shows overdominance. Two populations, A-1 and A-2, were set up in 1961 with the initial frequencies of *ew* 50 and 10 per cent, respectively. Each population consisting of 8 sub-populations has been maintained by so-called Pearl's method. The frequency of *ew* gene in each sub-population was examined in several generations after Generations 37 and 32 in Populations A-1 and A-2, respectively.

The results are given in Table 1. Although each population had 8 sub-populations, the gene frequencies of the highest (Sub-pop. 3 in A-1 and Sub-pop. 8 in A-2) and the lowest (Sub-pop. 6 in A-1 and Sub-pop. 1 in A-2) of all sub-populations between the 60th and the 70th generation, and the average in these 8 sub-populations are summarized in Table 1 for simple presentation. From Table 1, it can easily be seen that gene frequencies in both populations decreased not only on the average, but also in the frequencies of the highest and the lowest sub-population with increasing number of generations. All sub-populations had gene frequencies of more than 10 per cent in both populations (A-1 and A-2) until Generation 50. Between the 60th and the 70th generation, the highest gene frequencies were still more than 10 per cent in the respective populations, but the lowest ones were less than 4 per cent; namely, in early generations the differences in gene frequencies were not observed, but between the 60th and the 70th generation they became very clear. This phenomenon indicated that the *ew* heterozygotes showed adaptive

Table 1. Gene frequencies of the recessive gene *ew* in experimental populations.

Initial gene frequency		Generation				
			Gen. 32~46 (38.2)	Gen. 37~50 (42.4)	Gen. 62~66 (64)	Gen. 66~70 (68)
A-1	0.5	Average	—	0.1485	—	0.1099
		Sub-pop. 3 (the highest)	—	0.1940	—	0.1917
		Sub-pop. 6 (the lowest)	—	0.1167	—	0.0019
A-2	0.1	Average	0.2546	—	0.1347	—
		Sub-pop. 8 (the highest)	0.2892	—	0.2129	—
		Sub-pop. 1 (the lowest)	0.2062	—	0.0383	—

The figures in parentheses stand for the average generation number.

superiority due to linked genes in early generations, but later recombination occurred between them and the superiority of heterozygotes disappeared in some sub-populations (Sub-pop. 6 in A-1 and Sub-pop. 1 in A-2). On the other hand, in other sub-populations (Sub-pop. 3 in A-1 and Sub-pop. 8 in A-2) it has not disappeared because linkage has still remained between the 60th and the 70th generation and/or a new adaptive gene complex was developed around *ew* gene and this complex has been maintained by epistatic interaction. Therefore, it seems that *ew* gene in these populations has not been maintained on account of overdominance.

19. *Partition of fitness into components concerning the recessive mutant gene* ew *in two experimental populations of* Drosophila melanogaster

Tsuneyuki YAMAZAKI and Terumi MUKAI

A recessive mutant gene (*ew*) has been maintained without elimination in experimental populations almost for four years as described in a previous report (This Report, 18). However, great differences in *ew* gene frequencies have developed among the sub-populations. This work was designed to find out the reason for those differences. Several *ew*-carrying and wild-type chromosomes were extracted from each of the sub-populations showing the highest (Sub-pop. 8) and the lowest (Sub-pop. 1) gene frequency in Population A-2. Three components of fitness (viability, female fecundity and male mating ability) of their homo- and

Table 1. Relative values of viability, female fecundity, and male mating ability in Population A-2. The value of each fitness component for wild-type homozygotes (+/+) is taken to be unity. In male mating ability both genotypes, +/+ and +/*ew*, are assumed to be unity.

Sub-population	Genotype					
	+/*ew*			*ew*/*ew*		
	Viability	Female fecundity	Male mating ability	Viability	Female fecundity	Male mating ability
#1	1.0304	0.9556	1	0.9706	0.8170	0.6116
#8	1.0464	1.0866	1	0.9877	0.9462	1.0273

hetero-zygotes were examined using the above chromosomes.

The results of this experiment are summarized in Table 1. From this table, the heterozygotes (+/*ew*) in both sub-populations proved to be more viable than the corresponding homozygotes (+/+, *ew*/*ew*). On the other hand, an extreme difference was found between Sub-pop. 1 and Sub-pop. 8 concerning female fecundity and male mating ability; in Sub-pop. 8, female heterozygotes were more fecund than the corresponding homozygotes (+/*ew*>+/+>*ew*/*ew*), while in Sub-pop. 1, one of the homozygotes (+/+) was more fecund than the heterozygotes (+/+>+/*ew*>*ew*/*ew*). As to male mating ability, *ew*/*ew* homozygotes in Sub-pop. 8 were stronger than the other genotypes (*ew*/*ew*>+/+, +/*ew*), but the opposite result was obtained in Sub-pop. 1. Homozygous individuals with respect to *ew* gene in Sub-pop. 1 were weaker than the other genotypes (*ew*/*ew*<+/+, +/*ew*). From these results, the difference in gene frequencies between Sub-pop. 1 and Sub-pop. 8 seems to be mainly due to differences in male mating ability and female fecundity, but not in viability. As *ew* genes in these sub-populations were derived from a single mutant gene, it might be said that the differences in the magnitudes of fitness components between these two sub-populations are caused by the effects of the genetic background and/or interaction between the gene in question and its genetic background.

20. Disappearance of "Sex-Ratio" condition by double infection with two "Sex-Ratio" agents of different origin in Drosophila[1)]

Kugao OISHI and Bungo SAKAGUCHI

So-called maternally inherited "Sex-Ratio" (SR) condition is characterized by an extreme departure from the normal 1:1 sex ratio to give all or nearly all female offspring. The condition in *D. willistoni* and *D. nebulosa* is caused by the presence of infectious *Treponema*-like spirochetes (SR spirochetes) in the female bodies of the species hosting them (POULSON and SAKAGUCHI, 1961). Furthermore, the SR condition is interrupted when the two kinds of SR spirochetes, *i.e.*, from *D. willistoni* and from *D. nebulosa*, are combined in the same host (SAKAGUCHI, OISHI and KOBAYASHI, 1964). Such an experiment was carried out and it was tested whether the SR condition persists into subsequent generations. For this purpose a line of the Oregon-R strain of *D. melanogaster* was established by artificial transfer of *willistoni* spirochetes. This line will be referred to as ORSRB-3. The females of this line were superinfected, so as to bring together the *willistoni*, SRB-3, and the *nebulosa*, NebSR, spirochetes and were mated singly with males of normal Oregon-R strain.

In detail, 14 females of the ORSRB-3 were superinfected with *nebulosa* spirochetes and the sex ratio in successive broods was examined. Table 1 shows a striking effect that the proportion of males in the first generation was gradually increasing (after 1～3 days from the time of injection) and reached to about 50 per cent in the third to fifth broods, but it decreased again gradually from the sixth to the following broods. Furthermore, SR condition disappeared in the flies of the next generation except in the progenies of the first and the second broods. The SR condition was never restored in the subsequent generations.

In the control superinfected by the same SRB-3 spirochete (to which it was the host), or left without superinfection, no males were produced except in the first brood. The SR condition in this case was very stably transmitted into progenies as shown in Table 1.

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

Table 1. SR condition in the progenies of ORSRB-3+ females injected with the *nebulosa* and *willistoni* spirochetes.

Direction of injection			Brood, days after injection								
			0~3	4~6	7~9	10~12	13~15	16~18	19~21	22~24	25~27
			♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂
Host Donor	ORSRB-3+ NebSR*	A:	101:8	138:50	106:125	152:148	108:104	70:53	67:52	42:14	61:22
		B:	37	49	29	55	57	33	—	—	29
		C:	12	15	0	0	0	0	—	—	0
Host Donor	ORSRB-3 SRB-3**	A:	83:13	132:0	108:0	134:0	118:0	90:0	64:1	36:0	21:0
		B:	46	33	37	28	33	28	—	—	9
		C:	21	24	37	28	30	27	—	—	9
Host Donor	ORSRB-3 —	A:	81:0	53:0	31:0	39:0	39:0	26:0	15:0	9:0	20:0
		B:	25	24	15	24	15	17	—	—	17
		C:	11	23	15	24	15	17	—	—	17

A: Sex-ratio of the 1st generation flies.
B: No. of the 1st generation flies tested.
C: No. of SR.
\+ SR strain of *D. melanogaster*, Oregon-R, derived by transfer of SR from *D. willistoni* SRB-3 strain.
* SR strain of *D. nebulosa*.
** SR strain of *D. willistoni*.

21. *Nature of the substance produced by* nebulosa-*SR agent that kills* willistoni-*SR agent in* Drosophila[1)]

Bungo SAKAGUCHI and Kugao OISHI

So-called maternally inherited "Sex-Ratio" (SR) condition in *Drosophila willistoni* and *D. nebulosa* are caused by the presence of a *Treponema*-like spirochetes in the female bodies of the species hosting them (POULSON and SAKAGUCHI, 1961). When the two kinds of SR agents (SR spirochetes), *i.e.*, from *D. willistoni* and from *D. nebulosa*, were combined in females of Oregon-R (OR) strain of *D. melanogaster*, the expression of SR condition was temporarily interrupted. The phenomenon of interruption is the consequence of interaction between the two kinds of SR spirochetes, and finally SR spirochetes of *nebulosa* replace those of *willstoni* in the host's females. Furthermore, SR spirochetes of *willistoni* are always eliminated in the host in which the two kinds are combined (SAKAGUCHI, OISHI and KOBAYASHI, 1964).

In an attempt to gain a better understanding of the mechanism of the interaction between the two SR spirochetes, about one gram of OR females hosting *nebulosa* spirochetes was homogenized in 3 ml of 0.25 *M* sucrose or Drosophila Ringer solution and the homogenate was centrifuged at 5,000 rev/min for 15 minutes. The supernatant was again centrifuged at 30,000 rev/min (80,000 *g*) for one hour. A part of the supernatant which was almost free of spirochetes was injected into the test flies of OR females hosting *willistoni* spirochetes and the proportion of males and females in the successive broods from the injected flies was determined (control in Fig. 1). Another part of the supernatant was divided into three parts and each was then subjected to the following procedures. (1) The supernatant was treated by heating for 10 minutes at 60°C. After the treatment it was quickly chilled and centrifuged at 10,000 rev/min for 15 minutes. The supernatant was injected into the test flies and the sex ratios in the progenies of the injected flies were examined. (2) Another part of the supernatant was digested overnight with 0.3 percent trypsin at 37°C. The trypsin was then removed by saturation at 40 percent of ammonium sulfate. The digested fluid was also used for injection. (3) A third part was dialysed against distilled water with collodion membrane and the dialyzate solution was then used for injection. These results are shown in Fig. 1.

The proportions of females in the progeny of test flies injected with two fractions of nontreated supernatant (control) and those that were

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

heat treated were more than 90 percent in the first brood (1 to 3 days after injection), but suddenly decreased to about 50 percent in the third (6 to 9 days) and subsequent broods: SR condition was never restored. The hemolymph of adults was examined by phase-contrast microscopy.

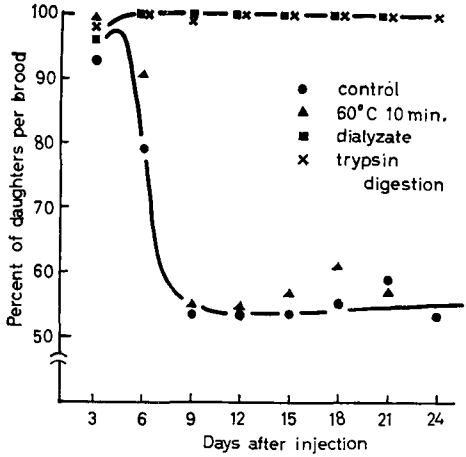

Fig. 1. Effects of various treatments of the substance produced by *nebulosa* spirochetes on *willistoni* SR condition.

SR spirochetes were observed in the first but not in the third and following broods. While no males were produced after injection of trypsin digested and dialyzate fluids. But, when nondialyzable solution was injected into test flies, the proportion of females was decreased in successive broods as in the control, though the result is not presented here.

These results indicate that the supernatant from homogenate of the SR females hosting *nebulosa* spirochetes contains some substance which is produced by *nebulosa* spirochetes and the substance may kill or incapacitate *willistoni* spirochetes. Furthermore, it is suggested that the nature of the substance is some kind of protein which might be called Spirocin.

An experiment to purify spirocin by means of column chromatography and electrophoresis is now under way.

22. *Multiplication of SR agents in* Drosophila[1]

Susumu KOBAYASHI and Bungo SAKAGUCHI

The SR agents in *D. willistoni* and *D. nebulosa* are *Treponema*-like spirochetes, about 4～16 microns in length and 0.1 microns in width (POULSON and SAKAGUCHI, 1961). A striking morphological feature revealed by electron microscope are vesicles which may occur at any point along the filament; they may form at least one to four granules (buds) which may develop into young spirochetes (A in Fig. 1). Another feature is branching of filaments at any point (A in Fig. 1). Free vesicles, about 0.3 microns in diameter, are also present in the hemolymph of host flies. They also form minute granules which may develop into young spirochetes (B, C and D in Fig. 1).

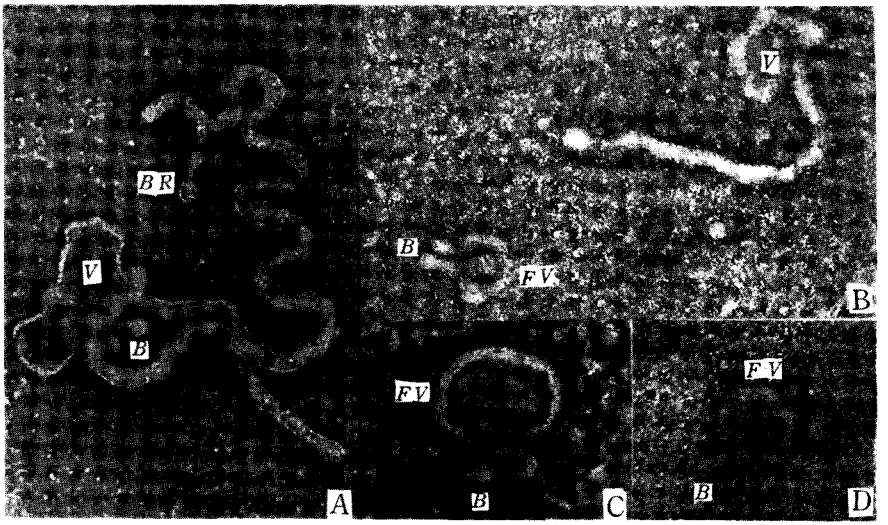

Fig. 1. Electron micrographs of SR spirochetes of *Drosophila nebulosa*. Negative contrast technique.

A. Mature SR spirochete. Large vesicle which occurs along the filament and two minute granucles (buds) which may have developed from the vesicle can be seen. A branch of filament can also be observed. ×24,000.

B, C and D. Free vesicle which may represent a stage in the life cycle of SR spirochetes. Note two granucles (buds) or a long tail attached to the vesicle. ×27,000.

B: bud; *BR*: branch; *FV*: free vesicle; *V*: vesicle.

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

These facts indicate the possibilities that the SR spirochetes may multiply by forming free vesicles which may represent different stages in the life cycle, by budding, or by separation of filament branches.

Experiments to determine the multiplication unit by a breakdown of the agent with sonic treatment and separation into elements of various size by centrifugation are now in progress. An autoradiographical attempt will be also made after the incorporation of isotopes into the SR agents. It is hoped that these studies will give the answer to the method of multiplication.

23. Cellular studies of antibody formation, IV. Variation in the kinetic indices of plasma cells in antibody response

Toshihiko SADO, Takashi MAKINODAN,[1] Martha R. LEONARD[1] and Carol J. CHADWICK[1]

Two of the major problems in the field of cytodynamics of antibody formation are (a) the fate of terminal plasma cells, and (b) the inter-compartmental transit time (ITT), or the time it takes for a cell to transform from one metabolically unique state into another. The present study was carried out in an attempt to resolve these problems by using H^3-thymidine as a cell marker.

A fixed number of primed spleen cells (2.4×10^7) were cultured in 0.1 μ porosity diffusion chambers together with an optimum dose of antigen ($\sim 50\,\mu g$ of bovine serum albumin). Autoradiographic and immuno-fluorescent methods were employed in detecting antibody-forming plasma cells. The results showed that mature antibody-forming cells in the stationary phase are derived from proliferating immature plasma cells in the late latent and log phases of response and that 70 % of all plasma cells at this time are immunofluorescent positive, antibody containing cells. This indicates that with use of this culture method it is possible to assess the kinetics of antibody-forming cells by direct plasma cell analysis. The evidence indicated that the life cycle of immature plasma cells was changing during the log-stationary phase of response, possibly due to a change in the G_1 phase of immature plasma cells, and that the ITT between immature and mature plasma cells was varying from 3 to 12 hrs. Finally, most of the mature plasma cells was shown to be dying exponentially with a $T_{\frac{1}{2}}$ of approximately 32 hrs. The number of degenerating plasma cells increased abruptly during the late log phase and remained relatively constant during the stationary phase.

These results indicate that because the ITT can vary from 3 to 12 hrs

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there must exist many "transitional" plasma cells that possess properties between a typical immature and mature plasma cells, and such cells have been observed by morphologists. The data also show that at any given time a newly formed mature plasma cell has an equal probability of dying as one that was formed much earlier. It follows then that senescence in the classical sense cannot be the primary cause for death of mature plasma cells. It is of interest to correlate these changes in kinetic indices of plasma cells with the drastic changes in cellular environment, *e.g.*, by increasing the concentration of antibody passively. Thus, in this manner we may be able to assess the role of specific antibody as a feedback regulator on the progenitor cells.

24. Chemical modification of DNA and its constituents

Tomotaka SHINODA

Attempts have been made to modify DNA and its individual constituents by using sodium trinitrobenzene sulfonate (TNBS). Under the several conditions employed, reaction with free bases and with nucleosides occurred only with guanine and its deoxyriboside, and none of the others were reactive towards the reagent. When purified calf thymus DNA was used in the reaction under a pH above 9 and the reaction was followed spectrophotometrically, the curve shown in Fig. 1 was obtained. The absorption maximum of modified DNA appearing in Fig. 1 disappeared in a medium whose pH was below 3. Trinitrophenylated guanine was obtained from an acid hydrolysate of modified DNA followed by paper chromatography by which it could be easily detected as a distinct yellow spot. The molar extinction coefficient of TNP-guanine was calculated to be 1.45×10^4 (λ_{max} 430 mμ at pH 10). This substance was rather stable in acid medium but it appeared to be unstable under alkaline condition. When the material was treated with *N* KOH at 100°C, most of it decomposed into picric acid and other brownish degradation products. A recovery of free guanine from the hydrolysate was somewhat poor, which might suggest that a considerable disintegration of the base ring was caused by the treatment. In contrast with the result obtained in the intact material, the modified substance was not affected by nitrous acid treatment. When the material was treated by usual alkylating agents such as dimethyl sulfate and ethylmethane sulfonate, similar results were obtained suggesting alkylation of guanine at position 7. This finding strongly suggests that a trinitrophenyl residue was located in the amino group of guanine at position 2.

Two interesting problems concerned with the investigation of the

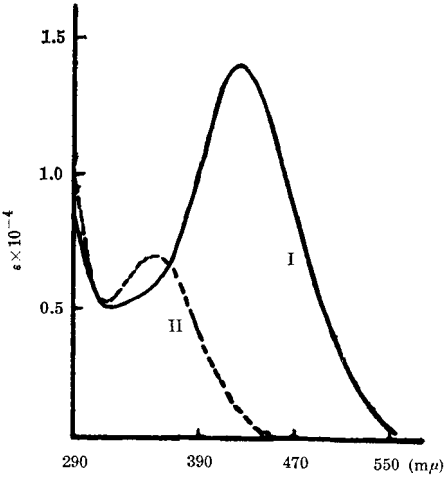


Fig. 1. Absorption spectra of TNP-DNA.
Curve I, at pH 10; Curve II, at pH 3.

chemical structure of DNA and of mutagenic effect induced by visible light other than UV have arisen from the utilization of the employed technique. One promising related problem is that substrate specificity of deoxyribonuclease was found to be limited by modification of DNA with TNBS. The other advantage is that in the analytical procedures of enzymatic digest of DNA oligonucleotides with TNP-guanine can be directly detectable by their intense yellow color, which no doubt is very convenient in the following analytical procedures.

25. *Isolation and characterization of the deoxyribonucleic acid from* Ephestia

Saburo NAWA

As previously reported (NAWA and CASPARI, Annual Report No. 14, 1963), a method to demonstrate transformation in *Ephestia* was developed. In order to achieve transformation, it was necessary to prepare pure, high molecular DNA from *Ephestia*. While methods for microorganisms and mammalian tissues are available and fairly routine, their application to insect has been accomplished only rarely. Whole animals were used for extraction of DNA, since it was difficult to separate a specific tissue of *Ephestia* in great quantities. Care was taken to avoid the degradation of DNA when homogenized. Larvae were gently homogenized with saline-citrate solution in a glass homogenizer by hand and then centrifuged.

DNA was extracted from the pellet with sodium dodecylsulfate according to the method of MARMUR. Protein denaturation was carried out by shaking with chloroform repeatedly. Ribonuclease was used to depolymerize RNA. The resulting preparaton was viscous high molecular DNA contaminated with not more than 2 % protein and 1 % RNA by weight. The slightly low phosphorus content (7 %) seems to be due to the presence of polysaccharides (probably mainly glycogen, since only glucose was found by hydrolysis with HCl and separation by paper chromatography). The molecular extinction coefficient at 260 mμ with respect to phosphorus was 6,800. The preparation showed a 30 % absorption increment at 260 mμ when treated with deoxyribonuclease or alkali. DNA was prepared by the same procedure from pupae and adults. It was, however, difficult to remove a trace of pigments (ommochromes) from adult DNA preparation. Phenol method was also available to get fibrous DNA's. Base compositions were determined by formic acid decomposition of DNA, following paper chromatography. There seems to be no difference of the base components among larval, pupal and adult DNA: Larval DNA; G, 16.4, A, 33.3, C, 18.2, T, 32.1. Pupal DNA; G, 17.4, A, 33.3, C, 18.2, T, 31.3. Adult DNA; G, 17.1, A, 32.8, G, 17.5, T, 32.5.

26. A new method for the study of chemical mutagenesis in the silkworm

Yataro TAZIMA and Kimiharu ONIMARU

With a purpose of exploiting a new technique for the study of chemical mutagenesis, a simple method of treating eggs of the silkworm has been studied. The egg shell of this insect is very porous having many spiracles on the surface. It is, however, uncertain whether or not a given chemical can pass through the spiracles and penetrate into the egg plasm. If the penetration of such chemical is confirmed, the experimenter could perform the experiment with comparative ease in the study of chemical mutagenesis.

In this regard a series of experiments have been carried out with some chemicals having lower molecular weight. The experiment to be reported here concerns mostly KNO_2, a known agent that reacts to purine and pyrimidine bases of DNA. Newly laid eggs were immersed in an aqueous solution of KNO_2 at 30°C. The effectiveness of the treatment was determined by the killing effect and/or mutagenicity. The eggs were approximately at the age of 30 minutes when the treatment was started. By this time egg- and sperm-nuclei are not fused yet. They are presumed to undergo syngamy around two hours after deposition. There would be

expected, therefore, an incidence of mosaic patches if the penetrating chemical causes mutation. The experimental conditions are given in detail in Table 1 together with the results.

Table 1. Experimental conditions of KNO_2 treatment and survival.

Group	Chemical	Concentration	pH	Sugar	Age after oviposit	Duration of treatment	Number of eggs observed	Survival in %
		Mol		Mol	min	min		
Cont. 1	Dist. Water					60	4,897	98.16
2	Dist. Water				30	120	7,585	98.26
K−2	S.A. Buffer		4.2			60	9,575	98.25
3	KNO_2	0.2	4.2		30	30	16,224	1.12
4					300		12,364	6.23
5	KNO_2	0.01	4.2		30	30	13,155	1.00
3		0.2					16,224	1.12
5	KNO_2	0.01	4.2		30	30	13,155	1.00
6			5.6				15,134	97.87
OP−1				0.0			15,410	14.18
2	KNO_2	0.2	4.2	0.25	30	30	14,040	39.84
3				1.0			4,285	54.03
OP−4				0.0			5,496	0.29
5	KNO_2	0.2	4.2	0.25	30	60	11,199	3.96
6				1.0			5,920	14.39
C−1						30	9,966	83.30
2	NKO_2	0.2	4.2		30	60	10,105	14.20
3						90	8,261	0.98

S.A.: sodium acetate

It has been known for T_2 phage that KNO_2 is most effective in its killing action at pH 4.2. Therefore, a similar pH condition was prepared by regulating the pH value with sodium acetate buffer. As clearly seen from the table the killing effect of KNO_2 treatment was remarkable at pH 4.2 but almost negligible at pH 5.6. This suggests that the chemical did act upon the egg cell after passing through the shell. However, it is not certain whether this reaction occurred due to the permeation of the chemical via aqueous solution.

This question could be answered by an experiment in which the osmotic pressure of the KNO_2 solution were regulated by adding cane sugar at various concentrations. The results of such experiment are given in the same table; they show that reaction to the chemical did actually occur due to its permeation.

From these results it can be inferred that any chemical, as far as its molecular weight is small, can be brought into the egg plasm by simply immersing them into an aqueous solution. Preliminary experiments, in which BU and BUdR were utilized, indicated that this method could conveniently be used in the study of chemical mutagenesis.

27. Purification of the three specific soluble chromoproteins from chromogranules in hypodermal cells of the silkworm larva

Mitsuo TSUJITA and Susumu SAKURAI

It was reported in our previous paper (TSUJITA and SAKURAI, 1963, 1964) that the chromogranules in hypodermal cells of silkworm larvae are an important factor participating in the manifestation of larval skin color or transparency. These chromogranules are composed of proteins, lipids, RNA and sugar. Two kinds of proteins exist in the chromogranules, that is, soluble protein of the inner part of the granules, and insoluble protein of granule pellicles. It was shown in our previous paper that the soluble protein combines with sepiapterin and can be separated from other proteins, such as those which combines with uric acid, or with isoxanthopterin. Therefore, we continued our experiments in order to remove some impurities from the protein that combines with sepiapterin. Moreover, experiments to separate the protein combining with uric acid from that combining with isoxanthopterin were carried out. In our report (TSUJITA and SAKURAI, 1964) these purification procedures were described.

28. Amino acid analysis of the three specific chromoproteins purified from chromogranules in hypodermal cells of the silkworm larva

Mitsuo TSUJITA and Susumu SAKURAI

It was reported in our previous paper that the three chromoproteins were separated and purified from the soluble proteins of chromogranules in hypodermal cells of silkworm larvae. In our report (TSUJITA and SAKURAI, 1964) absorption spectra of the purified chromoproteins and the experimental results of an amino acid analysis of those chromoproteins are described.

29. Amino acid analysis of the insoluble protein in chromogranule pellicles

Mitsuo TSUJITA and Susumu SAKURAI

Chromogranules collected from larval hypodermal cells of the normal strain (C124) was purified. Chromogranule pellicle samples prepared by removing their soluble proteins were analysed by Shibata's auto-amino acid analyser. Seventeen amino acids, lysine, arginine, histidine, aspartic acid, glutamic acid, threonine, serine, proline, glycine, alanine, cystine, valine, methionine, leucine, isoleucine, tyrosine and phenylalanine, were detected and quantitative analysis of each amino acid was carried out.

30. Lipid contents in chromogranules in hypodermal cells of the silkworm larva

Susumu SAKURAI and Mitsuo TSUJITA

The present experiments were undertaken in order to detect the lipid contents in the chromogranules of silkworm larval hypodermis. The chromogranules which were collected from the larval hypodermis of normal (C124 and Daizo) and mutant strains (*lem* and *d-lem*) were used as materials. Using thin layer chromatography, containing corn starch or calcium sulfate as the binder, a qualitative analysis of the lipid contents extracted from those granules by benzene-chloroform mixture or by benzene-petroleum ether-ethanol mixture was carried out.

When benzene-chloroform mixture was used as solvent, the normal strain samples, unlike the mutant strain samples, produced a supernumerary spot in addition to 5 common spots. On the contrary, when benzene-petroleum ether-ethanol mixture was used as solvent, the normal strain samples also produced a supernumerary spot in addition to 6 common spots. It is considered that 3 spots among the 6 common spots are palmitic, stealic and oleic acids. Judging from coloring reactions, the supernumerary spot observed in the chromatograph of the normal strain samples may be an unsaturated fatty acid.

The lipid composition may play an important role in the formation of the granules, especially in the binding of polypeptide chains in the granular pellicles.

31. *Multiple allelic genes for dilute lemon larval skin color of the silkworm*

Mitsuo TSUJITA and Susumu SAKURAI

Further studies on amino acid composition of the three specific proteins combining with pigments, *i.e.*, sepiapterin-protein, isoxanthopterin-protein and uric acid-protein complexes, are now under way.

For the purpose of studying the protein combining with sepiapterin several dilute lemon strains in addition to the normal lemon strain have been prepared. One of the dilute lemon strains is *d-lem* strain (TSUJITA, 1963) and the others are mutant strains artificially induced by X-rays. The method used for induction of the mutants with dilute lemon larval skin color was as follows. Female pupae of the normal lemon strain about 7 days after pupation were irradiated with X-rays at doses of 5,000~8,000 γ and were mated with *d-lem* males. Since mutants with dilute lemon larval skin color appeared in relatively high frequency among lemon larvae in the next generation, they were reared to imaginal stage and back-crossed to *d-lem* individuals. When new type dilute lemon larvae segregated in the next generation, they were reared. The characteristics of three new strains, A, B and C obtained in this manner, are as follows:

A. Mutant with remarkably dilute lemon larval skin. Although most of the larvae in 5th instar exhibit almost normal opaque skin color, a part show remarkably dilute lemon skin color. The activity of pterine reductase in this mutant individuals is weak just as in the *lem* and *d-lem* mutants. Besides, the content of isoxanthopterin in their hypodermal cells is smaller than in the normal lemon larvae.

B. These mutant larvae resemble A. In addition, the content of isoxanthopterin in larval hypodermal cells is rather smaller than in A mutant larvae, but we could not find any difference in the activity of pterine reductase.

C. These mutant larvae show dilute lemon skin color. Just like A and B the activity of pterine reductase is weak. However, the content of isoxanthopterin in larval hypodermal cells is to some extent larger than in the lemon larvae.

Hybrid larvae between 2 strains of the three dilute lemon mutants, A, B and C, show dilute lemon skin color. Moreover, hybrid larvae between *d-lem* individuals and those of one of the three dilute lemon mutants exhibit also dilute lemon skin color. Normal larvae, larvae with lemon skin color and those with dilute lemon skin color segregated in the F_2 generation of the crosses between individuals of the normal strain and those of one of the new dilute lemon mutants (Table 1).

Table 1. Segregation in the F_2 generation of the crosses between individuals of normal strain and those of one of the new dilute lemon mutants.

Genotype	No.	+	lemon	dilute lemon	Total
A × +(Daizo)	1	138	36	12	186
	2	280	78	20	378
	3	271	46	16	333
	4	240	48	16	304
	5	203	45	15	263
	Total	1,132	253	79	1,464
+(Daizo) × A	1	159	35	14	208
	2	137	39	14	190
	3	275	30	11	316
	4	229	35	11	275
	5	155	35	11	201
	Total	955	174	61	1,190
+(Daizo) × B	1	150	35	11	196
	2	152	32	10	194
	3	201	44	11	256
	4	172	32	10	214
	5	103	30	10	143
	Total	778	173	52	1,003
+(Daizo) × C	1	208	46	15	269
	2	240	56	11	307
	3	269	58	16	343
	4	197	50	14	261
	5	183	52	17	252
	Total	1,097	262	73	1,432

It was confirmed that *d-lem* belongs to the 2nd chromosome, the recombination value between *d-lem* and *Y* being 6.0 and between *d-lem* and *p* 30.2. It seems from the results of the crosses mentioned above that *d-lem* and the genes for A, B and C strains form a mutiple allelic series.

32. Variance and covariance analysis of egg weight, egg shape and body weight in the domestic fowl

Takatada KAWAHARA

Heritability of and genetic and phenotypic correlations among egg weight, egg width, egg length and body weight were investigated in a closed flock of White Leghorns. Data were collected from 479 pullets originated from 32 sires and 153 dams. Experimental records were taken for a period of approximately 11～12 months after hatching. The estimates of heritability from analysis of variance are shown in Table 1.

Table 2 presents the estimates of coefficients of genetic and phenotypic correlations between various traits.

It is found from Table 1 that the body weight and egg length are moderately heritable whereas the heritability of egg width is low.

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

Heritability	Egg weight	Egg width	Egg length	Body weight
h_s^2	0.336	0.303	0.447	0.791
h_d^2	0.324	0.177	0.455	0.526
h_{s+d}^2	0.330	0.240	0.466	0.658
c^2	−0.003	−0.031	0.005	−0.066

Table 2. Genetic and phenotypic correlations between body weight, egg weight, egg width and egg length.

Traits	Genetic correlation	Phenotypic correlation
Body weight-Egg weight	0.543	0.298**
Body weight-Egg width	0.476	0.263**
Body weight-Egg length	0.287	0.180**
Egg weight-Egg width	0.829	0.718**
Egg weight-Egg length	0.820	0.681**
Egg width-Egg length	0.309	0.271**

** Significant at the 1% level.

Notwithstanding the higher value of heritability of egg length than egg width, Table 2 shows that genetic correlation between egg length and body weight was as low as 0.287, while that between egg width and body weight was moderately high, *i.e.*, 0.476. It suggests that genes governing egg length are rather independent from those governing body weight and/or egg width.

33. Effect of heterosis and sex-linkage on sexual maturity in the domestic fowl

Takatada KAWAHARA

Three breeds, *i.e.*, White Leghorns (WL), Barred Plymouth Rocks (BPR) and Nagoyas (NG), and reciprocal F_1's and backcross hybrids between WL and the other two breeds were investigated for sexual maturity. Method of mating was such that a mixture of spermatozoa from two breeds was inseminated to the dams to produce purebreds and F_1 hybrids of the two breeds in paternal or maternal half-sib combinations. Reciprocal backcrosses were carried out between purebreds and F_1 hybrids in order to analyze sex-linked and maternal effects on sexual maturity. Several marker genes were used, namely for rapid (*k*) and slow (*K*) feathering, for dominant white (*I*) and barred (*B*) plumage, for melanin formation in the dermis (*id*) and its inhibitor (*Id*).

Results of this investigation are summarized as follows: The average number of days to attain sexual maturity was 189.05, 219.86, 192.55, for WL, BPR and NG, and 192.45, 178.34, 177.35 and 171.09 for WL ♀ × BPR ♂, BPR ♀ × WL ♂, WL ♀ × NG ♂ and NG ♀ × WL ♂, respectively. F_1 hybrids from the WL–BPR combination attained sexual maturity 19.06 days earlier than the corresponding mid-parents, and those from WL–NG combination 16.58 days earlier than the mid-parents, possibly due to heterosis. The BPR ♀ × WL ♂ F_1 hybrids showed sexual maturity 14.11 days earlier than the reciprocal cross, the difference being statistically significant. This finding was confirmed by the backcross experiment. The average number of days to attain sexual maturity was 188.36, 192.62, 189.04 and 194.78 for WL ♀ × F_1(WL–BPR, *k*) ♂, WL ♀ × F_1(WL–BPR, *K*) ♂, BPR ♀ × F_1(WL–BPR, *k*) ♂ and BPR ♀(WL–BPR, *K*) ♂, respectively. Age at sexual maturity in backcross progenies carrying WL sex-chromosome was 6.27 days earlier in WL ♀ × F_1 ♂ and in BPR ♀ × F_1 ♂ 5.74 days earlier than in those carrying BPR sex-chromosome, the differences being statistically significant.

Thus, it was concluded that the gene(s) located on the sex-chromosome, possibly the *k* allele, could be resposible for early sexual maturity.

34. Bilateral asymmetry in the ribs of cervical vertebrae in the domestic fowl

Kan-Ichi SAKAI and Takatada KAWAHARA

Bilateral asymmetry in the ribs of cervicae vertebrae in the domestic fowls was investigated. Carcasses of birds from inbred or non-inbred strains of three breeds, White Leghorns, Rhode Island Reds and Nagoyas, were treated with papain and the cervical vertebrae were carefully separated in boiling water. Measured were lengths of right and left ribs of five central of fourteen consecutive vertebrae. The degree of bilateral asymmetry was measured by the absolute difference between left and right rib of a vertebra. Analysis of variance revealed that strain differences were statistically significant. Table 1 presents mean values of bilateral asymmetry of six strains. Of interest is to find in Table 1 that

Table 1. Bilateral asymmetry of the ribs of cervical vertebrae in 5 inbred and one closed strains of three breeds of the domestic fowl.

Breed	Strain	Asymmetry (|Left side−Right side|) of cervical vertebrae rib length					
		5	6	7	8	9	Mean
White Leghorn	KO-1, inbred	0.332	0.291	0.236	0.282	0.300	0.288
White Leghorn	OW-2, inbred	0.463	0.438	0.300	0.650	0.413	0.428
White Leghorn	OW-4, inbred	0.214	0.257	0.329	0.286	0.300	0.277
White Leghorn	Closed flock	0.368	0.221	0.259	0.309	0.265	0.278
Rhode Island Red	KR-2, inbred	0.350	0.258	0.367	0.550	0.333	0.380
Nagoya	KN-1, inbred	0.391	0.182	0.364	0.355	0.318	0.323

among three inbred strains of White Leghorns, the OW-2 strain showed an unusually high asymmetry in comparison with others. These three inbred strains had approximately the same inbreeding coefficient, about 60 percent, whereas they differed with respect to the degree of inbreeding degeneration, the OW-2 strain being more vigorous and productive than the other two.

35. Bilateral asymmetry in the legs of the domestic fowl

Kan-Ichi SAKAI and Tohru FUJISHIMA

Bilateral asymmetry in the legs of chickens was investigated in 714 birds of two breeds, *i.e.*, 436 of White Leghorns and 278 of Rhode Island Reds. Lengths of shank and four toes were measured at 0, 3 and 6 weeks

of age with slide calipers, and the value, left minus right, was used as the index of bilateral asymmetry for each part.

It was found from this study that in one day old chickens the left side was generally longer than the right, except for the fourth toe, suggesting the presence of directional asymmetry (Table 1). Its degree was significantly different between breeds, suggesting presence of a genetic control.

Table 1. Bilateral asymmetries in legs of the domestic fowl.

Part	Wks of age	White Leghorn			Rhode Island Red			Breed diff. in asymmetry
		No. of birds	Left mean mm	Asymmetry σ mm	No. of birds	Left mean mm	Asymmetry σ mm	
Shank	0	436	28.78	$0.51^{**}\pm0.41$	278	28.30	$0.31^{**}\pm0.47$	P<0.01
	3	319	42.95	-0.05 ± 0.41	219	44.43	-0.01 ± 0.38	N. S.
	6	334	61.72	$-0.13^{**}\pm0.47$	220	61.63	$-0.17^{**}\pm0.45$	N. S.
1st toe	0	436	7.24	$0.47^{**}\pm0.52$	278	7.33	$0.27^{**}\pm0.49$	P<0.01
	3	319	11.57	$0.25^{**}\pm0.70$	219	12.25	$0.26^{**}\pm0.71$	N. S.
	6	334	16.35	$0.38^{**}\pm0.70$	220	17.01	$0.33^{**}\pm0.69$	N. S.
2nd toe	0	436	13.37	$0.26^{**}\pm0.44$	278	13.62	$0.42^{**}\pm0.44$	P<0.01
	3	319	19.02	$0.13^{**}\pm0.54$	219	20.17	$0.13^{*}\pm0.50$	N. S.
	6	334	26.07	$0.14^{**}\pm0.65$	220	27.02	$0.05^{**}\pm0.56$	N. S.
3rd toe	0	436	19.19	$0.14^{**}\pm0.45$	278	18.88	0.03 ± 0.50	P<0.01
	3	319	27.65	$-0.18^{**}\pm0.54$	219	28.85	$-0.31^{**}\pm0.50$	P<0.01
	6	334	37.47	$-0.29^{**}\pm0.60$	220	38.45	$-0.41^{**}\pm0.58$	P<0.01
4th toe	0	436	14.75	$-0.18^{**}\pm0.40$	278	14.94	$-0.23^{**}\pm0.42$	N. S.
	3	319	21.73	$0.17^{**}\pm0.47$	219	22.98	0.04 ± 0.59	P<0.01
	6	334	29.45	$0.34^{**}\pm0.59$	220	30.88	$0.18^{*}\pm0.51$	P<0.01

* Significant at the 5% level.
** Significant at the 1% level.
N. S. Non-significant.

The heritabilities of these asymmetries estimated were 0.36 for the shank, 0.00 for the first toe, 0.12 for the second toe, 0.09 for the third toe, and 0.19 for the fourth toe.

This directional asymmetry, however, seems to be interfered by later development. Thus, in 6 weeks old chickens, the shank and the third toe became longer in the right side, but the first, second and fourth toes were longer on the left side. Particularly, the asymmetries in the shank and the third toe changed in the negative direction, while that in the fourth toe

changed in the positive direction. Thus, the asymmetries in the third and fourth toes changed in the opposite direction with the growth of chickens.

36. Breed differences in competitive ability of growing chickens

Tohru FUJISHIMA

Experiments concerned with the competition during chick growth are very few, in spite of their importance in poultry husbandry, particularly in growing breeding birds or broilers. The present experiment was attempted to investigate differences, if any, between breeds in competitive ability of chickens, growth stage at which competition sets in and relationship between initial body weight and competitive capacity of domestic fowl.

Male and female chickens of three breeds, *i.e.*, White Leghron, Barred Plymouth Rock and Rhode Island Red, were divided into three single

Table 1. Body weights in single and mixed groups at 4, 5 and 6 weeks of age, and regression coefficients of gains in body weight (4~6 wks) on 4 wks body weight estimated within and among breeds.

Weeks of age	Breed	Male		Female	
		Single group (g)	Mixed group (g)	Single group (g)	Mixed group (g)
4	W L	191.1	191.8	177.9	176.4
	B P R	199.5	201.4	194.8	199.4
	R I R	208.9	200.8	191.6	191.1
5	W L	244.0	241.4	236.1	230.5
	B P R	245.7	256.0	239.4	250.7
	R I R	250.9	235.5	246.6	236.1
6	W L	300.8	309.2	291.8	287.3
	B P R	286.4	318.2	282.5	311.3
	R I R	311.2	293.9	298.3	294.4
Regres. coef. within breed	W L	0.398**	0.415*	0.417**	0.435*
	B P R	0.430*	0.953**	0.212	0.413
	R I R	0.463**	0.575	0.619**	0.515*
Regres. coef. among breeds		0.977	1.229	−0.260	0.605

* Significant at the 5% level.

** Significant at the 1% level.

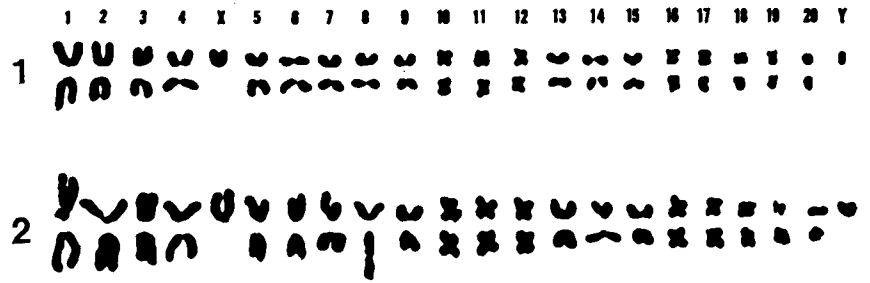
breed groups and a mixed group. Each single breed group contained 30 birds of the same sex and breed, while each mixed breed group contained 30 birds derived from all three breeds, each contributing 10 birds of the same sex. They were reared until the age of six weeks and were weighed every week. The experiment was repeated twice, and both replicates were kept under restricted feeding condition, except for drinking water.

The results of this experiment, as shown in Table 1, indicate that after the age of 5 weeks, difference in competitive ability becomes recognizable especially in males among these breeds, Barred Plymouth Rock being strongest competitor, White Leghorn intermediate and Rhode Island Red weakest. The regression coefficients estimated within and among breeds of gains in body weight on the body weight at 4 weeks of age, after which the competition set in, were not significantly different between mixed and single groups respectively, though they tended to be larger in the mixed groups. Therefore, it is concluded that body weight may be an essentially different character from competitive ability.

37. Studies on chromosomal polymorphism, I. Autosomal polymorphism in Rattus rattus *L. collected in Kusudomari and Misima*

Tosihide H. YOSIDA, Akira NAKAMURA and Takako FUKAYA

Chromosomes of *Rattus rattus* L., collected in Kusudomari (Nagasaki) and Misima (Sizuoka) were examined. The karyotype revealed a remarkable heteromorphism in chromosome No. 1. The homozygotic, *i.e.*, standard type, was characterized by 13 pairs of telocentric and 7 pairs of metacentric chromosomes. Chromosome pair No. 1 was telocentric. X



Figs. 1 and 2. Serial alignments of chromosomes of bone marrow cells in *Rattus rattus*. No. 1 chromosome pair in Fig. 1 is telocentric, while that in Fig. 2 is heteromorphic concerning telocentric and subtelocentric chromosomes.

and Y chromosomes were also telocentric (Fig. 1). The heteromorphic chromosome pair No. 1 was seen in 18.4 per cent of rats from Kusudomari and in 40 per cent from Misima. One chromosome of the heteromorphic pair was conspicuous by a subtelocentric centromere, while the other was telocentric (Fig. 2). The total length of the telocentric was almost the same as that of its subtelocentric partner. These facts indicate that the subtelocentric No. 1 chromosome might have arisen by a centromeric inversion of the telocentric partner. Individuals homozygous for subtelocentric No. 1 chromosome could not be found in either population. The difference in the frequency of the dimorphics collected in Kusudomari and Misima was statistically significant.

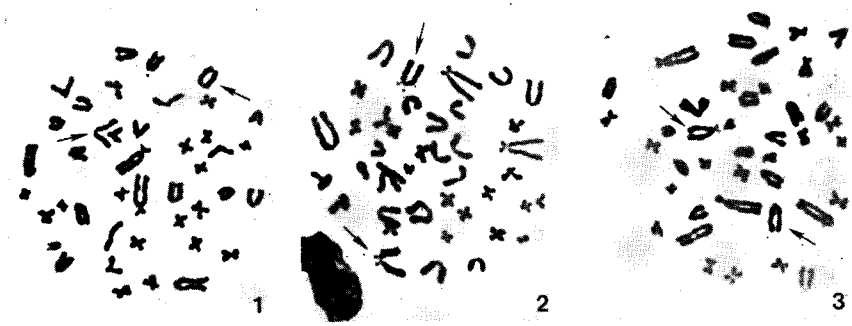
38. *Studies on chromosomal polymorphism, II. Autosomal polymorphism in Norway rats*, Rattus norvegicus

Tosihide H. YOSIDA and Kohachi AMANO

Polymorphism in chromosome pair No. 3 of *Rattus norvegicus* was found in laboratory strains and in wild rats. Some of the animals had a subtelocentric pair No. 3, while others had the same pair composed of two telocentrics. Fourteen inbred strains were classified into two types concerning the pair No. 3.

1. WIS-type (characterized by a telocentric pair No. 3): Donryu-, NIG-III-, W/T- and WIS-strains are included in this type (Fig. 1).

2. YOS-type (characterized by a subtelocentric pair No. 3): ACI-, Albany Buffalo , CW-, Fischer-, Long-Evans-, NIG-IV-, Wayne Pink-Eyed



Figs. 1, 2 and 3. Metaphase chromosomes of bone marrow cells in inbred rats (*Rattus norvegicus*). 1. WIS-strain rat with a telocentric No. 3 chromosome pair. 2. YOS-strain rat with a subtelocentric No. 3 pair. 3. F_1 hybrid between WIS- and YOS-strains, characterizing by telo- and subtelocentric No. 3 chromosomes. Arrows indicate the No. 3 autosome pair.

Hooded-, WKA- and YOS-strains belong to this type (Fig. 2).

F_1 hybrids between WIS- and YOS-strain rats had a heteromorphic pair No. 3 consisting of a telocentric and a subtelocentric chromosomes (Fig. 3).

Polymorphism in pair No. 3 was also found in 43 wild rats collected in Misima. They were classified into three types concerning pair No. 3. Among 43 rats, 17 were of WIS-type, 3 were of YOS-type and the remaining 23 were of hybrid-type.

39. *Further study on chromosomes of leukemias in the mouse induced with Moloney leukemogenic virus (MLV)*[1]

Tosihide H. YOSIDA and Lloyd W. LAW[2]

The present authors have already reported on the chromosome number of 19 Moloney mouse leukemias (YOSIDA and LAW, Annual Report No. 14, 1963). At that time 15 among them had 40 chromosomes as the modal number, 3 had 41 chromosomes and the remaining one showed bimodal distribution of chromosome numbers with the modes at 40 and 41. Last year, chromosomes of 10 Moloney mouse leukemias were newly observed. Among 10 leukemias 9 showed 40 chromosomes as the modal number, and only one had 41 chromosomes. Two leukemias have developed after thymectomy from C3Hf/Lw strain mice. Both had also 40 chromosomes in their stemline cells.

Chromosomes of 29 Moloney mouse leukemias in total were hitherto observed by us including the present study. Among them 23 leukemias (72.4 %) had 40 chromosomes as the modal number. On the other hand, about 50 % of spontaneous leukemias had 40 chromosomes and the remaining 50 % showed aneuploidy (STICH, 1960; KURITA and YOSIDA, 1961). The majority of mouse leukemias developed by chemical treatment had 41 chromosomes (STICH, 1961), and those developed by X-irradiation had various unusual karyotypes (FORD and MOLE, 1959; KURITA and YOSIDA, 1961; NODLER, 1962). From the above considerations it is assumed that most of virus leukemias develop without chromosomal alteration, and they may be due to the transformation of cells at molecular level by virus infection. Perhaps it would be well worthwhile to mention that the leukemias were from the artificially created high-leukemia lines G4 to G10 of several strains and all were lymphocytic except the 2 granulocytic leukemias.

[1] This work was supported by a research grant from the National Cancer Institute (CA 07798-01), Public Health Service, U.S.A.

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

40. Karyotypes in drug resistant sublines of mouse leukemia L. 1210[1)]

Tosihide H. YOSIDA and Kyoko OHARA

Chromosomes in drug sensitive and 5 drug resistant sublines in mouse leukemia L. 1210 were observed. They were established by Dr. LAW in the National Cancer Institute, Bethesda, U.S.A. The 5 sublines used in the present study were resistant to amethopterin (AM), 6-mercaptopurine and fluorouracil (6-MP/FU), 6-mercaptopurine, fluorouracil and amethopterin (6-MP/FU/AM), anti-leukemic agent NSC No. 38280 (38280) and thioguanine (TH).

1. Variation in chromosome number: Chromosome number in sensitive L. 1210 varied from 34 to 46 with the mode at 40. Resistant sublines to AM, 6-MP/FU, 6-MP/FU/AM and 38280 have also 40 chromosomes as the mode of variation fluctuating from 36 to 45. TH resistant line only had 41 chromosomes as modal number.

2. Marker chromosomes: A large metacentric chromosome was usually found to characterize the sensitive line. In none of resistant lines, however, this marker chromosome could be observed, while minute, small metacentric and subtelocentric chromosomes were often occurring in them.

41. Chromosomes in drug sensitive and resistant lines of plasma cell neoplasm 70429 of mice[1)]

Tosihide H. YOSIDA

Table 1. Chromosome number in sensitive and resistant lines of 70429 tumor.

No. of chromosomes	Sensitive	Resistant to		
		Azauridine	Azauracil	Azaserine
38	1	1	0	0
39	1	2	0	1
40	28 (84.9 %)	24 (75.0 %)	0	37 (90.2 %)
41	2	4	42 (95.5 %)	0
42	1	0	2	3
Total number of cells observed	33	32	44	41

1) This work was supported by a research grant from the National Cancer Institute (CA 07798-01), Public Health Service, U.S.A.

Chromosomes in sensitive and resistant lines of plasma cell neoplasm 70429 of mice were examined. All these sensitive and resistant lines were obtained from Dr. LAW's laboratory of the National Cancer Institute, Bethesda, U.S.A. The distribution of chromosome numbers is shown in Table 1.

As the table shows, sensitive and azauridine and azaserine resistant lines had 40 chromosomes in their stemline cells. Only the azauracil resistant line had 41 stemline chromosomes. All chromosomes were rod shaped in sensitive as well as in resistant lines.

42. Karyotypic differences in mouse ascites hepatoma MH 134 and MH 129P maintained in different places[1)]

Tosihide H. YOSIDA and Hirotami IMAI

Mouse ascites hepatomas, MH 134 and MH 129P, used in the present study were established in the National Institute of Health, Bethesda, U.S.A. in 1952 (named tentatively MH 134A and MH 129A), and were transferred to Tohoku University by Dr. SATO in 1956 (named MH 134J and MH 129J). MH 134 was transferred to Misima in 1961 (named MH 134M). The two strains were maintained in these different places by serial intraperitoneal transplantation to C3H mice. Their chromosome numbers were compared with the following result:

1. Frequency of near-diploid and near-tetraploid cells: Both MH 134A and J and MH 129A and J lines were characterized by higher occurrence of cells with near-diploid chromosomes. About 16% cells in MH 134A and J lines showed near-tetraploid chromosomes in their cell population and about 50% showed tetraploidy in MH 134M line. The frequencies of tetraploid cells in MH 129A and J lines were about 10%.

2. Karyotypes: MH 134A; The frequency of cells with 42 chromosomes was the highest showing fluctuation from 38 to 47. As markers, a large submedian metacentric chromosome and a minute one were observed. MH 134J; Cells with 40 chromosomes were of highest occurrence. A metacentric, a submetacentric and a minute chromosome were observed as markers. These karyotypes were also found in MH 134M line. MH 129A; Cells with 46 chromosomes occurred at highest frequency. As a marker, a large submetacentric chromosome with secondary constriction was observed. MH 129J; Cells with 45 chromosomes were of highest occurrence. A submedian metacentric, a submedian metacentric with secondary constriction and a median metacentric chromosome were

1) This work was supported by a research grant from the National Cancer Institute (CA 07798-01), Public Health Service, U.S.A.

observed as markers.

From the above investigations, it can be said that both lines, MH 134 and MH 129P kept in N. I. H., U.S.A., have considerably different karyotypes from those kept in Tohoku University.

43. *Autoradiographic analysis on the sensitive stage to 4-nitroquinoline 1-oxide during division cycle of Yoshida sarcoma cells*

Kazuo MORIWAKI, Yoshinori KURITA
and Tosihide H. YOSIDA

It has already been shown that 4-nitroquinoline 1-oxide (4NQO) exhibits a mutagenic activity in several kinds of micro-organisms (OKABAYASHI, 1955, etc.), in addition to its well-known carcinogenic and carcinostatic actions. The present study was undertaken to determine which stage of the division cycle of the Yoshida rat sarcoma cells is responsible for the occurrence of the chromatid breaks in the metaphase following the *in vivo* treatment with 4NQO. The findings in the micro-organisms mentioned above allow us to postulate that this drug might react preferentially with DNA or DNA-synthetic processes in mammalian cells as well. The present experiment, however, demonstrated that postsynthetic (G_2) stage is more sensitive to 4NQO than the DNA synthetic (S) stage. This can be shown as follows:

Chromatid breaks were observed about 4 hours after administration of 0.8 ml of $10^{-3}M$ 4NQO into the ascites tumor which cells seem to proliferate at random with an average mitotic index of 3%. The final concentration of 4NQO in the ascites fluid was estimated as approximately $10^{-5}M$. On the other hand, retardation of G_2 stage by 4NQO treatment was indicated by the autoradiographic analysis of the incorporation of tritiated thymidine into the chromatids. One hundred and sixty μc H^3-thymidine in 0.2 ml saline solution was injected into the intraperitoneal cavity of the tumor bearing rat. Grains on the autoradiographs of chromosomes appeared about 2.5 hours after this injection. The simultaneous 4NQO treatment delayed the appearance of the grains until about 6 hours after the injection.

These results suggest that this drug is able to give some structural change on the chromosomal materials during G_2 stage, and not during S stage. The precise mechanism of this action is yet to be studied.

44. *Chromatid aberrations induced in Yoshida sarcoma cells by the radiomimetic chemical, 4-nitroquinoline 1-oxide*

Yoshinori KURITA, Tosihide H. YOSIDA and Kazuo MORIWAKI

Chromosome aberrations induced in Yoshida sarcoma cells by the radiomimetic chemical, 4-nitroquinoline 1-oxide (4NQO), are only of the chromatid type. Chromatid intrachanges (breakage) occurred with maximum frequency 8 hours after the treatment, and their distribution among cells was nonrandom. The frequency of intrachanges was not proportional to the length of metaphase chromosomes. Chromatid interchanges (translocations) usually occurred most frequently 24 hours after treatment, and their distribution among cells was also nonrandom. The aberrations were mostly localized in the neighbourhood of the secondary constriction. Frequency of both, intrachanges and interchanges, increased proportionally with the doses 8 hours after treatment; the former increased with the square of the dose. However, the frequency of interchanges which occurred 24 hours after treatment showed no proportional relation to the dose.

The mechanism of aberration induction is supposed to be the result of cross-linking of the twin molecular chain of the chromonema, since complete lack of chromosome type aberration suggests that direct chromatid breakage in the cells of the G_1 phase at the time of treatment is very rare. Furthermore there is no evidence to support the possibility that the main function of the alkylating agents is hydrolysis of chromosomal macromolecules. The localized aberrations near the secondary constriction are supposed to be closely related to the location of nucleoli and of nucleoli organizers in the constrictions; the nucleoli are the sites of r-RNA synthesis which seems to be completely inhibited by the chemical treatment.

45. *Autoradiographic analysis of the mitotic cycle in Yoshida sarcoma cells*

Yoshinori KURITA, Kazuo MORIWAKI and Tosihide H. YOSIDA

The mitotic cycle of Yoshida sarcoma cells was studied by means of tritiated thymidine and autoradiography.

The duration of each phase of the mitotic cycle was estimated as follows:

(1) Early interphase (G_1) lasted about 5.5～6.0 hours.
(2) Phase of DNA synthesis (S) lasted about 9.0～9.5 hours.
(3) Antephase (G_2) was about 2.5 hours long.

The generation time of the sarcoma cells was determined to be about 18.5 hours, which is almost in complete agreement with FREYMANN's result obtained by X-ray experiment.

46. Strain difference of susceptibility to the teratogenic action of ethylurethane in mice

Kiyosi TUTIKAWA and Akira AKAHORI

It has been well recognized that the frequency of cleft palate following treatment of mice with cortisone varies with the genetic constitution of the mother and embryo. Recently, GOLDSTEIN *et al.* (1963), who used 6-aminonicotinamide as a teratogen, pointed out that cytoplasmic factors influencing the embryo's response to the teratogen may be active in the case of cleft palate, and may also exist for vertebral fusions.

The purpose of the study was to provide further information on the genetic basis for a strain difference in frequency of induced malformations following treatment of mice with ethylurethane. Mice of two inbred strains, CBA and C3H$_e$B/Fe, subsequently referred to as C and T, were

Table 1. Frequencies of late death (LD) and exencephaly following maternal treatment with ethylurethane on day 8.5 of gestation.

Cross	No. of females	CL	IMP	PIL	ED	LD	LE	LD/IMP	Percent of embryos with exencephaly
C×C	12	126	99	27	5	16	78	16.2	0
Control	6	59	44	15	1	2	41	4.6	0
T×T	10	107	100	7	4	40	56	40.0	21.9
Control	10	101	90	11	8	6	76	6.7	0
C×T	11	111	78	33	9	10	59	12.8	0
T×C	10	114	105	9	7	16	82	15.2	11.2
CT×CT	10	119	110	9	0	7	103	6.4	10.0
TC×TC	10	120	103	17	3	17	83	16.5	11.0
C×CT	13	137	127	10	15	25	87	19.7	0
C×TC	7	71	64	7	6	6	52	9.4	0
CT×C	3	35	31	4	1	6	24	19.4	0
TC×C	20	231	207	24	5	32	170	15.5	4.0
T×CT	12	137	118	19	4	21	93	17.8	12.3
T×TC	6	65	59	6	7	11	41	18.6	14.7
CT×T	3	36	32	4	4	7	21	21.9	10.7
TC×T	18	202	183	19	6	25	152	13.7	13.0

used in the present experiment. Pregnant females were injected with 1.5 mg/g of 10 % ethylurethane aqueous solution once on the 8.5 day of pregnancy and killed on day 17.5.

In treated mice, exencephaly was induced in 21.9 % of the susceptible T embryos and 0 % in the resistant C embryos. In reciprocal crosses between these two strains, the T×C embryos had a higher frequency of the defect than the C×T embryos. While, similar frequencies of exencephaly resulted from matings among the reciprocal F_1 themselves and from matings of the reciprocal F_1 females to susceptible T males (Table 1).

Similar result was obtained from the search for induction of posterior shift of thoracolumbar border following same maternal treatment. Thus the factors controlling the susceptibility of an embryo to ethylurethane are not cytoplasmically transmitted, and involve the interaction of fetal and maternal genotypes.

47. Further studies on water soluble proteins in mouse skin following the application of a tumor promoting agent

Yoshito OGAWA

In a previous preliminary note (OGAWA and KOBAYASHI, Annual Report No. 14, 1963), the effect was described of Sweet Orange Oil on the water soluble proteins in the skin of the mouse. Sweet Orange Oil which is a component of the peal of *Citurus sinensis* aroused the interest of cancer researchers as a promoting agent in experimental carcinogenesis. The present communication corrects some errors that occurred in our previous note resulting from the use of denaturalized materials due to refrigeration and describes the changes in those soluble proteins following the application of Sweet Orange Oil (B.P.C. grade).

0.4 g of Sweet Orange Oil was applied individually to the dorsal skin of mice with two day intervals, and the treated part of the skin was cut off with three day intervals after treatment. The cut skin tissue was 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 clear supernatant was made at room temperature on cellulose acetate strips (OXOID) of 2.5×9.0 cm size by using veronal buffer (pH 8.6, $\mu = 0.06$) at 0.7 mA/cm for two hours. Nigrosin staining was used for the estimation of the analyzed proteins. A non-treated group was prepared for control.

The analytical findings are given in Table 1. The total protein concentration increases immediately after the application of Sweet Orange

Table 1. Effect of Sweet Orange Oil on the water soluble proteins in mice skin tissue (mg/g skin tissue weight).

Day after treatment	Total protein	A (Albumin)	F	G	B	H	D	E	I
Normal (Control)	12.52	3.25	1.40	1.06	1.90	0.00	2.28	2.16	0.47
3	20.66	5.23	1.44	1.18	5.10	0.00	4.18	3.01	0.52
6	23.21	4.24	2.25	2.25	4.74	0.00	8.98	0.00	0.75
9	31.30	8.21	0.00	0.00	11.20	0.00	7.31	2.06	2.52
12	19.07	4.46	0.00	0.00	3.25	5.41	3.11	1.90	0.94
15	16.58	3.85	2.02	1.45	4.34	0.00	2.22	1.93	0.77

Oil. It was 250% higher than in the control on the 9th day after the treatment. This finding was closely related to the progress of the mitotic index in the skin tissue. The most remarkable increase of the mitotic index was recognized on the 9th day and then it decreased gradually with the lapse of time. The soluble proteins extracted from normal skin tissue were separated into seven fractions (A, F, G, B, D, E and I). Fraction C of our previous note was not recognized in fresh materials either in normal or in treated skin. No remarkable change was found in the components of soluble proteins during the first three days after treatment. But on the 6th day, fraction E disappeared temporarily, and fractions F and G disappeared from 9th to 12th day after treatment. On the contrary, a new fraction H was recognized on the 12th day. This transient fraction resulted from the treatment with Sweet Orange Oil.

The physiological and chemical nature of this new fraction is now studied.

48. Chromosome observation in Japanese ants

Hirotami IMAI and Tosihide H. YOSIDA

Since there have been made only a few studies on the chromosomes of ants, taxonomists could not utilize cytological data in evolutional considerations of this insect. The present authors made an attempt to coordinate karyological data with problems of ant phylogeny. As the first step, the authors made a detailed analysis of their chromosomes by using the hyaluronidase-aceto-orcein squash method in combination with the drying-method, and obtained suitable preparations. The present paper reports the chromosome numbers of 22 Japanese species belonging to 4 subfamilies (Table 1).

From these results, it became clear that the variation of the chromo-

Table 1. Chromosome numbers of Japanese ants.

	n	$2n$
Subfamily PONERINAE		
Brachyponera luteipes SMITH	11	22
Cryptopone sauteri WHEELER		28
Subfamily DOLICHODERINAE		
Iridomyrmex itoi FOREL	14	28
Subfamily MYRMICINAE		
Pheidole fervida SMITH	10	20
Monomorium pharaonis LINNÉ	11	
Pristomyrmex pungens MAYR	12	24
Leptothorax spinosior FOREL		24
Crematogaster laboriosa SMITH		26
Tetramorium caespitum jacoti WHEELER	14	28
Aphaenogaster sp.	16	
Aphaenogaster famelica SMITH	17	34
Vollenhovia emeryi WHEELER		36
Messor aciculatum SMITH	22	44
Subfamily FORMICINAE		
Camponotus sp.	9	18
Camponotus kiusiuensis SANTSCHI		28
Camponotus japonicus MAYR	14	
Lasius niger LINNÉ		30
Lasius talpa WILSON		30
Formica sanguinea LATREILLE	26	52
Formica truncorum yessensis FOREL	26	52
Formica japonica MOTSCHULSKY	27	54
Polyergus samurai YANO	27	54

some numbers is very wide among the genera of the same subfamily, namely, from $n = 4$ to $n = 27$ (including the data of PEACOCK *et al.* (1954) and HAUSCHTECK (1961, 1962)). Striking heteroploidy has been developed in two subfamilies, Formicinae ($n = 8 \sim 27$) and Myrmicinae ($n = 4 \sim 22$). Based on this fact, it is presumed that both subfamilies evolved independently from a very early evolutional stage in the direction toward increasing the chromosome number and developing heteroploidy. This assumption agrees with the opinion of BROWN (1954) who divided the family Formicidae into two large groups based on morphological traits, *i.e.*, Poneroid-complex including Myrmicinae and Myrmecioid-complex including Formicinae. The assumption is also supported by their wide distribution and mode of reproduction. WHEELER (1928) thought that the origin of ants is tropical, but both those subfamilies became distributed all over

the world. Therefore it may be assumed that the heteroploidy occurred in various genera, one after another, in the course of their migration to new environments. At the same time, their complicated system of parthenogenetical reproduction (haploid or diploid parthenogenesis of eggs laid by the queen or especially by the workers) may have also contributed to the development of heteroploidy as know in many other parthenogenetical animals.

49. Abnormal taster to phenyl-thio-carbamide (PTC) in Japanese population

Yoshito OGAWA

This report furnishes a few data on abnormal tasters for PTC among the Japanese population, namely those with sweet, puckery, salty, pungent and other taste responses.

Within the age range from 15 to 18 years, 2,304 persons residing in Matsumura (Niigata Pref. : 354 persons), Chiba (Chiba Pref. : 109 persons), Okazaki (Aichi Pref. : 141 persons), Katsuyama (Fukui Pref. : 166 persons), Hamada (Shimane Pref. : 246 persons), Kure (Hiroshima Pref. : 429 persons), Nakamura (Kochi Pref. : 310 persons), Miyazaki (Miyazaki Pref. : 176 persons) and Tokyo (Tokyo : 373 persons) were examined for their taste reaction to PTC solutions prepared in accordance with Hartman's report (1939).

In distilled water diluted solutions (1:2, 1:4, 1:8, etc.) were prepared from a stock solution (0.16 %) of PTC. According to their strength, the solutions were numbered from 1 to 12, 1 being the stock solution and 12 the weakest solution (0.78125^{-4} %). The threshold value of taste sensitivity, irrespective of the kind of taste, was investigated twice in each individual. When different results were obtained between first and second test, the examination was made again next day and only sure results were used in this report.

According to the taste response to PTC, the subjects were divided into two groups. One represented the normal tasters (simple tasters) and the other the abnormal tasters (complicated tasters). The normal group showed only bitter response to every concentration of PTC, also in respect of their taste threshold, and non-tasters even when the stock solution of PTC was used. This group consisted of 1,987 persons (86.24 %). The abnormal group, comprising 317 persons (13.76 %), showed some other taste response, *e.g.* sweet, puckery, etc., on the taste threshold point. And some of the people in this group showed often bitter response to stronger PTC solutions.

In the group of abnormal tasters, 25.24 percent (80 persons) showed no bitter taste response even to No. 2 solution of PTC. But in the group of normal tasters, only 8.91 percent (177 persons) were non-tasters. The distribution of bitter taste threshold values between these two groups is clearly different ($X^2 = 38.52$, $DF = 7$, $P < 0.01$) (Table 1).

Table 1. Distribution of threshold values of bitter taste response in normal and abnormal taster groups.

Group	Non-tasters <1, 1, 2	Tasters 3	4	5	6	7	8	9	10–12	Total
Normal tasters	177	56	66	127	224	415	393	325	204	1,987
Abnormal tasters	80	21	19	25	26	47	49	34	16	317

Moreover the distribution of threshold values of taste response in the group of abnormal tasters, irrespective of the kind of taste, was also significantly different from that of the threshold value of bitter taste response of the normal group as showed in Table 2 ($X^2 = 267.89$, $DF = 9$, $P < 0.01$).

Table 2. Distribution of threshold values of taste response in normal and abnormal tasters.*

Group	Non-tasters <1, 1, 2	Tasters 3	4	5	6	7	8	9	10	11	12	Total
Normal tasters	177	56	66	127	224	415	393	325	108	68	28	1,987
Abnormal tasters	3	4	11	12	18	23	44	47	51	49	55	317

* Irrespective of the kind of taste response to PTC solution.

These results indicate that the threshold value of taste response in the abnormal group does not correspond to that of the normal group. The abnormal taster group must be regarded as a different population from that of normal tasters.

B. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF PLANTS

50. *"Germless grain", a new effect of* caudata *cytoplasm on the manifestation of wheat genomes*

Hitoshi KIHARA and Koichiro TSUNEWAKI

It is already known that *caudata* cytoplasm exerts various important effects on the manifestation of wheat genomes, such as induction of male sterility or pistillody of stamens, reduction of plant vigor, and increased occurrence of haploids and twin seedlings (KIHARA, 1949~1964; KIHARA and TSUNEWAKI, 1961~1964). Recently it has been found to increase the occurrence of germless grains which is here reported.

Male-sterile plants of *Triticum aestivum* ssp. *vulgare* var. *erythrospermum* (Abbrev. *T. v. e.*) with *caudata* cytoplasm were interplanted with normal plants of the same variety. About 40 spikes of both male-sterile and normal lines were emasculated before flowering and then artificially pollinated with normal pollen grains. The hand-pollinated spikes were harvested together with some open pollinated spikes, and the caryopsis was examined for the presence of an embryo. The result is summarized in Table 1.

Table 1. Frequency of germless grains among seeds produced by male-sterile *T. v. e.* with *caudata* cytoplasm and the normal line.

Lines	Type of pollination	No. of seeds examined	No. of germ-less grains	%
Normal	Artificial	593	1	0.2
	Open	15,520	17	0.1
Male-sterile	Artificial	506	48	9.5
	Open	1,607	276	17.2

The frequency of germless grains in normal *T. v. e.* was, on the average, 0.1 %, and no difference was found between artificial and open pollination. On the contrary, germless grains were found in abundance among seeds set on male-sterile plants. Their frequency was higher when the seeds were produced by open pollination than when they were obtained from hand pollination. From those results, it can be said that *caudata* cytoplasm increases the frequency of germless grains and that open pollination of male-sterile plants further increases their frequency probably due to inadequate timing of pollen transfer. In order to test

the germination ability of the germless grains, they were seeded on moist filter paper and their germination was compared to that of normal grains. In this test all normal grains germinated but none of the germless grains, as shown in Fig. 1.

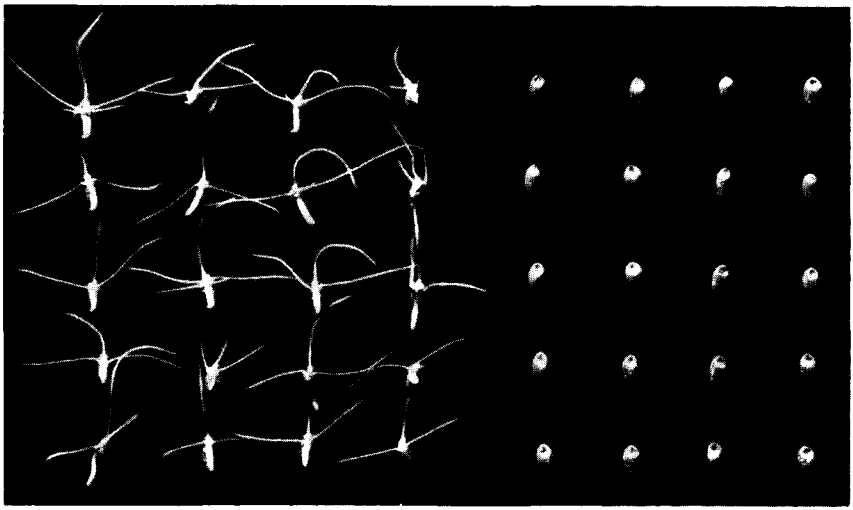


Fig. 1. Germination test of normal and germless grains obtained from male-sterile *T. v. e.*
Left, normal; right, germless.

51. *An effective restorer to male-sterile* ovata *cytoplasm*

Hitoshi KIHARA and Koichiro TSUNEWAKI

Since the work of FUKASAWA (1953) the cytoplasm of *Aegilops ovata* is known to cause male sterility in wheat. In order to find restorers for this cytoplasm, WILSON and Ross (1961) crossed 112 varieties or strains of common wheat to FUKASAWA's male-sterile Norin 26 and tested the fertility of the F_1 hybrids. They could not find any restorer that would recover high fertility in heterozygotes.

The authors crossed FUKASAWA's male-sterile Norin 26 as the female parent to three known restorers of *caudata* cytoplasm, *i.e.*, P 168, compactum 44 and ABD-13. Restoring genes of the last two did not function effectively in *ovata* cytoplasm. In contrast, F_1 hybrids with P 168 restored fertility almost completely, *i.e.*, their selfed seed fertility, on the average, was 91.3%, which is well comparable to that of normal Norin 26 (97.5%) or P 168 (85.2%). Spikes of the F_1 and its parents are

Fig. 1. Spikes of male-fertile F_1 from the cross, male-sterile Norin 26×P 168. From left to right: normal Norin 26, male-sterile Norin 26, (male-sterile Norin 26×P 168) F_1 and P 168.

shown in Fig. 1. This restorer in combination with *ovata* cytoplasm seems to be promising as a new material for hybrid wheat breeding.

52. Gene transfer between di-, tetra- and hexaploid wheats, I. Transfer of v *gene from common wheat to emmer wheat*

Koichiro TSUNEWAKI

A virescent gene of common wheat that does not affect germination in homozygous condition was found by NEATBY (1933). It was later designated by *v* and located on chromosome 3B by SEARS (1954). At a temperature of 13～16°C, seedlings of common wheat homozygous for *v* are devoid of chlorophyll in the first and second leaves, and die at the third or fourth leaf stage. However, if they are grown at 21～24°C, the extreme tip of the first leaf shows a trace of chlorophyll. All later leaves are pale at first but become gradually green.

Since *v* is located on chromosome 3B, transfer of this gene to tetraploid emmer wheat should be possible. For this purpose, heterozygous plants (*Vv*) of common wheat were crossed to an emmer wheat, *Triticum carthlicum* var. *stramineum*. The pentaploid F_1 hybrids were backcrossed to the emmer parent. In the B_1 generation plants having 14 bivalents were selected and again backcrossed to the same emmer. At the same time, the B_1 plants were self-pollinated and their offspring were investigated

Table 1. Germination and segregation of virescent plants in the selfed progeny of B_1, *T. aestivum* (*Vv*)×*T. carthlicum* (*VV*)[2].

B_1 lines	Genotype of B_1	No. seeds sown	No. seeds germinated	Per cent germination	No. normals	No. virescents
1145-3	*Vv*	20	14	70	13*	1
1149-1	〃	20	14	70	14*	0
1149-3	〃	20	14	70	13*	1
Total		60	42	70	40	2
1147-1	*VV*	20	19	95	19	0
1147-2	〃	20	18	90	18	0
1149-2	〃	20	19	95	19	0
1151-3	〃	20	17	85	17	0
Total		80	73	91	73	0

* Some showed a tendency to virescence but distinction between *Vv* and *VV* was difficult.

for the segregation of virescent plants. The result is summarized in Table 1.

Only two virescent seedlings were obtained among 42 offspring of the *Vv* plants. The frequency (less than 5%) was much lower than the expected 25%. In addition, germination rate of the selfed progeny (B_1 line) of *Vv* plants was only 70%, whereas that of *VV* was higher than 90%. These facts clearly indicate that *vv* homozygotes of emmer wheat are mostly seed-lethal. Since the *v* gene, in common wheat, causes virescence without affecting germination, it can be said that the gene upsets the physiology of embryos more drastically on the tetraploid than on the hexaploid level.

53. Geographical distribution of necrosis genes in common wheat, I. Genetic basis of necrosis

Koichiro TSUNEWAKI

Based on F_2 and B_1 data of a cross, Chinese Spring×Macha sub., necrosis (NISHIKAWA's type 1) has been assumed by TSUNEWAKI and KIHARA (1961) to be controlled by three complementary genes, Ne_1, Ne_2 and Ne_3. Furthermore, two distinct types of expression have been noticed, which were thought to be due to different alleles of Ne_1, namely, Ne_1^{sy} for systemic type and Ne_1^{ep} for epidemic type (NISHIKAWA, 1963).

However, recent results of the author obtained by test crosses of F_2 and F_3 of the same hybrid, Chinese Spring×Macha sub., indicated that the two types of necrosis are under control of two independent complementary gene systems. For simplicity the systemic type of necrosis will be here called "chlorosis", because of prominent chlorosis of leaves prior to necrosis, and the term "necrosis" will be retained for the epidemic type.

Chlorosis is caused by two complementary genes, Ch_1 located in A or B genome (chromosome is yet undetermined) and Ch_2 (former Ne_3) on chromosome 3D, while necrosis is due to Ne_1 on chromosome 5B and Ne_2 on chromosome 2A. The results of a detailed gene analysis revealed the following genotypes in haploid phase for the three testers previously offered for necrosis:

Jones Fife	$ne_1Ne_2ch_1Ch_2$
Prelude	$Ne_1ne_2ch_1Ch_2$
Macha sub.	$Ne_1ne_2Ch_1ch_2$

For the two complementary gene systems, nine genotypes are expected as Table 1 shows. The phenotypes of F_1 hybrids between three

Table 1. Genotype of three testers and phenotypes of F_1 hybrids between them and unknown varieties with each of nine possible genotypes.

Nine possible genotypes	Testers		
	Jones Fife ($ne_1Ne_2ch_1Ch_2$)	Prelude ($Ne_1ne_2ch_1Ch_2$)	Macha sub. ($Ne_1ne_2Ch_1ch_2$)
1. $Ne_1ne_2Ch_1ch_2$	chlorotic	chlorotic	normal
2. $Ne_1ne_2ch_1Ch_2$	necrotic	normal	chlorotic
3. $Ne_1ne_2ch_1ch_2$	necrotic	normal	normal
4. $ne_1Ne_2Ch_1ch_2$	chlorotic	chlorotic	necrotic
5. $ne_1Ne_2ch_1Ch_2$	normal	necrotic	chlorotic
6. $ne_1Ne_2ch_1ch_2$	normal	necrotic	necrotic
7. $ne_1ne_2Ch_1ch_2$	chlorotic	chlorotic	normal
8. $ne_1ne_2ch_1Ch_2$	normal	normal	chlorotic
9. $ne_1ne_2ch_1ch_2$	normal	normal	normal

testers and an unknown variety with each of the nine genotypes are also shown in Table 1. It must be noted that chlorosis is epistatic over necrosis.

From this table, it is evident that genotypes 1 and 7 can not be distinguished by the use of the present tester series, while all the other seven are distinguishable from each other.

54. *Geographical distribution of necrosis genes in common wheat, II. Distribution in Japanese local varieties*

Koichiro TSUNEWAKI and Yasuo NAKAI

There are about 250 varieties in Japan which can be called local. Of those 194 varieties were crossed to the testers for necrosis and their genotypes were determined. The result is summarized in Table 1.

About one-third of Japanese varieties carry Ne_1 gene, while Ne_2 is found in less than one-tenth of them. The frequency of the former is much higher in South Kanto-Sansei-Shin-etsu district than in all other districts. A great majority of Ne_2-carriers are found in northern Japan, mostly in Hokkaido. This fact seems to suggest that they have been originated from American or European varieties.

Table 1. Frequencies of various genotypes and alleles for necrosis in Japanese local wheat varieties.

	District						Total
	Kyushu	Chugoku, Shikoku	Kinki, Tokai, Hokuriku	S. Kanto, Sansei, Shin-etsu	N. Kanto, Tohoku, Hokkaido	Unknown	
Freq. of each genotype (No. of varieties)							
$Ne_1ne_2ch_1Ch_2$	13	5	6	25	8	3	60
$ne_1Ne_2ch_1Ch_2$	0	3	0	1	12	1	17
$ne_1ne_2ch_1Ch_2$	35	15	18	18	25	5	116
$ne_1ne_2ch_1ch_2$	0	0	0	0	1	0	1
Total	48	23	24	44	46	9	194
Freq. of each dominant allele (Relative freq. in per cent)							
Ne_1	27	22	25	57	17	33	31
Ne_2	0	13	0	2	26	11	9
Ch_1	0	0	0	0	0	0	0
Ch_2	100	100	100	100	98	100	99

55. Geographical distribution of necrosis genes in common wheat, III. Distribution in Pakistan, Afghanistan and Iran

Koichiro TSUNEWAKI and Yasuo NAKAI

More than 240 strains of common wheat were collected in Pakistan, Afghanistan and Iran by two members (Dr. H. KIHARA and Dr. K. YAMASHITA) of the Kyoto University Scientific Expedition (KUSE) to the Karakoram and Hindukush, 1955. Of those 36 strains from Pakistan, 40 from Afghanistan and 143 from Iran, making 219 in total, were crossed to the testers of necrosis genes and their genotypes were determined. In some strains one of the three test crosses was unsuccessful and, consequently, the genotype was only partially determined. The result is summarized in Table 1.

Ne_1 gene was very common in northern Iran, *i.e.*, from Gorgan to Ardabil and Tabriz, while its occurrence was very rare in all other districts including Pakistan and Afghanistan. None of the Ne_2-carriers could be found. Ch_1 was also rare. Ch_2 occurred rather commonly. From this result it can be safely concluded that the wheat population of northern Iran is distinctly different from the other populations regarding the very high frequency of Ne_1 gene.

Table 1. Frequencies of various genotypes and alleles for necrosis in KUSE wheat.

	Country and district							
	Pakistan	Afghanistan	Iran					
			Subzuwar	Isfahan	Tehran	Gorgan	Ardabil, Tabriz	Total
Freq. of each genotype (No. of varieties)								
$Ne_1ne_2ch_1Ch_2$	1	1	1	3	2	14	35	55
Ne_1 ? ch_1Ch_2	0	1	0	0	0	0	1	1
$Ne_1ne_2ch_1$?	0	0	0	0	0	0	1	1
$ne_1Ne_2ch_1Ch_2$	0	0	0	0	0	0	0	0
$ne_1ne_2ch_1Ch_2$	13	9	14	16	15	0	0	45
ne_1 ? ch_1Ch_2	0	2	2	0	1	0	1	4
? $ne_2ch_1Ch_2$	0	1	0	0	0	0	0	0
? $ne_2Ch_1ch_2$	0	0	0	1	0	0	0	1
$ne_1ne_2ch_1ch_2$	19	26	3	7	8	4	8	30
$ne_1ne_2ch_1$?	3	0	1	2	2	1	0	6
Total	36	40	21	29	28	19	46	143
Freq. of each dominant allele (Relative freq. in per cent)								
Ne_1	3	5	5	11	7	74	80	40
Ne_2	0	0	0	0	0	0	0	0
Ch_1	0	0	0	3	0	0	0	1
Ch_2	42	35	85	70	69	78	82	77

56. *Taxonomical and ecological studies on* Agropyron humidum *Ohwi et Sakamoto*

Jisaburo Ohwi and Sadao Sakamoto

Extensive studies of *Agropyron* species in Japan were first undertaken by Dr. M. Honda. In 1927 he described a new species, *Ag. mayebaranum* Honda, based on the herbarium specimens collected in Kyushu by Mr. K. Mayebara.

Recently the present junior author reexamined the morphology and pollen fertility of the specimens collected by Mr. Mayebara. He found that the specimens collected by him are natural interspecific hybrids between *Ag. tsukushiense* var. *transiens* Ohwi and another hitherto unknown species to which a new name was given; namely *Ag. humidum* Ohwi et Sakamoto. The hybrids have intermediate characters between parental species.

Adaptation to moist environment is one of the most pronounced characteristics of *Ag. humidum*. Two distinct characters of this new species imply high adaptiveness to the habitat of fallowing paddy fields. One is the formation of an abscission layer at maturity in the node below the flag leaf. Thus, from this node on upward the spike is easily separated from the rest of the plant by wind or other physical forces. The other is the perennialization of the culm with the exception of the uppermost internode and spike. New shoots and roots are produced very easily from the nodes when the conditions are favorable.

The two characteristics mentioned above have not been found in any species belonging to tribe Triticeae which are mostly adapted to rather dry habitats. With these two adaptive traits seeds and clones of this species are dispersed uniformly in the paddy field during the preparation for rice planting in June. This seems to be the reason why it occurs in swarms and uniform populations in fallowing paddy fields as observed in the suburbs of Misima.

57. *Genome analysis of Japanese and Nepalese* Agropyron

Sadao SAKAMOTO

Morphological, physiological and cytogenetical studies were carried out in 14 different interspecific *Agropyron* hybrid combinations among five Japanese species, *i.e.*, three tetraploids, *Ag. ciliare* (TRIN.) FRANCH., *Ag. gmelini* (LEDEB.) SCRIB. et SMITH var. *tenuisetum* OHWI and *Ag. yezoense* HONDA, and two hexaploids, *Ag. humidum* OHWI et SAKAMOTO and *Ag. tsukushiense* (HONDA) OHWI, two Nepalese tetraploids, *i.e.*, *Ag. semicostatum* NEES and *Ag. gmelini*, and one American tetraploid, *Ag. trachycaulum* (LINK) MALTE.

The main characteristics of the hybrids were as follows: (1) Growth of hybrids was vigorous, and tillering, heading and flowering were normal. (2) The general appearance of many characters was intermediate between the parents or superior to both or similar to that of one of the parents, particularly in so far as quantitative characters are concerned. (3) Complete pollen sterility and high seed sterility were the rule in all hybrid combinations. The anthers were non-dehiscent at the flowering time and the pollen grains were completely abortive.

Chromosome pairing in hybrids is summarized in Table 1. From these results, the following conclusions are drawn: (1) The Japanese species are cytologically closely related with each other; two homologous genomes are shared by three tetraploid and two hexaploid species with an added third genome to the latter. (2) The genomes of Japanese and Nepalese

Table 1. Chromosome pairing in interspecific hybrids of Japanese, Nepalese and American *Agropyron*.

Cross combination (♀ × ♂)	No. of cells examined	No. of bivalents		Average chromosome pairing				
		Range	Mode	V	IV	III	II	I
Japanese 4x×4x:								
Ag. ciliare×*Ag. yezoense*	45	10~14	14		0.36	0.20	11.29	2.29
Japanese 4x×Nepalese 4x:								
Ag. ciliare×*Ag. semicostatum*	408	5~14	12	0.002	0.14	0.15	11.34	4.20
Ag. semicostatum×*Ag. yezoense*	69	10~14	12		0.04	0.04	12.26	3.17
Japanese 4x×American 4x:								
Ag. ciliare×*Ag. trachycaulum*	325	2~ 9	5		0.02	0.07	5.33	17.10
Japanese 4x×6x:								
Ag. tsukushiense×*Ag. ciliare*	107	9~15	14		0.06	0.08	13.06	8.44
Ag. tsukushiense×*Ag. yezoense*	87	7~15	14		0.15	0.33	12.24	8.81
Ag. gmelini (Japan)×*Ag. tsukushiense*	50	11~15	13		0.18	0.16	12.28	8.84
Ag. humidum×*Ag. ciliare*	53	12~16	14		0.09	0.02	13.17	6.45
Nepalese 4x×Japanese 6x:								
Ag. tsukushiense×*Ag. semicostatum*	155	6~14	11		0.11	0.16	9.80	14.05
Ag. humidum×*Ag. semicostatum*	47	8~14	10			0.02	10.66	13.62
Ag. gmelini (Nepal)×*Ag. tsukushiense*	97	3~15	12		0.11	0.11	11.30	11.57
Ag. gmelini (Nepal)×*Ag. humidum*	25	9~15	11		0.04	0.08	11.92	10.76
Japanese 6x×6x:								
Ag. humidum×*Ag. tsukushiense*	46	20~21	21			0.02	20.74	0.46

Ag. gmelini are truly homologous except for a slight but definite reduction in pairing; this result was obtained indirectly through crosses of both, Japanese and Nepalese *Ag. gmelini*, with *Ag. tsukushiense*. (3) The genomes of the Nepalese species, *Ag. semicostatum*, are basically homologous to those found in Japanese tetraploid species, but some degree of genomic differentiation which reduces the number of bivalents and increases that of univalents in Japanese 4x×Nepalese 4x and Nepalese 4x×Japanese 6x hybrids has taken place. (4) The genomes of a tetraploid American species, *Ag. trachycaulum*, are partially homologous to those found in a Japanese tetraploid species, *Ag. ciliare*.

58. *Natural interspecific hybrids in Japanese* Agropyron

Sadao SAKAMOTO

Two cases of natural interspecific hybridization in Japanese *Agropyron* were studied in Misima. One is the pentaploid hybrid between *Ag. ciliare* (TRIN.) FRANCH. ($2n = 28$) and *Ag. tsukushiense* (HONDA) OHWI ($2n = 42$), and the other is the hexaploid hybrid between *Ag. humidum* OHWI et SAKAMOTO ($2n = 42$) and *Ag. tsukushiense*. Clones of the former, *Ag. ciliare*×*Ag. tsukushiense*, were found mainly in the hills and in the river dikes in the suburbs of Misima.

No seed setting was observed in this hybrid due to extremely high sterility of both male and female organs. It is assumed that in this hybrid combination introgressive hybridization would not occur in natural conditions.

As to hexaploid natural hybrids, two cases were observed; *i.e.*, one allopatric and the other sympatric. In the allopatric case *Ag. humidum* and the common type of *Ag. tsukushiense* were involved, while, in the sympatric case *Ag. humidum* and the early ecotype of *Ag. tsukushiense* participated (SAKAMOTO, 1961). In both cases low seed setting was observed from examination of a considerable number of clones growing on foot-paths of paddy fields of the former hybrid and in fallowing paddy fields of the latter. From progeny tests of backcrossed plants obtained from the sympatric natural hybrids between *Ag. humidum* and the early ecotype of *Ag. tsukushiense*, the following conclusions are drawn: It is quite possible that introgression of characters from one species to the other, such as waxiness, might have occurred in the natural populations through backcrossing to the parental species of natural F_1 hybrids followed by segregation of the characters concerned. Rather quick restoration of fertility in the hybrid progenies makes the establishment of hybrid swarms in a state of nature possible, having intermingled characteristics

of the parental species. However, so far no introgressants have been found yet in the natural sympatric populations of these two species. High sterility of F_1 bybrids might be a major cause of the restriction of introgression.

59. *Morphological and cytological studies of two intergeneric hybrids of* Elymus sibiricus *Linn. and* El. dahuricus *Turcz. with* Agropyron tsukushiense *var.* transiens *Ohwi*

Sadao Sakamoto

Among the genera of the tribe Triticeae, the phylogenetic relationships between *Agropyron* and *Elymus* are very complicated, because both genera are quite large comprising various and complex polyploid species which distribute widely in the temperate regions of the world where many natural hybrids between them have been found.

Exploring cytogenetical relationships of these two genera, two species of Asiatic *Elymus*, *El. sibiricus* ($2n=28$) and *El. dahuricus* ($2n=42$) were crossed with a Japanese *Agropyron*, *Ag. tsukushiense* ($2n=42$). Pentaploid as well as hexaploid intergeneric F_1 offspring were more vigorous than the parental species. Shape of the spikes of F_1 was of *Elymus* type. One of characteristics distinguishing *Elymus* from *Agropyron* is the production of two spikelets at most nodes of the rachis. This character was in F_1 expressed only in 1/3~1/4 of all nodes of a rachis. All pollen grains of both hybrids were completely abortive.

Average chromosome pairing at MI of PMCs in F_1 was 0.01_{IV} (0~1) $+0.25_{III}$ (0~1)$+8.93_{II}$ (4~13)$+16.38_{I}$ (9~27) in *El. sibiricus*×*Ag. tsukushiense* and 0.04_{V} (0~1)$+0.28_{IV}$ (0~2)$+0.32_{III}$ (0~2)$+17.67_{II}$ (13~21)$+4.41_{I}$ (0~16) in *El. dahuricus*×*Ag. tsukushiense*. (The parentheses indicate the range of respective pairing.) *Ag. tsukushiense* is an allohexaploid whose genome constitution comprises three different genomes (Sakamoto, 1964). From the present results, it is assumed that *El. sibiricus* and *El. dahuricus* used in this experiment contain at least one genome in the former and two in the latter that are homologous to those of *Ag. tsukushiense*, and that the third genome of *El. dahuricus* might be partially homologous to the remaining genome of *Ag. tsukushiense*

60. *Further observations on the flowering of* Oryza *species*[1]

Tadao C. KATAYAMA

Investigated were date of flowering, order of flowering among the spikelets of a panicle, number of spikelets which open on successive days and time of the day when they open. All characters are important in practical breeding as well as in ecological and phylogenetic studies of rice. Some data have already been reported in 1960 and 1962. This year, further observations were carried out using four wild species, *O. australiensis*, *O. brachyantha*, *O. tisseranti* and *O. longiglumis*, which were newly added to our collection.

Several strains of these species were grown in the greenhouse. Observations were made from September 15 to November 16. Time of flowering was determined by the time of opening of the lemma and palea of the first spikelet in a panicle. The duration of flowering was indicated by the interval between opening and closing of a given spikelet.

Results of observations concerning the time of the day were as follows: *O. australiensis* flowered in the evening. Its behavior was very different from that of cultivated species and their close relatives, the wild *Perennis* group. On the other hand, the behavior of *O. brachyantha* and *O. tisseranti* was quite similar; they started to flower from 10 to 11 a.m. The spikelets of *O. longiglumis* opened very early in the morning as observed also in *O. ridleyi*. The results obtained in four years indicated that time and duration of flowering of taxonomically related species are similar. Generally the time was in any species gradually delayed from September to November.

The opening of the spikelets in a panicle takes place in all species in a regular sequence; they start to open on the uppermost branch of a panicle continuing to the lower branches in succession. However, some differences in the order of opening spikelets in a branch are found among the species. In *O. australiensis* and *O. longiglumis*, flowering occurs first in the uppermost spikelet of a branch, followed by the lowest one and then proceeds again toward the upper spikelets in successive order. Consequently, the opening of the second spikelet from the top is the latest in a branch. In other two species, *O. brachyantha* and *O. tisseranti*, flowering starts at the top of a branch, proceeding straight downward.

In *O. australiensis*, *O. brachyantha* and *O. tisseranti*, flowering is continuous. In *O. longiglumis* and *O. ridleyi*, a several days interval was observed between the opening of the uppermost spikelet (always

[1] This work was supported by the Grant RF 62027 from the Rockefeller Foundation.

male-sterile) and that of the second one in a branch. However, this behavior was not detected in the remaining three species, *O. australiensis*, *O. brachyantha* and *O. tisseranti*.

61. *Diallel crosses in Sikkimese rice*[1)]

Tadao C. KATAYAMA

KIHARA *et al.* (1960 and 1964) reported the occurrence of *indica* and *japonica* types in Sikkimese rice based on the examination of morphological characters, phenol reaction, pollen and seed fertility and hybridization. Most important for the classification of cultivated rice into *indica* and *japonica* types is the hybrid sterility between the two types. Therefore, in order to reconfirm our classification of Sikkimese rice and to clarify the relationships between its strains, diallel crosses were carried out using sixteen strains, namely, fourteen from Sikkimese rice, one *indica* type strain and one of *japonica* type.

Most of crosses were successful and 1,583 hybrid seeds were obtained. These seeds will be sown next year for the analysis of pollen and seed fertility. The observed crossability fairly well indicated a considerable degree of intervarietal differentiation among Sikkimese strains.

62. *Preliminary studies on tillering in the genus* Oryza

Tadao C. KATAYAMA

Investigated were date of initiation and shooting of tillers, order of tillering, developmental state of tillers, whether vegetative or reproductive, and order of shooting of tillers after the main stalk had changed to reproductive state. The study was undertaken in order to provide information on the factors governing the type of growth habit, whether annual or perennial, a character of great importance in rice breeding and cultivation. This year 15 strains covering 8 species were used and the plants were grown in wooden boxes. Observations of tillering were made several times between June 15 and December 7.

In *O. glaberrima*, *O. breviligulata* and *O. stapfii*, tillering was detected only from the first tiller of the main stalk. In the remaining five species, *O. sativa*, *O. perennis*, *O. barthii*, *O. officinalis* and *O. subulata*, however, tillering was observed in the first, second and higher tillers of the main stalk. The primordia of the tillers were generally initiated

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

in regular sequence during the life history of a plant.

As soon as the main stalk after forming the panicle was cut off, primordia at the second or third node counted from the top developed at once but none at the first node. Later, the lower primordia developed gradually. An exceptional case was observed in *O. subulata*; in this species only the first and second primordia from the top developed into shoots, but not the lower ones.

Most of the tiller primordia were fixed after the main stalk had changed to reproductive state. Fixed materials were studied anatomically under dissecting microscope. In perennial species, such as *O. sativa*, *O. perennis* or *O. officinalis*, many primordia in relatively lower nodes were found to remain in vegetative state. Such primordia could play an important role in maintaining the plants as perennials. On the contrary, in annual species, such as *O. glaberrima* or *O. breviligulata*, all primordia observed were reproductive and no vegetative primordia were found. It is concluded that the anatomical difference between annual and perennial species in *Oryza* depends on the developmental state of tiller primordia, whether a change to reproduction occurs or they remain vegetative, when the primordium of the main stalk changed from the vegetative into the reproductive state.

63. *Preliminary studies on embryo transplantation in the genus* Oryza

Tadao C. KATAYAMA

Embryo transplantation between several rice species was attempted. This year, the technical possibility of embryo transplantation was explored using 10 strains of five species of *Oryza*, *O. sativa*, *O. sativa* var. *spontanea*, *O. perennis*, *O. glaberrima* and *O. breviligulata*. The dormant seeds were sterilized for 30 minutes in 10% chlorinated lime solution and washed twice in sterilized water. The seeds were divided by a sharp knife into embryo and endosperm. Four kinds of experimental materials were used, namely, intact seeds, embryos alone, embryos transplanted to their own endosperm (homogeneous transplantation) and embryos transplanted to alien endosperm (heterogeneous transplantation) of other strains or species. The materials were placed on aseptic agar and kept under continuous dark condition at 25°C. Observations were made from 30 to 60 days after incubation.

In general, the transplantation was successful not only in intraspecific, but also in some interspecific combinations. Furthermore, fusion between tissue of embryo and that of endosperm was ascertained in some intra-

specific combinations. The growth of seedlings obtained from homogeneous transplantations was more vigorous than from heterogeneous ones.

64. *Photoperiodic responses of* Oryza *species, VII*[1)]

Tadao C. KATAYAMA

Several factors are responsible for different photoperiodic sensitivities among various *Oryza* strains. The acceleration degree, which was expressed by differential heading acceleration under short day condition, was dealt with in a previous paper. This year, the acceleration degree is reported for several low latitude strains of *O. sativa*, *O. sativa* var. *spontanea*, *O. perennis* and *O. officinalis*, which were recently collected in Borneo and Java. Several cultivated and wild strains from India and Japan were also used for comparison. The acceleration degree of heading date was expressed by three different methods as previously reported (KATAYAMA, 1963).

Heading date of the strains depended strongly upon day length. According to the data reported in 1963, a high correlation was found between geographical distribution and sensitivity, namely, strains distributed in low latitudes showed in general greater sensitivity than those of high latitudes. The data obtained confirmed the validity of this correlation. This result answers the question, why photoperiodically sensitive strains predominate even in low latitudes. It is assumed that the response of these strains to a small change of day length gives them a selective advantage in natural condition.

One of the factors influencing photoperiodic sensitivity is the so-called accumulation effect. The accumulation effect is shown by the photoperiodic effectiveness of short day treatment interrupted by long day condition. Accumulation effect of 20 strains of *O. sativa*, *O. sativa* var. *spontanea*, *O. perennis* and *O. glaberrima* were analyzed this year. A combination of 12^h30^m light+11^h30^m dark was selected as short day condition, whereas natural day length from June to August was used as long day treatment.

The effectiveness was measured by the degree of flowering induction in relation to the frequency of short day plus long day treatment. Generally, 10 day interruption of long day condition in short day treatment cancelled out thoroughly the effect of short day treatment. It was clear that the shorter was the long day condition inserted between two short day treatments, the more prominent was the accumulation effect.

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

Also, it was found that the lower was the latitude from where the strains were originated, the smaller was the accumulation effect.

65. *Genetic studies on developmental instability in* Oryza sativa

Kan-Ichi SAKAI

Investigations have been carried out cooperatively by the staff members of the writer's laboratory. They are still under way, but a brief account is given.

By comparing control and X-rayed progeny lines of 6th generation after irradiation, the genetic effect of X-rays was investigated on developmental instability in leaves. It was found that the flag leaf and the third leaf counted from it downward showed an increase in genetic variation for intraplant variability. The mutation was observed to occur toward higher instability. The next leaf to the flag, however, showed at least no increase in genetic variation by X-rays. The mutation in length of those three leaves was found to behave in a similar way.

In parallel with the above experiment, inheritance of intraplot variability or developmental instability has been investigated on plant height, plant weight, muber of culms per plant, seed yield and panicle length. It was found that correlation between two consecutive generations was statistically significant and as high as 0.57 to 0.34 for the characters given above except for the culm number per plant. Further study is required in order to draw a definite conclusion.

66. *Survey of F_1 sterility relationships between strains of* Oryza perennis

Hiroko MORISHIMA and Hiko-Ichi OKA

We have been engaged in this work since 1958. This year, several strains from New Guinea were observed in addition to other materials. Some of them are of spontanea type (small annual plants), while the others are of perennis type. In their natural habitats, they are completely isolated from cultivated rice. This may prove that the spontanea type is not necessarily a hybrid derivative from *O. perennis* and *O. sativa*. The New Guinean spontanea strains showed a high F_1 sterility when crossed with strains of Asian, American and African origins, but the perennis strains generally produced fertile F_1 hybrids. The survey is still under way.

67. *Variation in characters related to seed dispersion in* Oryza perennis

Hiko-Ichi OKA and Hiroko MORISHIMA

In wild grasses, the awn might be the organ responsible for seed dispersion. Its length, diameter (at the 1 cm point from the apiculus) and the length of bristles on its surface (at the same place as above) were measured in a number of strains of *Oryza perennis*. The three measurements, each varying within a range among strains, were found to be inter-correlated. Their standardized values were then summed up to obtain an index of seed dispersability. Among the strains used, the index was positively correlated with grain shedding and seed dormancy, and negatively with the regenerating ability of stem cuttings.

68. *Deterioration of F_1 embryos in hybrids between subsp.* barthii *and other forms of* Oryza perennis

Yaw-En CHU and Hiko-Ichi OKA

Oryza perennis subsp. *barthii* is a wild form distributed in Africa. When cross-pollination was made between it and other forms of *O. perennis* in either direction, a part of the F_1 embryos were found to deteriorate five to six days after fertilization. The frequency of deteriorated embryos differs according to cross-combination. It was found that when deterioration occurs, the differentiation of embryonic cells into plumula and seminal root primordia could be blocked. This phenomenon may be regarded as a type of hybrid inviability taking place in young embryos.

69. *An observation of cultivation pressure on seed germination*

Hiko-Ichi OKA

When a population of wild plants is cultivated, genes expressing characters of cultivated forms will have an advantage under the pressure of selection due to cultivation, which we call "cultivation pressure". Five hybrid populations between *Oryza perennis* and *O. sativa* were each divided into two plots; in one of them, seeds were harvested at maturity and were sown next spring on puddled soil, while in the other plot seeds were not harvested and the naturally shed seeds germinated after the remaining plants of the previous generation were removed. From a comparison between them, it was found that plants in the seeded plots tended to resemble cultivated rice, while those of the naturally

germinating plots had many traits of wild rice, *e.g.*, spreading panicle-branches, a high percentage of grain shedding, etc. This indicates that when seeds are sown, certain cultivated types are selected, while characters discriminating between wild and cultivated types are genetically inter-correlated. This experiment is continued in Chung-Hsing University, Taichung, Taiwan.

70. Pachytene analysis and an observation of chromosome associaion in haploid rice

Yaw-En CHU and Yô TAKENAKA

The pachytene chromosomes of haploid plants of cultivated rice (*O. sativa*) could be divided into 3 types according to their length and arm ratio. When they were numbered in the order of length, chromosomes 3, 4 and 7 were of median type, and chromosome 11 of sub-terminal type, while the rest were of sub-median type. In most cells, cromosomes 10 and 12 were attached to the nucleolus, the former with a higher frequency than the latter. Chromosome 1 was 2.8 times as long as chromosome 12.

At diakinesis and metaphase I, 30 different association types were found, their number ranging from 0 to 8. The average number of associations per cell was 3.23, 2.90 and 2.48 at pachytene, diakinesis and metaphase I, respectively. The maximum configuration of associating haploid chromosomes has been regarded to be $2(3)+3(2)$, namely association of 7, by many researchers. But such configurations as including 3(3) and 1(4) were found, with the frequency of 36/680. It seems that $2(3)+3(2)$ is not maximum.

If the association of chromosomes in a haploid cell is due to a specific affinity between partially homologous segments, it should occur between definite chromosomes. Distinction of individual pachytene chromosomes was attempted on the basis of their length and arm ratio, and the position of association in each chromosome was recorded. Distinguishing between chromosomes 5 and 6, and 9 and 10 was difficult, but chromosome 1 could be easily identified. It showed association with 3 different chromosomes at different positions. This suggests that chromosome 1 can simultaneously associate with three others, forming a group of four. In general, the distal part of chromosomes had more associations than the proximal part. Some association configurations appeared as if a chiasma was formed. For estimating the residual homology between chromosomes, it might be more useful to study their distribution frequency in the associations than determining the maximum association which represents the extreme case of variation.

We may take it for granted that the association in haploid cells indicates the presence of homologous segments in chromosomes, but the pattern of association actually observed might be influenced somewhat by the other conditions.

71. *The genetic analysis of developmental instability in leaves of* Nicotiana tabacum *by diallel crosses*

Yoshiya SHIMAMOTO and Kan-Ichi SAKAI

Developmental instability in leaves of *N. tabacum* was investigated by the method of diallel crosses. Six varieties, Ambalema, T. I. 448A, Bright Yellow, Daruma, Sumatra and Connecticut Broad Leaf and their F_1 hybrids were grown in randomized blocks with two replications. Eight plants were selected at random from each strain and bilateral asymmetry of leaf blade and vein distance variability were measured in five leaves of eace plant.

Bilateral asymmetry was measured by the absolute difference between left and right sides of blade, divided by the total width. The vein distance variability was measured by intraleaf standard deviation of vein distance from the mid-rib divided by average vein distance. The figures thus obtained are understood to be the indices of developmental instability of tobacco leaves.

Experimental results generally showed no sign of heterosis in both instabilities, whereas the degree of dominance of genes governing bilateral asymmetry and vein distance variability was 0.6278 and 0.6347, respectively. The dominance was toward instability for bilateral asymmetry, but toward stability for vein distance variability.

Analysis of variance proved that additive gene effect, dominance effect in specific combinations, maternal effect and reciprocal differences in specific crosses were statistically significant.

72. *Tumor formation in interspecific hybrids of* Nicotiana, *especially in relation to injury*

Yô TAKENAKA and Yoshiaki YONEDA

Among many specific hybrids in *Nicotiana* which were made for cytotaxonomical researches, we observed 11 tumorous ones, namely, F_1 *N. glauca*×*N. langsdorffii*, F_1 *N. glauca*×*N. longiflora*, F_1 *N. glauca*×*N. plumbaginifolia*, F_1 *N. paniculata*×*N. langsdorffii*, F_1 *N. rustica*×*N. cavanillesii*, F_1 *N. suaveolens*×*N. langsdorffii*, F_1 *N. suaveolens*×*N. plumbaginifolia*, F_1 *N. suaveolens*×*N. longiflora*, F_1 *N. gossei*×*N. alata*, F_1 *N.*

gossei×*N. longiflora* and F_1 4x *N. tabacum*×*N. alata*. Among them, 3 hybrids are newly found by us, namely, F_1 *N. gossei*×*N. alata*, F_1 *N. gossei*×*N. longiflora* and F_1 *N. suaveolens*×*N. plumbaginifolia*.

The tumor shapes ranged from teratoma-type to massive-type. In F_1 *N. glauca*×*N. langsdorffii* whose tumor formation is most striking, spontaneous stem tumors begin to grow at the end of flowering. Their location was: a) cicatriculae of flowers and leaves, b) apexes of lateral shoots, c) internodes and d) various parts of roots.

Injury of leaf veins and stems of F_1 *N. glauca*×*N. langsdorffii* was observed to stimulate tumor formation. Pricking experiments showed that tumors were more frequently induced in the upper part of the stem than in the lower one.

73. *Hereditary tumor in Japanese morning glory*

Yô TAKENAKA and Yoshiaki YONEDA

For fourteen years we have cultured many strains and hybrids of Japanese morning glory (*Pharbitis Nil*). Among the offspring of strain 056 which is assumed to have the dominant gene *Blown* (*B*) and recessive genes *delicate* (*dl*) and *feathered* (*fe*) and also those of strain 058 assumed to have the genes *B* and *fe*, neither producing seeds in homozygous condition, we found many individuals showing tumor-like shoot development at cotyledonary stage. As the result of our observation of the external morphology of cotyledons and normal parts of those individuals, the co-existence of *B* and *fe* genes in homozygous condition is considered to be connected with the occurrence of the tumor.

In *in vivo* tumor tissues, we found various irregular configurations of vascular elements.

In vitro tumor tissues grew slowly on modified Whites' agar medium but the addition of yeast extract stimulated the growth considerably. Those cultured tissues showed frequently remarkable high polyploidy.

74. *Chromosomes of some species of genus* Paspalum

Yasumichi TERADA and Yô TAKENAKA

As the first step for finding good pasture species of genus *Paspalum*, chromosome numbers and meiotic behavior of those grasses were observed. The results are summarized in Table 1, together with other researchers' results.

The somatic chromosome number of *P. notatum* was reported to be 20 or 40 by SAURA (1948). The authors counted 20 somatic chromosomes

Table 1. Somatic and meiotic chromosome number of genus *Paspalum*.

Species	Strain or origin	Chromosome number 2n	n	Meiotic behavior	Researcher
P. notatum		20,40			SAURA, 1948
〃	Pensacola F. C 33195	20	10	10 II	
〃	Pensacola BN 1309~57	20	10	10 II	
〃	Tifhi-1	20	10	10 II	
〃	Argentina	—	10***		
〃	Paraguay	—	10***		
〃	Common	40	20	20 II	
P. dilatatum		50			KRISHNASWAMY, 1940
〃		40			BROWN, 1948
〃	L-B 230 F. C 32960	50	30	20II+10I	
〃	L-B 230 Po×46777	50	30	20II+10I	
〃	Common	50	30	20II+10I	
〃	Yellow anther A & B*	50	30	20II+10I	
〃	Local A & B**	50	30	20II+10I	
P. dilatatum var. *paucicilatum*		40			PORODI, 1946
〃	Prostrate	40	not defined	10II+10I+ 10I****	
P. thunbergii		20			MORIYA and KONDO, 1950
〃		40			ONO and TATEOKA, 1953
〃	Kumamoto	40	—		
P. malacophyllum		40			BURTON, 1940
〃	Okinawa	40	20	20 II	

* Found in the collection of Kyushu Agri. Ex. Sta.
** Collected in Kumamoto, Kyushu, Japan.
*** 20 chromosomes are rarely found at diakinesis and MI.
**** Not yet confirmed.

and 10 bivalents in 3 strains, and 40 somatic chromosomes and 20 bivalents in one strain.

The somatic chromosome number in *P. dilatatum* was observed to be 50 by KRISHNASWAMY (1940) but 40 by BROWN (1948). The authors counted 50 somatic and about 30 meiotic chromosomes at diakinesis and MI, being generally the configuration of 20 bivalents and 10 univalents.

PORODI (1946) reported 40 somatic chromosomes in *P. dilatatum* var. *prucicilatum*. The authors also found 40 somatic chromosomes, but they

could not define the meiotic chromosome number, although they found rarely 10 bivalents and 20 univalents at MI of PMC.

In *P. thunbergii*, MORIYA and KONDO (1950) counted 20 somatic chromosomes but ONO and TAKEOKA (1953) counted 40. The authors also found 40 somatic chromosomes in a strain collected in Kumamoto. BURTON (1940) reported 40 somatic chromosomes in *P. malacophyllum* and the authors found also 40 somatic chromosomes and 20 bivalents at MI of PMC in a strain brought from Okinawa.

At present genome analyses of the genus are underway.

75. The origin of Prunus yedoensis, *II*

Yô TAKENAKA

The external morphology of about 130 seedlings of *P. yedoensis* was observed. A few typical *P. lannesiana* var. *speciosa* and *P. subhirtella* var. *pendula* form *ascendens* were found among them and many others ranged from *speciosa* to *ascendens* with wide distribution of various character combinations. Needless to say a few *P. yedoensis*-like seedlings were also found.

Fourteen hybrids between *speciosa* and *ascendens* grew well. Although they showed minor differences from each other, they belonged all to the *yedoensis* group from the morphological point of view. But they had larger leaves and flowers and more stamens than *P. yedoensis* and the flower color of all plants except one was pink fainter than that of *P. yedoensis*.

Hybrids between *P. lannesiana* var. *speciosa* and *P. subhirtella* var. *pendula* had more delicate branches and a little smaller leaves and flowers than *P. yedoensis*. In general, *P. yedoensis* may be assumed to be a hybrid between *speciosa* and *ascendens* rather than between *speciosa* and *pendula*.

Nineteen hybrids between *speciosa* and *yedoensis* were observed. In the hybrids between *speciosa* and *ascendens*, hairiness of leaves, peduncles and calyxes and umbel inflorescence were assumed to be dominant to hairless and corymb respectively. In all mentioned hybrids, these dominancies were also recognized.

Funabara-yoshino, which was found on Funabara-pass in Izu, had somewhat larger flowers and leaves and a few more stamens than *P. yedoensis*, but was very similar in the other characters to *P. yedoensis* and showed heterosis like *P. yedoensis*. Therefore this flowering cherry tree is also assumed to be a new natural hybrid between *speciosa* and *ascendens*.

Kurama-zakura, which has been cultivated in Kumamoto since long

ago, has leaves and flowers somewhat larger than those of *yedoensis* and its branches are slightly bent. But the other characters and appearance of heterosis are the same as in *yedoensis*. Accordingly this tree may be another hybrid between *speciosa* and *ascensens*.

Midori-yoshino (*P. yedoensis* var. *Nikaii* HONDA) has been cultivated in Hagi from Edo-period but is not encountered there now. By identification from specimens, all characters are identical with those of *yedoensis*, except one character, namely white petal color. This plant is also assumed to be a hybrid between *speciosa* and *ascendens* and might have originated in Izu and have spread as a garden plant from there.

I found some trees which could presumably be the offspring of *yedoensis* in Izu, Boso and Noto. Similar plants were found by some taxonomists. Some of them may be the offspring of *yedoensis* itself and the others may be that of the other hybrids of the same origin.

76. Method of estimating genetic parameters in a forest where inter-tree competition is occurring

Kan-Ichi SAKAI and Hiromasa MUKAIDE

SAKAI and HATAKEYAMA have published in 1963 a paper dealing with a new method of estimating the genetic parameters in a standing forest of *Abies*. The method was based upon the principle of random distribution of genotypes in a forest, in contrast to the heterogeneous pattern of distribution due to environment. In a further study on *Cryptomeria*, however, the method was found not to be appropriate because of inter-tree competition in a forest, on the one hand, and of the necessity of estimating the genetic parameters in face of competitive pressure, on the other.

Inter-tree competition is concluded to occur: (1) if correlation between two adjoining trees is zero or negative, (2) if variance of difference between two adjoining trees is equal to or larger than the two-fold variance of single trees, (3) if correlation between the difference between adjoining i and j trees and the difference between adjoining j and k trees, is equal to or smaller than -0.5 or (4) if variance of difference between $(i-j)$ and $(j-k)$ trees is equal to or larger than the six-fold variance of single trees.

When we detect the occurrence of inter-tree competition in a forest, estimation of genetic parameters can be made in the following way: Variance of average value of x trees in each cluster is expected to involve genotypic, environmental and competitional variances as follows:

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

where G, E and C stand for genotypic, environmental and competitional variances, respectively, while b is a constant lying between 0 and 1 which relates to soil heterogeneity, and T_x is a coefficient of C which varies in accord with x. If we take various sizes of clusters in a forest and compute variances, then we can solve the following set of simultaneous equations for B, G, E and C, which show the best fit between observed and expected variances.

$$\begin{aligned}
V_{(1)} &= G + E + C, \\
2V_{(2)} &= G + 2^B E + 0.600C, \\
3V_{(3)} &= G + 3^B E + 0.535C, \\
4V_{(4)} &= G + 4^B E + 0.300C, \\
5V_{(5)} &= G + 5^B E + 0.340C, \\
6V_{(6)} &= G + 6^B E + 0.235C, \\
7V_{(7)} &= G + 7^B E + 0.270C, \\
8V_{(8)} &= G + 8^B E + 0.215C, \\
9V_{(9)} &= G + 9^B E + 0.173C,
\end{aligned}$$

where $B=1-b$. Finding those G, E and C values allows us to compute heritability. Genetic correlations between two traits are also computed by finding covariances in place of variances.

Investigation in actual forests of *Cryptomeria* has proved this method to be useful. Heritability values for stem diameter and tree height of *Cryptomeria japonica* estimated by this study are so low as 0.1 to 0.4, while genetic correlation between them is 0.6 to 0.9. An important finding in the course of this study was that trees in an isogenic forest (a clone) do not compete against each other, which deserves a further study. Details will be published before long.

C. MATHEMATICAL GENETICS

77. The number of alleles that can be maintained in a finite population

Motoo KIMURA

It is not known for certain whether the so-called wild type gene in a natural population is usually a single entity or a complex consisting of numerous isoalleles that are indistinguishable by any ordinary means. Since each gene is made of a large number of nucleotide pairs, the latter possibility can not be excluded. In the present work the number of alleles that may be maintained in a finite population was calculated assuming three models: (1) A system of selective neutral isoalleles whose

frequency in the population is determined by the mutation rate and by random drift. (2) A system of mutually heterotic alleles. (3) A mixture of heterotic and harmful mutants.

The work was carried out in collaboration with Dr. J. F. CROW of the University of Wisconsin.

In the present calculation it is assumed that all homozygotes are equally deleterious and that each new mutant is an allele that does not already exist in the population. Also random mating is assumed.

When there is no selection at all, the effective number of isoalleles maintained in the population is approximately $4N_eu+1$, where N_e is the effective population number and u is the mutation rate. The effective number of alleles is defined as the reciprocal of the sum of squares of allelic frequencies in a population. Thus, if $4N_e$ is much less than the reciprocal of the mutation rate, most individuals in the population will be homozygous for this locus.

When there is overdominance, the result is more complicated, but, the relationship between the effective number of alleles and the population number is given graphically for various combinations of mutation rate and selection coefficient.

For details, see KIMURA and CROW (1964).

78. The mutational load with an epistatic interaction

Motoo KIMURA

In this report we will consider the following situation: Mutations are deleterious in heterozygous as well as homozygous condition but the harmful effect of each mutant gene is small. The mutation rate per locus, however, is much smaller than the selection coefficient against each mutant gene, with the result that its equilibrium frequency is very low. Because of a large number of loci involved, the total mutation rate per individual may nevertheless be appreciable and the number of mutant genes accumulated in each individual may be much larger than unity. The population is very large and random mating is practiced.

Under such conditions, most of the mutant genes may be held in heterozygous condition, while in each locus the homozygous condition is so rare that its effect may be neglected in the following calculation.

The main purpose of the present calculation is to investigate the mutational load when the deleterious effect of mutant genes against an individual is proportional to the second order polynomial of the number of mutant genes (x) contained in the individual.

Let W_x be the selective value of an individual having x mutant genes

($x=0, 1, 2, \ldots.$), assuming $W_x=e^{-h_1x-h_2x^2}$.

It is difficult to work out the exact probability distribution of x among individuals at equilibrium, but under free recombination and slow selection, it may be approximated with the normal distribution, unless the mean number of mutant genes per individual is very small: Let λ and σ^2 be the mean and the variance of such distribution, then $\xi=(x-\lambda)/\sigma$ is distributed normally with mean 0 and variance unity, where $\sigma=\sqrt{\lambda}$:

$$f(\xi)=\frac{1}{\sqrt{2\pi}}e^{-\xi^2/2}.$$

Here $f(\xi)$ represents the distribution of mutant genes at fertilization, *i.e.* after recombination but before selection. The selection coefficient of a mutant gene may be obtained from

$$-h=\int(w_{x+1}-w_x)f(\xi)d\xi\Big/\int w_xf(\xi)d\xi.$$

Thus

$$h=1-\exp\left\{-\frac{h_1+h_2+2h_2\lambda}{1+2h_2\lambda}\right\}.$$

Let p be the frequency of the mutant gene in a locus, then $\lambda=\sum 2p$, where the summation is over all relevant loci.

At equilibrium in which mutation and selection balance each other,

$$hp=\mu,$$

where μ is the mutation rate in the locus. This leads approximately to

$$(1)\quad 2h_2\lambda^2+(h_1+h_2-2h_2M)\lambda-M=0,$$

where $M=\sum 2\mu$ is the total mutation rate per individual and λ is obtained as a solution of equation (1). With this λ, the mutational load is

$$(2)\quad L=1-\frac{1}{\sqrt{1+2h_2\lambda}}\exp\left\{-\frac{(h_1+h_2\lambda)}{1+2h_2\lambda}\right\}.$$

If $h_2\lambda\ll 1$, (1) and (2) may be substituted by much simpler relations:

$$(1')\quad 2h_2\lambda^2+h_1\lambda-M=0$$
$$(2')\quad L=1-e^{-(h_1+h_2\lambda)\lambda}.$$

In the special case of $h_1=0$, *i. e.*, if the decrease of fitness of an individual is proportional to the square of the number of mutant genes within the individual,

$$L=1-e^{-M/2}.$$

Namely, the mutational load is roughly equal to half the total mutation rate.

On the other hand, if $h_2=0$, *i. e.*, if the decrease of fitness is proportional to the number of mutant genes,

$$L=1-e^{-M}\ .$$

This is the well-known case of mutational load being roughly equal to the total mutation rate.

The above calculation is based on the normal approximation of distribution of x. It is probable that, because of the epistasis and linkage, σ of the actual distribution is slightly smaller than $\sqrt{\bar{\lambda}}$. Therefore, it is interesting to note that at the limit of $\sigma=0$ the load is exactly given by (1′) and (2′). This means that these two relations (1′) and (2′) may be used to calculate the mutational load with good approximation for most of the cases.

79. *Theory of the gene frequency cline in a habitat of finite length*

Motoo KIMURA

In the mathematical theories of cline developed by HALDANE and FISHER, the length of habitat is assumed to be infinite. The purpose of the present study is to investigate the gene frequency cline assuming a finite length of habitat.

Consider a linear habitat represented by a finite interval $[L_1, L_2]$ on one dimensional coordinate axis and designate by X a position on the habitat. Let y be the distance between the point of birth of an individual and that of its parent. We assume that y is distributed with mean 0 and variance $\sigma_m{}^2$. Let $p(X)$ be the frequency of a gene A_1 at point X, and assume that it may be changed by migration and selection. The rate of change by selection is assumed to be represented by $sp(1-p)f(p, X)$ where s is a constant and $f(p, X)$ is a function of p and X. When equilibrium is reached in the gene frequency cline, $p(X)$ may be given by the solution of

$$(1)\qquad \frac{\sigma_m{}^2}{2}\cdot\frac{d^2p}{dX^2}+sp(1-p)f(p, X)=0\ ,\quad (L_1\leqq X\leqq L_2)$$

that satisfies the boundary conditions

$$(2)\qquad \frac{dp}{dX}=0$$

both at $X=L_1$ and $X=L_2$.

Thus

$$\int_{L_1}^{L_2}p(1-p)f(p, X)dX=0\ .$$

If the cline is mild, $p(X)$ may be expressed by the power series of X. For example, in the case of no dominance between a pair of alleles, A_1

and A_2, if A_1 is advantageous on the left side of point X_0 and disadvantageous on the right side of X_0, and if the selection coefficient is proportional to the distance from X_0, then

$$f(p,\ X)=-(X-X_0)\ .$$

In this case,

$$X_0=\int_{L_1}^{L_2} Xp(1-p)dX \Big/ \int_{L_1}^{L_2} p(1-p)dX\ .$$

Let $x=(X-X_0)/\alpha$, where $\alpha^3=\sigma_m{}^2/(2s)$, then (1) and (2) becomes respectively

(1′) $p''=xp(1-p)$

and

(2′) $p'=0$ at $x=l_1$ and l_2, $(l_1<0,\ l_2>0)$,

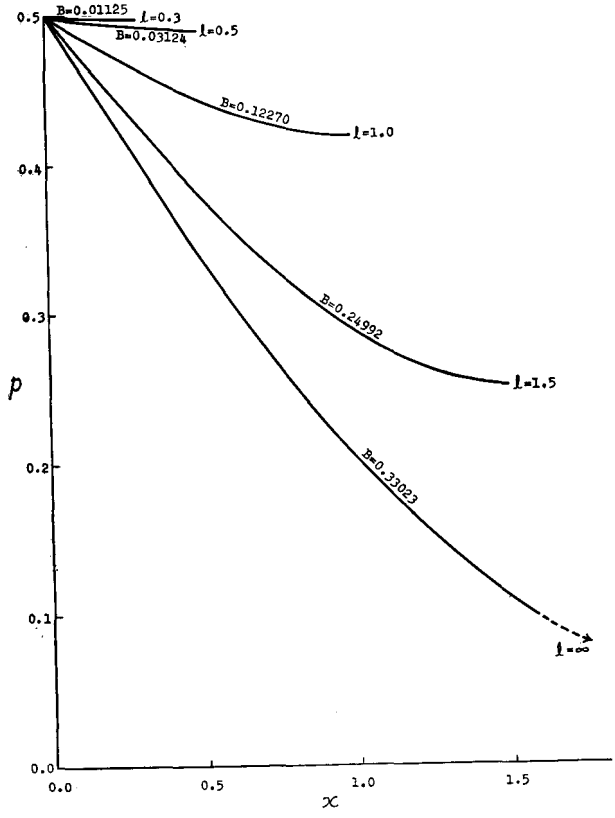


Fig. 1. Relation between x and p.

where $l_1=(L_1-X_0)/\alpha$ and $l_2=(L_2-X_0)/\alpha$.
The solution is given by

$$(3)\quad p=A-Bx+\frac{A(1-A)}{2\cdot3}x^3+\frac{B(2A-1)}{3\cdot4}x^4-\frac{B^2}{4\cdot5}x^5+\cdots,$$

where constants A and B are determined by (2'). For the symmetrical case of $-l_1=l_2=l$, we have $A=1/2$, $B=\frac{1}{8}l^2-\frac{11}{4608}l^5+\cdots$.

The power series expansion (3) seems to be quite accurate within the range of $-1.5<x<1.5$. Relation between x and p is graphically given in Fig. 1 for several values of l. Treatments for more general cases are under investigation.

D. GENETICS AND BIOCHEMISTRY OF MICROORGANISMS

80. Hybridization between Salmonella abony *and* S. abortus-equi[1)]

Tetsuo IINO and Yasuko SUZUKI

SJ457 is a non-flagellated (*fla*$^-$), consequently non-motile, mutant originated from a slow motile Hfr strain SW1391 (*gal*$^-$, *sm*^{r}) of *S. abony*. Motility of SW1391 is characterized by the formation of 'flare' on semisolid plates, in contrast with 'swarm' which is formed by motile clones.

Mating experiments were carried out between SJ457 and a strain of *S. abortus-equi* SJ152 (*fla-F*$^-$, *gal*$^+$, *sm*^{s}). When semisolid plates were used as selective media, flares as well as swarms appeared from mixture of the cells of both strains. Each of both, flares and swarms, was isolated, and genetic traits of the component cells were examined growing them on indicator plates. As a result, swarms were found to be *fla*$^+$ recombinant clones between SJ457 and SJ152. The flares were grouped into two types, namely stable and segregational. When cells of the stable type flares were dispersed in saline and spread on a semisolid plate, the colonies grown on the plate were all flares; while those from the segregational type dissociated into compact colonies, swarms and flares. The compact colonies were composed of non-flagellated cells and their genetic markers were mostly those of SJ152. The swarms were composed of the stable *fla*$^+$ hybrid cells. The flares were of segregational type or occasionally stable.

1) This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (AI-02872), Public Health Service, U. S. A.

From those observations, it is assumed that a flare of stable type is a recombinant clone which received a slow motility gene from an Hfr cell, and that of the segregational type is a hemizygous clone currently segregating recipient type cells and recombinants in its offspring.

81. *Mutation of flagellar antigen*-1.2 *in* Salmonella[1)]

Tetsuo IINO and Michiko MITANI

SW577 is a curly mutant strain derived from *S. typhimurium* TM2. Its phase-1 flagella are curly-*i* type and those of phase-2 are normal-*1.2* (IINO, Annual Report No. 9, 1958). Cells of SW577 grown on semisolid plates containing anti-*1.2* serum are inhibited in their motility by the contained antiserum, and cannot form swarms on the plates. Among the bacterial cultures, a mutant which can spread and form a swarm appears on the plates at the frequency of 10^{-6} to 10^{-7} per cell. Eighteen such mutant clones were isolated and their antigenicities were examined by slide agglutination. They were all found to belong to *1.2*-type. The agglutination titers of anti-*1.2* serum, which was prepared against SW577, given to each of these mutant clones were measured before and after absorption with each of the mutant cells in all pairwise combinations. From the absorption-agglutination test, it was found that each of the mutants had lost a fraction of *1.2*-antigen. Antigenic subunits lost by the mutants are schematically represented in Fig. 1.

Agglutination test with anti-*1* and anti-*2* sera indicated that the antigenic loss of clone-1 covers both antigen-*1* and -*2*, while others are de-

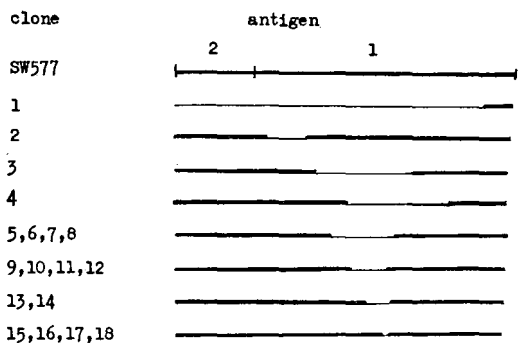


Fig. 1. Antigenic fractions lost in the mutant clones of SW577.

1) This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (AI-02872), Public Health Service, U.S.A.

ficient only in antigen-*1*. It was further indicated that antigen-*1* is composed of at least eight subunits.

82. *Flagellar mutants from a curly flagellar strain of* Salmonella[1)]

Michiko MITANI and Tetsuo IINO

Curly mutant cells have flagella with wavelength half of that of normal type (IINO, Annual Report No. 9, 1958). They cannot spread on semisolid plates and their growth is confined to the region of inoculation. After prolonged incubation on the plate, subclones which can spread and form swarms appear among the cultures. The edges of seven such swarms of independent origin were isolated from the culture of a curly strain of *S. abortus-equi*, SJ30 (phase-2 monophasic, (*a*): *enx*), and the characteristics of those subclones were compared with each other and with the curly type. From the observation of motility by hanging drop method under optical microscope, of swarm types spreading on semisolid agar plates and of flagellar morphology under electron microscope, it was found that they were divided into five groups as shown in Table 1.

Table 1. Five types of flagellar mutants originated from a curly strain, SJ30.

Type	Rate of spreading*	Movement in broth	Flagellar shape
Wild	1.00	translation	normal
I	2.45	translation wiggle	normal and curly
II	0.53	translation	small amplitude
III	0.44	circular translation wiggle	curly
IV	0.15	wiggle rotation	short
V	0.08	translation wiggle	hooked curly
Curly	0.00	rotation	curly

* Speed of spreading of the wild clone on a semisolid plate at 37°C was taken as 1.00.

1) This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (AI-02872), Public Health Service, U.S.A.

The structure gene of phase-2 flagellin, H_2, was transduced with P22-phage lysate from the five representative clones to the phase-1 monophasic strain of *S. typhimurium* SW1166 ((*i*):*1.2*). Motility and flagellar shape of the transductional clones in phase-2 showed the property of each donor. These results indicate that each of the motile subclones appeared by a mutation presumably in H_2; the mutation may have caused a structural alteration of flagellar protein resulting in the change of flagellar shape and consequently of cellular motility.

83. *Bundled flagella of curly mutant cells in* Salmonella[1]

Michiko MITANI

Flagellar morphology and motility of a moving bacterium were already observed by microcinematography under dark field microscope by A. PIJPER (1946). A swimming bacterium of a normal and a curly flagellar strain appears as a bright particle trailing the helically bundled flagella

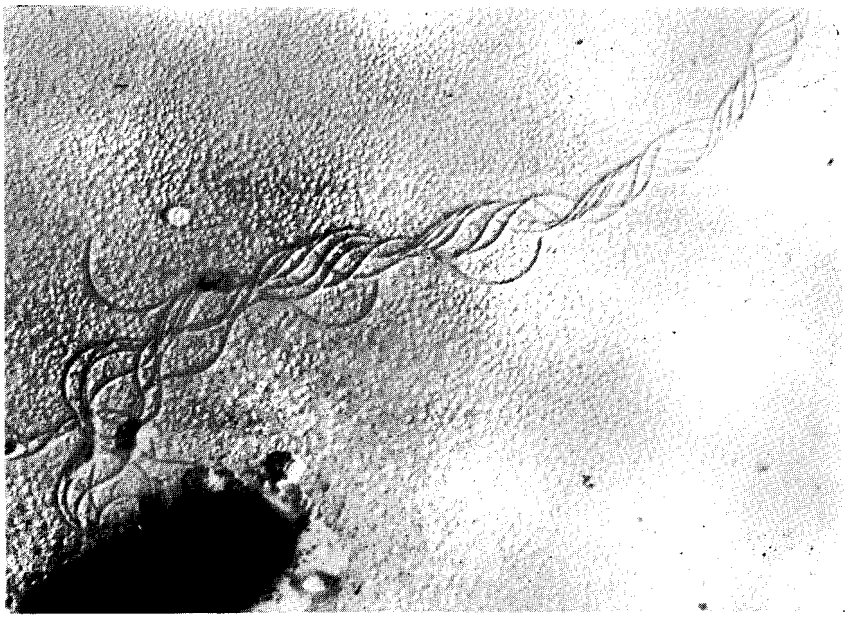


Fig. 1. Bundled flagella of a curly mutant cell observed by chromium shadowing.

[1] This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (AI-02872), Public Health Service, U.S.A., bestowed on Dr. T. IINO.

and moving forward by their screwing motion in the aqueous solution of 0.5% methylcellulose. Flagella of the normal type observed by electron microscope dissociated from each other distributing around the bacterial surface, and no image of bundled flagella was demonstrated under dark field microscope. During an investigation of flagellar shape, bundled, not dissociated, flagella have been observed under electron microscope on cells having curly flagella. Observed in a chromium shadowed sample, they were regularly wound about each other helically (Fig. 1). By the extensive observation, bundled flagella have also been found on normal flagellar cells, but at far less frequency than on those with curly flagella. The difference between the two might be ascribed to the difference in the flagellar shape itself. This state of flagella was expected to reveal their details in a moving bacterium. The connection between bundle formation of flagella and bacterial movement and the genetics of flagellar shape are now investigated.

84. A pauci-*flagellated mutant in* Salmonella typhimurium[1]

Masatoshi ENOMOTO

A slow motile mutant, SJ399, was isolated from a strain of *S. typhimurium*, TM2, on semisolid medium. Its cells were motile in broth culture at 37°C and their speed of movement and mode of locomotion were indistinguishable from those of the wild type, TM2, while its cells grown on semisolid medium did not give rise to any normal swarm even after 40 hour incubation at 37°C, and produced a narrow band of swarms at the margin of the inoculation site. From experiments on the swarm's progression on media containing a low agar concentration, it was found that the motility of this strain was hindered by the viscosity of the semisolid medium. H-antigenicity and morphological characters of this strain were the same as those of the wild type. The response to motility phage, chi, was somewhat different between SJ399 and the wild type. Plaques on cells of SJ399 were small and smeary, while those on the wild type cells were clear and large, and the efficiency of plating was 0.36 for SJ399. Moreover, it was found from the observations of stained preparations of flagella that their number per bacterium decreased to about one third of that of the wild type. The reduction in their number was also ascertained by biochemical measurement of flagellar protein which was purified after deflagellation of the cells. The content

[1] This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (AI-02872), Public Health Service, U.S.A., bestowed on Dr. T. IINO.

of flagellin in SJ399 was about 32% of that of the wild type. The mutant SJ399 was designated as "*pauci*-flagellated" mutant.

Genetic analyses by means of P22 phage mediated transductions from SJ399 to non-flagellated (*fla*$^-$) and paralysed (*mot*$^-$) mutants showed that the mutant, SJ399, complemented 7 *fla*$^-$s and 3 *mot*$^-$s which were representative mutants of the flagellation- and motility-cistrons, respectively. By recombination test it was found that the site of SJ399 was co-transduced with both A and B motility cistrons. Further, by experiments of two point crosses with mutants in motility A and B cistrons and a cross with a deletion type of B cistron, the site of SJ399 was found to be located in the latter.

85. *Sensitivity of* g*-group* Salmonella *to M8-phage, a host range mutant of chi-phage*[1]

Shigeru YAMAGUCHI

A Salmonella phage, chi, has been known to be unable to attack Salmonella serotypes which have the *g*-group flagellar antigens, but several host range mutants of chi which can attack some of them have been isolated. Among the mutants, M8 has the widest host range on the *g*-group Salmonella serotypes, but the degree of their sensitivity to M8 varies with strains.

In regard to the response to M8 in a spot test, the *g*-group Salmonella serotypes tested were classified into three types; that is, type-I which produces clear plaques with high efficiency of plating (e.o.p.), to which *S. hato* (*gms*:-), *S. banana* (*mt*:-) and *S. dublin* (*gp*:-) belong, type-II which produces turbid plaques with the e.o.p. of 10^{-1} to 10^{-3} of that of the type-I, to which *S. kingston* (*fg*:-), *S. enteritidis* (*gm*:-) and *S. blegdam* (*gmq*:-) belong and type-III which produces a vague patch when high titer lysate is spotted and does not produce any plaque with low titer lysate, to which *S. budapest* (*gt*:-), and *S. rostock* (*gpu*:-) belong. In type-III cells the phage cannot multiply.

From each of these *g*-group Salmonella serotypes, the antigentype-determining gene was transduced to *S. typhimurium* TM2 (*i*: *1.2*) and *S. abortus-equi* SJ241 (*a*: (*enx*)), which are sensitive to both chi and M8. Regardless of the type of the donor strains, all the transductants obtained were sensitive to M8 and produced clear plaques with high e.o.p., but were fully resistant to chi. Conversely, when the *i*-antigen-determining

[1] This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (AI-02872), Public Health Service, U.S.A., bestowed on Dr. T. IINO.

gene was transduced to the *g*-group *Salmonella*, each transductant showed almost the same response to both M8 and chi as that of the recipient to M8.

These results indicate that M8 can attach to all *g*-group antigenic flagella, and the differences in the response to M8 among the *g*-group Salmonella serotypes depend on some factors not associated with the determinant of the flagellar antigen, and also indicate that the host range mutant, M8, differs from the original phage, chi, only in the ability to attach to the flagella having *g*-group antigens.

86. *Effect of arginine added at various growth stages to the cultures of* arg-s *mutant of* Salmonella typhimurium

Jun-ichi ISHIDSU

The growth inhibition of an arginine sensitive mutant, *arg-s-1*, of *S. typhimurium* caused by arginine is promptly removed by the addition of uracil (ISHIDSU, Annual Report No. 14, 1963). On the contrary, the addition of arginine to a normally growing culture of this mutant did not cause immediate growth inhibition.

For example, when arginine was added to a culture growing normally in synthetic minimal medium at early log phase (O.D. 0.07 at 660 mμ, 2 hours after the cultivation was started) so as to get the final concentration of 10^{-3} *M*, the optical density of the culture continued to increase to 0.62 in 6 hours, being followed by a sudden drop (Fig. 1). Viable cell count and observation under a microscope showed that the residual O.D. increase was brought about first by cell division and then by cell elongation (Fig. 2). Elongation of cells starts about two hours after the addition of arginine and, at its maximum (in about six hours), they become as long as 6 to 7 times of the normal cell length. At the same time cell agglutination proceeds which causes, at its maximum, a sudden decrease in optical density.

When arginine was added at middle log phase (O.D. 0.5, 4.5 hours after the start of the cultivation), the inhibitory effect was far less pronounced and resulted in a slight delay only in O.D. increase and lowering of a stationary level (Fig. 1).

These findings suggest that during the normal growth period uracil itself or uracil synthesizing enzymes, or systems for synthesizing those enzymes are established and accumulated to a level high enough to permit cells to grow residually upon arginine addition. Also it is clear from Fig. 2 that arginine's action inhibits the growth of this mutant but does not kill it at least for the first 10 hours.

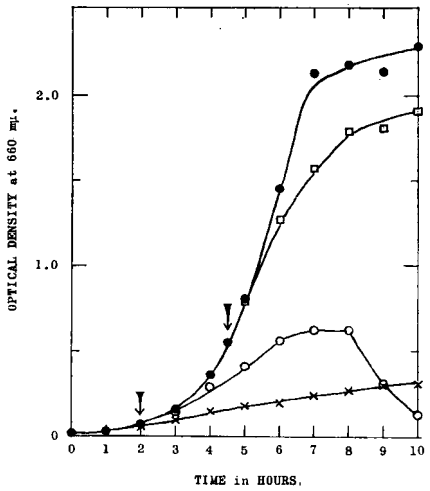


Fig. 1. Effect of arginine added at various growth stages.
↓ : Arginine addition.
●—●: Growth in minimal medium.
×—×: Growth under the presence of 10^{-3} *M* arginine.
○—○: Arginine was added at early log phase (at 2 hours).
□—□: Arginine was added at middle log phase (at 4.5 hours).

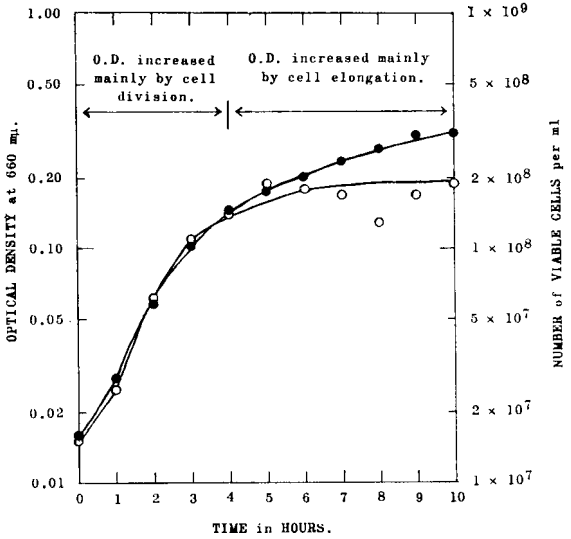


Fig. 2. Comparison of increases in O.D. and in number of viable cells.
●—●: Optical density.
○—○: Number of viable cells.

87. Effect of uracil together with arginine in the preculture of arg-s *mutant of* Salmonella typhimurium

Jun-ichi ISHIDSU

Addition of arginine to a normally growing culture of *arg-s-1* of *S. typhimurium* failed to inhibit immediately the growth of this arginine sensitive mutant (This Report, **86**). This made it very troublesome to investigate direct effect of arginine on the metabolic systems of the mutant from the biochemical point of view.

Conditions under which the growth of *arg-s-1* is immediately and absolutely inhibited upon arginine addition were searched for. Washing cells by saline, or starvation in minimal medium without carbon source but supplemented with 10^{-3} *M* arginine for 1 hour at 37°C was not effective enough. Even after these treatments, cells showed considerable residual growth when transferred to arginine medium.

Treatment with chloramphenicol (200 γ/ml) for 2 hours cut out this residual growth upon transfer to arginine medium that did not contain chloramphenicol. Successive dilution and cultivation method was also tried. Arginine was added to a normally growing culture at middle log phase (O.D. 0.55) and the culture was diluted into fresh arginine medium to the O.D. of 0.18 when the O.D. reached 0.70. Incubation was continued under shaking, and similar dilutions and cultivations were repeated several times successively. Step by step with repeated dilution, the slopes of growth curves became gentler suggesting that the accumulation of whatever it may be supporting the growth of the mutant even in the presence of arginine was being exhausted without being synthesized after the addition of arginine.

Absolute and immediate inhibition was observed when the mutant was first grown in a synthetic medium containing both arginine (10^{-3} *M*) and uracil (10^{-3} *M*) and then transferred to the arginine medium. Fig. 1 shows that, even when this transfer is made at middle log phase (O.D. 0.56), the residual growth can be neglected. In the case of transfer from the culture grown in minimal medium at a similar phase resulted in continuation of almost normal growth (This Report, **86**). As has been reported before (ISHIDSU, Annual Report No. 14, 1963), this mutant can grow quite normally in the medium supplemented with both arginine and uracil just as in the minimal medium. But the metabolic situation of cells under these two conditions is quite different. The following conclusions can be drawn from these experiments. (1) Arginine inhibition is caused by its inhibition of uracil synthesizing systems and resulting uracil starvation. (2) Uracil, when supplied together with arginine, can

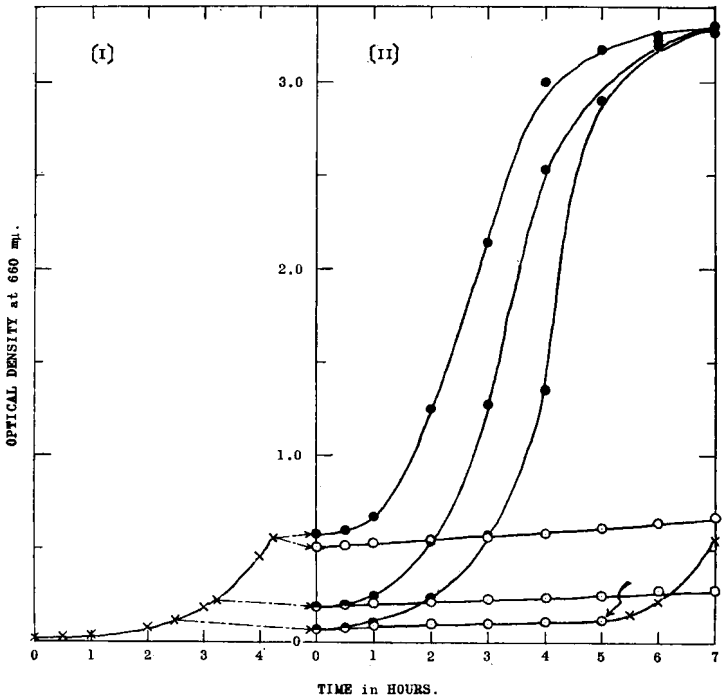


Fig. 1. Growth curves of *arg-s-1* first grown in a synthetic medium containing both arginine (10^{-3} *M*) and uracil (10^{-3} *M*) (×—×) and then transferred to minimal medium (●—●) or to the same medium containing only arginine (10^{-3} *M*) (○—○).

[I]: First culture. [II]: Second culture.
—→: Washing procedure. ↙: Uracil addition (10^{-3}*M*).

support the growth of *arg-s-1* but represses at least some part of uracil synthesizing systems. (3) Cells first grown in the medium containing both arginine and uracil suffer immediate growth inhibition when transferred to arginine medium since they lack established uracil synthesizing systems. (4) Cells first grown in the minimal medium do not suffer immediate growth inhibition upon transfer to arginine medium because of their established uracil synthesizing systems rather than because of accumulation of uracil itself.

88. Ribosomal RNA synthesis in the early stages of sporogenesis

Hideho SUZUKI

In a glucose-containing culture of *Bacillus cereus* strain T, pH of the medium decreases as the exponential growth proceeds and then rapidly increases accompanied by the initiation of the sporulation cycle (NAKATA and HALVORSON, 1960). A similar phenomenon was observed in the culture of *B. cereus* NTCT 569[1] grown in media containing glucose. Moreover, the growth curve of the culture, as depicted by intermittent logarithmic increases in absorbancy at 660 mμ (E_{660}), revealed the existence of three phases prior to the stationary phase. The first phase of growth (phase I) corresponds to exponential growth accompanied by dissimilation of glucose and pH decrease in the medium. In the second phase (phase II), E_{660} increased again logarithmically accompanied by pH rise and morphological changes characteristic of the early sporulating process. This was followed by an additional increase of E_{660} in the third phase (phase III).

The cells in the late stages of each phase were fed with ^{14}C-uracil (1 μc/100 ml medium) for 20 min. The nucleic acids were extracted with phenol from these cells after grinding them with quartz sand in the cold and were analyzed by sucrose density gradient centrifugation. The profiles of the radio activity of phase II in the centrifuge pattern were scarcely different from those of phase I, the exponential growth phase, that is, the cells that had started the sporulation cycle synthesized 16s and 23s RNA as well as soluble RNA; whereas in the nucleic acids from the cells at phase III there was no incorporation of radio-activity, suggesting the absence of RNA synthesis. This is in accordance with the observation of the ability of *Clostridium botulinum* to sporulate without net synthesis of RNA in the stationary phase (DAY and COSTILOW, 1964), though the possibility cannot be eliminated that the cell membrane at phase III might be impermeable to uracil. From these observations it is suggested that in *B. cereus* NTCT 569 the synthesis of ribosomal RNA is not turned off by the initiation of the sporulation cycle but it ceases at the transitional stage from phase III to the stationary phase. It is in contrast to *B. subtilis* which appears to synthesize ribosomal RNA during the stationary phase (DOI and IGARASHI, 1964; BALASSA, 1964).

[1] Derived from a stock culture of Prof. H. CHANTRENNE at Université libre de Bruxelles, Belgique, and obtained through Dr. S. OSAWA of Hiroshima University.

89. *Changes in γ-ray sensitivity with time after ultraviolet irradiation of several strains of* Escherichia coli

Mituo IKENAGA and Sohei KONDO

Ultraviolet light (UV) prevents in bacteria colony formation by forming thymine dimers in their DNA. But UV irradiated bacteria regain their colony forming ability under some appropriate post-irradiation conditions. Among these post-treatment recovery phenomena, dark recovery is quite interesting. To interpret the mechanism of repair of DNA in bacteria during incubation in the dark after UV irradiation it was proposed that the cutting of thymine dimers together with DNA backbone leaves a gap, *i.e.*, a partially single stranded part in DNA, which is followed by

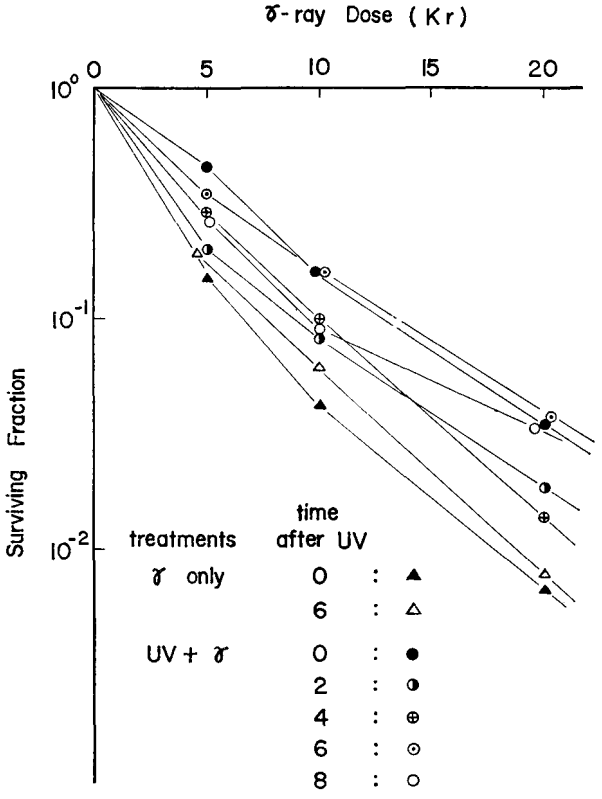


Fig. 1. Changes in time of sensitivity to γ-rays of *Escherichia coli* B phr⁻ after UV irradiation.

reconstruction of twin helical DNA due to partial DNA synthesis (SETLOW and CARRIER, 1964; BOYCE and HOWARD-FLANDERS, 1964; PETTIJOHN and HANAWALT, 1964). If this is true, bacteria some time after UV exposure should have some single stranded parts in DNA and may be expected to be more sensitive to additional agents, such as ionizing radiation, than fully recovered bacteria long time after UV irradiation. With this possibility in mind, we studied γ-ray sensitivity of UV irradiated *E. coli* at two hour intervals after irradiation. The used strains were *E. coli* B, B phr$^-$ (mutant of B, lacking in ability of photoreactivation), B/r (radiation resistant mutant of B), B/r phr$^-$ (H/r) and B_{s-1} (extremely UV sensitive mutant of B, which lacks the ability to excise thymine dimer from its DNA). After 254 mμ UV irradiation to the extent of a small survival percentage, the bacteria were diluted in a 1/15 *M* phosphate buffer and held in the dark at 21°C. After several two hour intervals, a part of them was irradiated with ^{137}Cs γ-rays and then the survivors were estimated by colony counting. Fig. 1 is a typical example for *E. coli* B phr$^-$ which shows a depression in the resistance to γ-rays 2$\sim$4 hours after UV irradiation. This may partly fit the UV recovery model mentioned above, though it can not explain why UV irradiated B phr$^-$ was more resistant to γ-rays than the unirradiated one, as judged from the slopes of the curves. On the contrary, the UV irradiated B/r and H/r showed soon after UV exposure a considerable depression in the resistance to γ-rays and then gradually became resistant to γ-rays and the slopes of their survival curves finally reached to control level. The B_{s-1} showed no change in γ-ray sensitivity at any time interval after UV irradiation. So far the obtained data are rather complicated, but it may be at least concluded that there are correlations between the dark recovering ability and γ-ray sensitivity changes after UV irradiation.

E. RADIATION GENETICS OF ANIMALS

90. Effects of X-ray induced mutations on several components of fitness

Yuichiro HIRAIZUMI

Second chromosomes of *D. melanogaster* were X-rayed (sperm: 100 r/min for 5 min) and the effects of induced mutations on female fecundity, rate of development and larval stage viability were examined. In order to eliminate the effects of possible heterogeneity among the genetic backgrounds of the stocks, the whole matings were repeated without

X-irradiation (=control set). Results obtained during the past three years are summarized as follows.

1. Female fecundity

One hundred and sixty-nine X-rayed (77 in the control set) chromosome lines (four, sometimes three or two females per line) were tested. Fecundity was measured by the total number of progenies produced during the first 9 days. Results are given in Table 1, where ⊕ represents X-rayed second chromosome. In order to minimize the effects of environmental fluctuations, experiments were designed such that comparisons among genotypes could be made pairwise. For each pair, Δ_1=(+/+)−(⊕/+) and Δ_2=(+/+)−(⊕/⊕).

Table 1. Female fecundity.

Set	+/+	⊕/+	⊕/⊕	$\bar{\Delta}_1$	$\bar{\Delta}_2$
X-rayed	143.43	139.25	131.70	4.17±2.30	11.72±2.90
Control	139.88	142.16	140.14	−2.27±4.00	−0.25±4.00

In the X-rayed set, $\bar{\Delta}_2$ was significantly and $\bar{\Delta}_1$ nearly significantly larger than zero indicating that female fecundities of ⊕/+ and ⊕/⊕ were reduced. In the control set, the sign of $\bar{\Delta}_1$ was negative, suggesting that the genetic backgrounds of the stocks used in this experiment were not completely homogeneous.

2. Developmental rate

Two (sometimes more) fertilized females of a genotype in question, and two fertilized, standard, *cn bw* females were put together into a food vial and allowed to lay eggs for 24 hours, and their progeny flies were counted once a day until all the flies had eclosed. The average time from egg laying to eclosion was then computed for each genotype separately in the two sexes. Let x and y be the eclosion time (in day) of *cn bw* strain and of the genotype in question, taking the unweighted average for the two sexes. $z=y-x$ was then computed for each culture, and the results are summarized in Table 2 (190 lines in X-rayed, and 81 in the control set. Larger value of z represents slower developmental rate). In Table 2, Δ_1 (or Δ_2)=z of +/+ minus z of ⊕/+ (or ⊕/⊕).

Table 2. Developmental rate.

Set	$\bar{\Delta}_1$	$\bar{\Delta}_2$
X-rayed	0.0499±0.017	−0.0312±0.020
Control	0.0088±0.025	0.0190±0.040

In the X-rayed set, $\bar{\Delta}_1$ was significantly larger than zero, or the developmental rate of $\oplus/+$ was significantly faster than $+/+$. Since there is a positive correlation between rate of development and viability, this suggests that the viability of $\oplus/+$ increased. Although not statistically significant, $\oplus/\oplus$ revealed slower developmental rate than $+/+$. No clear deviation was seen in the control set.

3. Larval stage viability

Two hundred and seven chromosomes (148 in the control set) were tested. Matings were: $+/Cy$ ♀ × $+/L^2$ ♂, $+/Cy$ ♀ × $\oplus/L^2$ ♂ and $\oplus/Cy$ ♀ × $\oplus/L^2$ ♂. In the F_1 of these matings, the number of wild flies was divided by the number of $+/L^2$ flies plus one (for Δ_1) and of $+/Cy$ flies plus one (for Δ_3) to estimate the relative viabilities of the three genotypes. Results are summarized in Table 3, where $\Delta_3=(\oplus/+)-(\oplus/\oplus)$.

Table 3. Larval stage viability.

Set	Standard genotype $=+/L_2$	Standard genotype $=\oplus/Cy$
X-rayed	$\bar{\Delta}_1=-0.0168\pm0.030$	$\bar{\Delta}_3=0.0405\pm0.021$
Control	$\bar{\Delta}_1=-0.0105\pm0.035$	$\bar{\Delta}_3=-0.0119\pm0.026$

In the X-rayed set, $\bar{\Delta}_3$ was significantly larger than zero. This suggests that either the viability of $\oplus/+$ increased or that of $\oplus/\ominus$ decreased (or both). Further statistical analyses are in progress.

91. Heterozygous effects of radiation-induced mutations on viability in a synthetic homozygous genetic background

Terumi MUKAI, Isao YOSHIKAWA and Tsuneyuki YAMAZAKI

It has been generally accepted that specific co-adapted gene complexes have been established within natural populations as a result of natural selection (*e.g.*, see WALLACE and VETUKHIV, 1955). In order to test whether or not the manifestation of overdominance of radiation-induced mutations previously reported (MUKAI, YOSHIKAWA and YAMAZAKI, Annual Report No. 14, 1964) is a characteristic of co-adapted gene complexes at a chromosomal level, namely, if the nature of overdominance expression is the product of natural selection (DOBZHANSKY, 1950), this experiment was conducted.

An isogenic line (W180) was synthesized (abbreviated as *MM*) using entirely unrelated 64 wild-type stocks collected from almost all over the world. This synthesis was conducted so as to bring about their equal contribution to the isogenic line. After irradiating W180 males by X-

rays at the dose of 500 r, heterozygotes with respect to radiation induced mutations were produced by the aid of *Cy/Pm Sb/Ubx* strain. The relative viabilities of irradiated heterozygotes with respect to the second chromosome (*MM'*) were estimated in comparison with $Cy/+_M$. The irradiated X and third chromosomes were completely substituted by the unirradiated ones of W180 before the estimation of viability. The control experiments were conducted in the same manner without irradiation. The results are presented in Table 1, together with the results in homozygous genetic background (A_1A_1) which was directly extracted from a random mating population.

From Table 1, it can be seen that radiation-induced mutations which occurred in synthetic homozygous individuals were not overdominant in contrast with the mutations induced in homozygous individuals extracted from a random mating population. In conclusion, it might be said that the overdominance manifested by mutations induced in otherwise homozygous genetic background of the origin of an equilibrium random mating population is caused by interaction with their genetic background. This might be a product of natural selection.

Table 1. Heterozygous effects of radiation-induced mutations on viability in *Drosophila melanogaster* (500 r, 2nd chromosome).

Genotype	No. of chromosome lines tested	Average number of flies that emerged in each chromosome line	Average viability
MM	315	1,694.76	1.0099
MM'	306	1,683.74	1.0096
Difference		(−11.02±12.47)	(−0.0003±0.0053)
A_1A_1	287	766.63	1.0141
A_1A_1'	286	789.74	1.0412
Difference		(+23.11*±12.89)	(+0.0271**±0.0101)

' Indicates irradiated chromosomes.
* Significant at the 5% level.
** Significant at the 1% level.

92. *Radiation effect of the "Sex-Ratio" agents of* D. willistoni *and* D. nebulosa[1)]

Bungo SAKAGUCHI, Susumu KOBAYASHI and Kugao OISHI

It was demonstrated by POULSON and SAKAGUCHI (1961) that the "Sex-

1) This work has been supported by U. S. Public Health Service Research Grant GM 10238 from the Division of General Medical Sciences, and Grant in Aid Scientific Research of Ministry of Education (95212).

Ratio" (SR) agents are the etiologic factor in the production of maternally transmitted SR condition in *Drosophila* and that they are *Treponema*-like spirochetes (SR spirochetes). They were found in high concentration in the hemolymph of adult females of SR strains in *D. nebulosa*, in *D. willistoni* and in *D. melanogaster* to which the SR condition has been artificially transferred.

In order to make clear the inactivation and recovery of the function of SR spirochetes by γ-ray irradiation, the present experiments were carried out.

Two SR lines of the Oregon-R (OR) strain of *D. melanogaster* were established by artificial transfer, one hosting *willistoni* and the other *nebulosa* spirochetes. Females of these two preinfected OR lines were exposed to Cs-137 γ-ray with 30 kr dose. After one week from the time of irradiation, the hemolymph containing a large amount of irradiated SR spirochetes was injected into females of normal OR strain. Direction of the injection was always from one donor to one host. The injected females were then mated singly with males of the same OR strain and the sex ratios in the progenies were examined. Injection and examination were repeated several times as shown in Fig. 1.

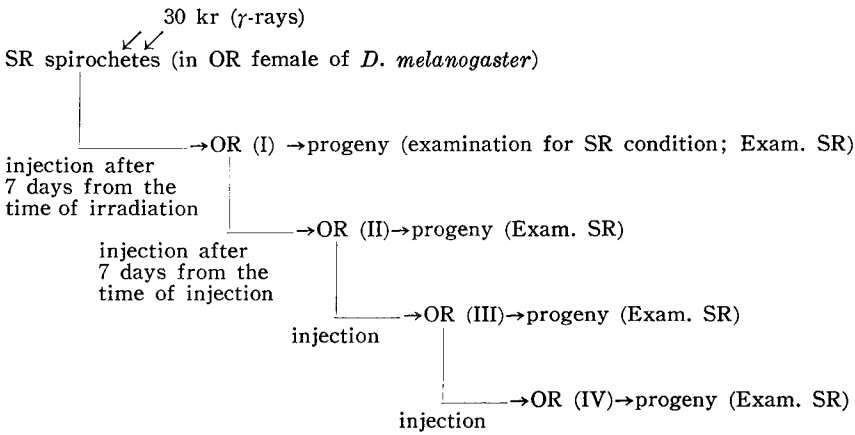


Fig. 1. Diagram representing the method for testing inactivation and recovery of irradiated SR spirochetes.

The results are summarised in Table 1.

In the series of injections with irradiated *willistoni* spirochetes, only one in each of II and III injections produced SR progenies. While in the injection with irradiated *nebulosa* spirochetes, several flies produced SR progenies from two to eight in first and second injection and finally

all injected flies produced SR progenies in the following injection steps.

These results indicate that the sensitivity against γ-ray irradiation of *willistoni* spirochetes is considerably higher than that of *nebulosa* spirochetes, while multiplication after irradiation of *nebulosa* spirochetes is faster than that of *willistoni*.

It is of interest that SR spirochetes were observed in the hemolymph of a part of normal sex ratio progenies from the injected flies (Table 1).

Table 1. The manifestation of SR condition in successive injections of irradiated SR spirochetes.

Irradiated SR spirochetes	*willistoni*			*nebulosa*			
Successive** injection	I	II	III	I	II	III	IV
No. of injected flies	11	11	10	12	11	8	6
No. of SR condition	0	1	1	2	8	8	6
No. of normal sex ratio	8	8*	8*	3*	2*	0	0
No. of sterile	3	2	1	7	1	0	0

* SR spirochetes were observed in the hemolymph of a part of males and females.

** See in Fig. 1.

93. *Mechanisms controlling two types of dose-rate dependence of radiation-induced mutation frequencies in silkworm gonia*[1]

Yataro TAZIMA

The dose-rate effect of radiation in the induction of mutation has been studied extensively for the past five years with silkworm gonia. The results so far obtained are summarized and a new explanation is proposed.

As far as X- and γ-rays are concerned, the mutational response of young germ cells of the silkworm, mostly at gonial stage, is not simple. At least two types of dose-rate dependence, one being the reverse of the other, have been found in this insect. In one type, the mutagenic effectiveness of chronic irradiation (0.1 r/minute) is lower than that of acute irradiation (100~300 r/minute) (Type I), and in the other, mutagenic effectiveness is higher for chronic than for acute irradiation (Type II). Which type appears solely depends upon the developmental stage of the irradiated larvae. Irradiation of newly hatched larvae results in Type I dose-rate dependence, while irradiation at a later stage, around the time

[1] Abstract of a paper presented before the conference on Dose-Rate Effect of Radiation at Genetic and Cellular Levels, Oiso, 1964.

when the larvae are eight days old, gives completely reversed results.

The complexity of the dose-rate effect is entirely due to a drastic change in mutagenic effectiveness of acute irradiation according to the developmental stage. The dose-mutation frequency curve shows the highest peak at hatching time but decreases rapidly thereafter. In contrast, the mutagenic effectiveness of chronic irradiation remains almost the same.

In order to interpret the reverse type of dose-rate effect, a hypothesis involving selective cell killing was proposed. The hypothesis was based on the finding that the homogeneous germ cell population which exists at hatching time becomes gradually heterogeneous with the development of the larvae and the gonial cells at an advanced stage are highly sensitive to the killing action of radiation. It was also assumed that cell killing is more drastic in acute than in chronic exposure and that cells sensitive to killing are also sensitive to having mutations induced in them. However, we failed to obtain cytological evidence to support this hypothesis.

Later a differential repopulation hypothesis was proposed but this was again found to be not satisfactory.

Since then, efforts have been made to obtain some decisive evidences which may provide a clue to the solution of the problem. Extensive experiments with fractionated doses and with neutrons were carried out. The dose-fractionation experiments yielded very suggestive information for the interpretation of the dose-rate effect. When 1,000 r acute irradiation was delivered in two fractions, 500 r+500 r, separated by various time intervals from 2 to 48 hours, a remarkable increase in mutation frequency was observed throughout the period examined, showing the peak if the second dose was given 18 or 24 hours after the initial exposure. Several possibilities have been considered for interpreting this effect. Among them the most promising was the idea that metastasis produced by the exposure depresses for some while the repair of pre-mutational damage, whose duration depends on the metabolic activity of the cell. The same idea reasonably explained the drastic change in the mutational response to acute irradiation during the progressing development of the larvae.

A new hypothesis, based on this idea, has been proposed for the interpretation of the complicated features of the dose-rate effect as follows:

(a) a large part of the radiation-induced pre-mutational damage is repaired during the post-irradiation recovery process,

(b) the degree and extent of repair primarily depend upon the total dose and dose-rate,

(c) the occurrence of repair also depends upon the cellular conditions,

presumably metabolic activity, of the irradiated cell,

(d) the final magnitude of the observed mutation frequency is determined by both factors combined.

With these assumptions, the two types of dose-rate dependence, one being the reverse of the other, have been interpreted consistently.

94. *Nature of post-irradiation metastasis as revealed by fractionated γ-irradiation and application of high or low temperature between the exposures*

Yataro TAZIMA and Kimiharu ONIMARU

As a possible interpretation of the enhancing effect of mutation frequency after two-fraction γ-irradiation was administered from 2 to 48 hours apart, the following assumption has been proposed (TAZIMA, 1964): namely, a kind of temporary metastatic state, presumably a disturbance of metabolism, was assumed to be produced in the cell that hinders the repair of pre-mutational damage until it disappears after a certain while. If the second irradiation is given before the completion of repair, most pre-mutational damages cannot be repaired so that higher mutation frequencies are the result.

In order to look into the nature of post-irradiation metastasis, an experiment was carried out by subjecting irradiated larvae to different temperatures, 15°C, 25°C and 30°C, between the two exposures. Total 1,000 r was divided into two halves and each 500 r was given to the

Table 1. Results of temperature treatment, performed in order to elucidate the duration of the post-irradiation metastasis.* (643)

	Treatment				Oögonia			Spermatogonia		
	0hr	24hr	48hr	Temp.	Observed	Mut. freq. ($\times 10^{-5}$)		Observed	Mut. freq. ($\times 10^{-5}$)	
						pe	*re*		*pe*	*re*
F 1	500 r		500 r	15°C	160,146	133.6	82.4	133,420	152.2	72.0
F 2	500 r	500 r			159,278	148.8	89.2	120,594	141.0	53.1
F 3	500 r		500 r		150,598	150.7	72.4	116,714	142.2	50.6
F 4	500 r	500 r		25°C	165,788	176.7	119.4	115,520	190.4	84.8
F 5	1,000 r				179,676	67.9	50.1	115,520	111.7	39.8
F 6	500 r		500 r	30°C	167,958	62.5	31.6	112,535	78.2	50.7
F 7	500 r	500 r			183,148	139.8	92.8	114,326	105.0	66.5

* Data for Type I stage.

larvae 24 or 48 hours apart. The first dose was delivered immediately after hatching. Except for experimental treatments, all larvae were raised at 25°C throughout their life cycle. The material used was wild type C108 strain. For the estimation of mutation frequency we used specific loci method with egg color marker genes, *pe* and *re*.

The results are shown in Table 1.

It has been known from the previous experiments that under the standard rearing temperature, 25°C, the maximum increase in the mutation frequency due to two-fraction irradiation appears in both sexes about 24 hours after initial irradiation. The present data for 25°C are consistent with the previous ones as far as the stage of appearance of the peak is concerned.

It may be seen from the table that an enhancing effect of mutation frequency disappears rapidly in 30°C group but persists fairly long when irradiated larvae are kept at 15°C. Even 48 hours after initial exposure mutation frequency was still high in 15°C group. These results suggest that the length of the period during which the metastatic state persists is temperature dependent.

The observed phenomenon can also be interpreted by assuming simply a repair of pre-mutational damage. According to this assumption the length of the period during which damage persists is taken to be temperature dependent. The assumption, however, can not explain the following finding. It has been known for acutely γ-irradiated gonia at late stage that the dose-mutation frequency relation is linear up to 1,000 r, increasing very slowly with increasing doses (TAZIMA and KONDO, 1962), but turns into rapid non-linear increase at higher doses than 1,500 r (MURAKAMI and KONDO, 1964). The discrepancy between the two findings can only be interpreted by introducing another factor in addition to pre-mutational damage produced in genetic material. This factor is supposed to be a disturbance of cellular metabolism produced by radiation.

It can be inferred from these findings that radiation produces in the cell at least two kinds of disturbance, a pre-mutational damage in genetic material on one hand and a disturbance in metabolic system on the other and that both factors in combination determine the final manifestation of a mutation.

95. Modification of radiation-induced mutation frequency by post-treatment with KCN, chloramphenicol, high and low temperatures and nitrogen gas

Yataro TAZIMA and Kimiharu ONIMARU

As described in the preceding article (This Report, **94**), a post-irradiation metastasis of the cell has been assumed. If this is actually the case, it is foreseen that post-treatment of irradiated cells with agents that affect cellular metabolism would result either in an increase or in a decrease of mutation frequency.

Experiments have, therefore, been carried out to confirm this assumption by applying, immediately after γ-irradiation, various agents: namely a known respiratory poison KCN, chloramphenicol, high and low temperatures and oxygen and nitrogen gases.

Prior to those treatments larvae were irradiated with 1,000 r γ-rays from a Cs-137 source at a dose-rate of 100 r per minute. Both females and males of wild type C108 strain were exposed to radiation. For the determination of mutation frequency the specific loci method was utilized with use of egg color genes, *pe* and *re*, as markers.

Treatment with KCN and chloramphenicol was performed by feeding the larvae on leaves supplemented with these chemicals. The administered dose of the chemical was determined by taking into account the results of a preliminary experiment on the adverse effect on viability of the larvae; it happened to be the same for both chemicals; *i.e.*, 30 mg and 50 mg per 1,000 larvae for newly hatched and 7 days old larvae, respectively.

The temperature treatment was carried out by exposing irradiated larvae to three different temperatures, 15°C for 48 hours, 25°C for 24

Table 1. Results of post-irradiation treatment of 7 days old larvae. (641)

Treatment	Oögonia			Spermatogonia		
	Observ. number	Mut. freq. ($\times 10^{-5}$)		Observ. number	Mut. freq. ($\times 10^{-5}$)	
		pe	*re*		*pe*	*re*
1,000 r	273,178	9.9	9.9	191,079	36.1	12.0
1,000 r+KCN (50 mg, 1 %, 6 hrs)	241,773	14.1	11.2	187,985	43.1	12.2
1,000 r+CMP (50 mg, 1 %, 6 hrs)	263,707	19.0	12.1	184,890	46.0	12.4
1,000 r+15°C (48 hrs)	265,701	16.2	13.2	184,890	61.1	12.4
1,000 r+30°C (24 hrs)	275,671	14.9	5.8	187,598	33.0	7.5
0 r	266,698	7.9	6.0	182,570	10.4	0.0

^{137}Cs-γ-rays, 100 r/min CMP: Chloramphenicol

Table 2. Results of post-irradiation treatment of newly hatched larvae. (642)

Treatment	Oögonia			Spermatogonia		
	Observ. number	Mut. freq. ($\times 10^{-5}$)		Observ. number	Mut. freq. ($\times 10^{-5}$)	
		pe	*re*		*pe*	*re*
1,000 r	187,200	57.2	52.4	158,900	62.9	41.5
1,000 r+KCN (30 mg, 1 %, 6 hrs)	144,000	54.2	35.2	162,050	66.6	51.8
1,000 r+CMP (30 mg, 1 %, 6 hrs)	158,400	51.8	44.8	148,400	69.4	52.6
1,000 r+15°C	129,600	42.4	32.4	142,100	114.7	66.2
1,000 r+30°C	143,550	50.9	26.5	157,850	51.9	33.6
0 r	158,850	15.1	5.7	154,000	8.4	0.6

^{137}Cs-γ-rays, 100 r/min

Table 3. Results of post-irradiation treatment of newly hatched larvae with oxygen and nitrogen gases. (643)

Treatment	Oögonia			Spermatogonia		
	Observ. number	Mut. freq. ($\times 10^{-5}$)		Observ. number	Mut. freq. ($\times 10^{-5}$)	
		pe	*re*		*pe*	*re*
1,000 r	189,900	57.9	45.3	135,450	70.1	36.2
1,000 r+O_2 (24 hrs)	191,250	57.0	44.4	133,000	64.7	48.1
1,000 r+$\begin{cases} N_2\ 50\,\% \\ \text{air}\ 50\,\% \end{cases}$ (24 hrs)	182,700	52.5	43.2	136,850	46.8	27.8
1,000 r+N_2 (24 hrs)	131,850	73.6	97.1	152,950	94.8	27.5

^{137}Cs-γ-rays, 100 r/min

(Added at proof reading: N_2 gas was later on found not pure.)

hours or 30°C for 24 hours. All three groups were incubated and raised in the same room at 25°C except during a treatment.

Gas treatment was performed in a glass cylinder. Irradiated larvae were transferred immediately after irradiation into cylinders where either oxygen or nitrogen gas was kept running for 24 hours. The gases used were obtained commercially.

The results are given in Table 1~3.

Throughout three different experiments, treatment with agents that are known to depress metabolic activity of the cell resulted in an increase in mutation frequency. Namely, treatment with KCN, chloramphenicol, low temperature and nitrogen gas gave higher mutation frequencies than γ-irradiation only, high temperature or oxygen gas treatment. These results are consistent with the expectation that radiation produced

metastasis is intensified or prolonged by agents which depress or slow down the metabolic activity of the cell. However, this does not necessarily imply that this is the only mechanism that underlies the present findings.

96. Relative biological effectiveness of 14 MeV neutrons to gamma-rays in the induction of mutations in germ cells of hibernating silkworm embryos

Akio MURAKAMI and Yataro TAZIMA

It was reported in our previous paper that mutational RBE values of both 14 MeV neutrons and fission neutrons for gonial cells of the silkworm differed between two early larval stages. This finding was explained by assuming a difference in metabolic condition of germ cells at the two stages. It is, therefore, necessary, in order to obtain the intrinsic mutational RBE, to utilize an ideal cell system where the interaction of metabolic condition can be ignored. In this regard an experiment has been carried out by using germ cells of hibernating silkworm embryos as material. This is because in these cells metabolic activity is regarded to be very low, if not negligible.

The method of testing mutation frequency is similar to that used in our previous work. Hibernating embryos were treated with 14 MeV neutrons from T(d, n)He reaction with the average dose rate, 8.7 rad per minute, in four dosage groups: 320, 660, 900 and 1,300 rad. For the sake of comparison of effectiveness, ^{137}Cs gamma-irradiation was performed in parallel, in which irradiated larvae were treated similarly except for irradiation. In γ-ray experiment seven different doses were administered with dose-rate 100 r per minute; *i. e.* 250, 500, 1,000, 1,500, 2,000, 2,500 and 3,000 r.

The results are shown in Fig. 1 only for the male sex. It can be seen from this figure that the dose-mutation frequency curve for 14 MeV neutrons increases exponentially up to 600 rad and then turns into increasing gradually up to about 1,300 rad, whereas, the gamma-ray curve increased approximately linearly below about 2,400 rad and then decreased against the dose. The mutation frequencies for 14 MeV neutrons were significantly higher than those for gamma-rays throughout the whole dose-range examined.

It was, however, difficult to obtain definite RBE value, because of the non-linear relation of the obtained curves for both radiations. Consequently, RBE value was obtained tentatively by comparing the estimated doses for each radiation at an arbitrary mutation frequency level of 10^{-3}.

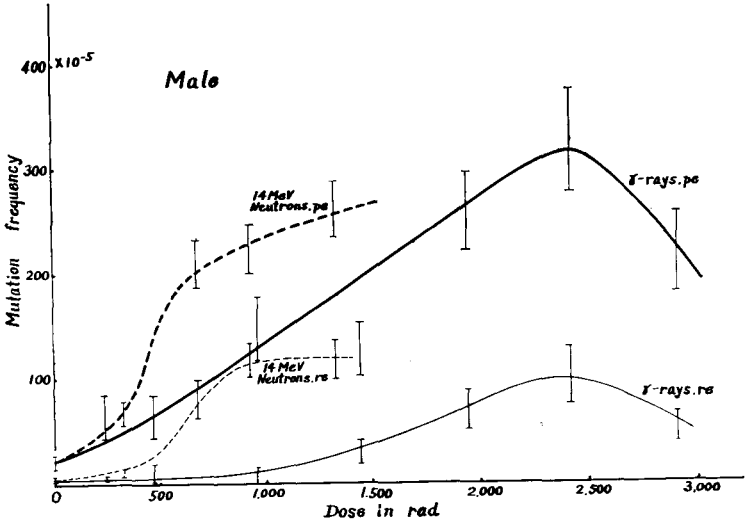


Fig. 1. Dose-mutation frequency curves obtained after 14 MeV neutron and ^{137}Cs γ-ray irradiation of male hibernating embryo of the silkworm.

At this level RBE values of 14 MeV neutrons are 1.8 and 2.9 for *pe* and *re* locus, respectively. But the value decreases to a considerable extent at higher dose levels.

The RBE values obtained in this manner are higher than those for newly hatched larvae, but almost similar to those for 7 days old larvae, in which the metabolic activity is presumed to be high. From these comparisons, it can be inferred that germ cells of hibernating embryos cannot be regarded as an ideal model for getting intrinsic RBE. Perhaps there are some unknown biological factors affecting mutation frequency.

97. Induction of visible recessive mutations by X-radiation in silkworm germ cells at pre-embryonic stage

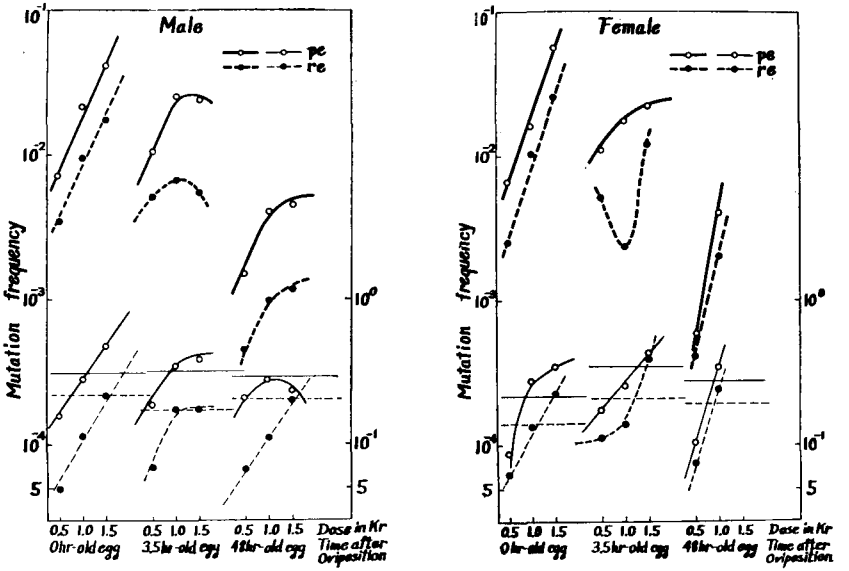
Akio MURAKAMI and Yataro TAZIMA

It has been known in *Drosophila* that germ cells are mutagenically most sensitive to radiation around the time of fertilization (OSTER, 1958). A similar phenomenon has been observed by NAKAO in the silkworm. In order to study the relation more precisely and to elucidate the relevant mechanisms, the present experiment has been undertaken.

The egg material was obtained from actively laying wild type moths of C108 strain. After allowing oviposition for one hour at 25°C eggs

were collected and subjected to X-ray treatment. The conditions for X-irradiation were 20 mA, 175 kVp and 50 cm in target distance. The doses given were 500, 1,000 and 1,500 r with a dose-rate of 60 r per minute. Irradiated insects were crossed, after emergence, to partners of the marker strain, *pe* and *re*, in order to examine the mutation frequency.

It is generally accepted from cytological evidences that syngamy of female- and male-pronuclei occurs in this insect about two hours after oviposition at 25°C. In the present experiment irradiation was administered at three different stages; 0~1 hour-old eggs (before fertilization), 3.5~4.5 hour-old eggs (soon after fertilization) and 48~49 hour-old eggs (early embryonic stage).



Figs. 1-2. Variation in X-ray induced mutation frequencies at the earliest developmental stages of silkworm eggs.

The results are shown in Figs. 1 and 2 for males and females, separately. As seen from the figures, dose-response curves for mutation frequencies are essentially similar for both sexes, but differ depending on the irradiated stage. Dose-response curves for 3.5 hour-old eggs and 48 hour-old eggs represent a saturation effect, while no such effect is observed for 0 hour-old eggs. The discrepancy seems to be correlated with the extent of the killing effect of radiation. Indeed it has been observed that the hatchability of 3.5 and 48 hour-old groups was markedly lower than that of 0 hour-old group.

The mutation frequency obtained for 0 hour-old and 3.5 hour-old eggs is approximately seven times as high as the maximum frequencies obtained for spermatids and mature oocytes. However, it decreased very rapidly with the progress of embryogenesis and fell to about the same level as that of the maximum frequencies obtained for spermatids and mature oocytes at 48-hour age.

However, when the mutation frequency was calculated per batch base a very interesting information was obtained regarding the sensitivity to mutation. Mutation frequencies given in Figs. 1 and 2 were obtained in accordance with the ordinary method from pooled data, although the original data were recorded for each batch. From these records mutation frequency per batch base was calculated by dividing the number of mutation yielding batches by their total number. There was no significant difference in the frequency among the three egg stages.

During the course of the analysis, however, it was found that a difference existed among those groups in the number of mutants per batch. The size of mutant clusters recovered in the same batch was markedly larger in 0 hour-old and 3.5 hour-old groups than in 48 hour-old group.

98. Proliferation kinetics of spermatogonia of the silkworm during chronic γ-irradiations

Toshihiko SADO

During the past few years, attention has been directed in this laboratory to study the mechanism involved in the dose-rate effects on the frequency of radiation-induced mutations. The type II dose-rate dependence (*i. e.*, a higher mutation rate after chronic than after acute irradiation) is the characteristic of the dose-rate effect observed in the silkworm gonia and was interpreted initially on the basis of a selective cell killing hypothesis (TAZIMA, KONDO and SADO, 1961). This hypothesis, however, was not favored by the subsequent cytological observations; *e. g.*, a substantial difference was not detected between acute and chronic group in the number of gonial cells which survived the exposure. Secondary spermatogonia, which are extremely sensitive to the killing action of X-rays, were also found to be killed after chronic irradiations (SADO, 1962). This observation led the author to postulate that synchronization of cells at some mutagenically sensitive phase of the cell cycle during the chronic exposure may be the primary factor involved in this dose-rate dependence. This implies that the difference in mutation frequency between acute

and chronic exposure is primarily a reflection of mutation rates at random *versus* selected-sensitive phase(s) of the cell cycle.

In an attempt to gain a better insight into this problem, kinetics of proliferation and killing of spermatogonia of the young larvae of this insect (3~8 days after the hatching) during chronic exposure (0.142 r/min, total doses 1,000 r in 5 days) were studied. The following information was obtained. 1) The number of spermatogonia at the beginning and at the end of the chronic exposure was essentially the same. 2) The mitotic index of spermatogonia determined every 24 hours after the onset of exposure was approximately the same as that of the control, and at some intervals it was even higher in chronic than in the control group. 3) Degeneration of spermatogonia was observed during the exposure. There was a gradual rise in the frequency of pycnotic spermatogonia of 1~2 cell stages during the first 48 hours, followed by a sharp drop during the subsequent 24 hours. Among the more advanced spermatogonia (4~32 cell stages) which began to appear 72 hours after the onset of the exposure, 13 percent were pycnotic.

These data emphasize the following. Under this experimental condition mitotic inhibition of spermatogonia is not taking place, suggesting that spermatogonia are proliferating apparently at a normal rate during the chronic exposure. A considerable fraction of spermatogonia are dying, however, during the exposure. Thus, the dynamic equilibrium is established between the newly occurring and dying cells. The experiments to reconfirm and extend these findings are now in progress.

99. *Correction factor (K) for estimating the number of spermatozoa bundles from serial sections of a testis of the silkworm*

Toshihiko SADO

For the evaluation of radiation-induced mutation frequencies in relation to the irradiated stages of the silkworm, or the stages of germ cells at which irradiations were given, it is often desired to know the number of spermatozoa produced by the irradiated insects in relation to the number of germ cells which survived the exposure. This is particularly important when the cluster of mutations is taken into consideration (KONDO, 1961). The number of spermatozoa produced per testis can be obtained by multiplying the number of spermatozoa bundles produced by 256 because a single spermatozoa bundle contains 256 spermatozoa. For the estimation of number of spermatozoa bundles per testis, Machida's method (MACHIDA, 1929) has been used in which the total number of spermatozoa bundles in each testis was scored on the serial sections (20μ)

only at the portion where the mass of nuclei were observed. A possible source of error in this method was that a single mass of nuclei might be scored two or more times. Therefore, the total number scored by this method was corrected by the equation, $N=KA$, where N and A represent scored and actual number, respectively, while K is a proportionality constant dependent on the size and form of the mass of nuclei of a spermatozoa bundle and the thickness of the sections which was calculated theoretically to be 2.0 for eupyrene, or typical, spermatozoa (MACHIDA, 1929). This indicates that, in general, a single spermatozoa bundle may be scored twice on the serial sections of 20 μ thick. This value has been used in the past to assess the number of spermatozoa bundles produced (KOGURE and NAKAJIMA, 1958; SADO, 1961, 1963). However, it was felt that this number was overestimated. An attempt was made, therefore, to reexamine the validity of this value.

The number of mass of spermatozoa nuclei, regardless of the size, was scored on the $20\sim30$ successive sections of a serial paraffin sections (20 μ) of a testis of middle and late pupal stages on the one hand, and its actual number was determined on the other. This was done by eliminating the doubly scored spermatozoa bundles with the use of serial microphotographs taken from the corresponding sections. K was then determined, using $N=KA$, or $K=N/A$. The result indicated that K was 1.22 and 1.28, respectively, for testes from middle and late pupal stages, the mean being 1.25. This supports the notion of MACHIDA (1929) that K might be smaller than 2.0.

Using this value, the number of spermatozoa bundles produced in a testis was estimated to be approximately $4,000\sim5,000$ (recalculated from MACHIDA, 1929; SADO, 1961, 1963). This indicates that one individual male of this insect produces approximately $2.0\sim2.5\times10^6$ spermatozoa per generation. Available data at hands suggest that 1,000 r of X- or γ-rays given to the larvae before the third instar, independent of the dose-rate, apparently does not affect the number of spermatozoa produced, even though almost complete loss of the secondary spermatogonia was observed within a few days after the irradiation. However, it has been shown previously that irradiation at later stages, *e. g.*, early fifth inster, significantly reduced the number of spermatozoa produced (SADO, 1961, 1963).

100. Further studies of ^{32}P induced mutations in the silkworm[1)]

Mituo IKENAGA and Sohei KONDO

In the previous paper (IKENAGA and KONDO, Annual Report No. 14, 1963) the relative mutagenic efficiencies of γ-rays and disintegrating ^{32}P ingested to larvae were compared, and the mutation frequency of egg color at an equivalent dose of γ-rays and ^{32}P β-rays was found to be the same for male groups but different for female groups. The latter discrepancy, *i. e.*, existence of transmutation effects of ^{32}P, could be accounted for a dosimetric error involved in the female group, since meiosis occurs in females two weeks after ^{32}P ingestion.

To prove the above assumption, we carried out similar experiments with ^{32}P injection given to the silkworm at the middle pupal stage, about one week before meiosis in females. ^{32}P induced mutation frequencies were compared with those induced by daily irradiation with γ-rays so as to fit the daily ^{32}P dose in the silkworm gonads. The gonad dose of ^{32}P β-rays was rather easily obtained, because a pupa is a closed system

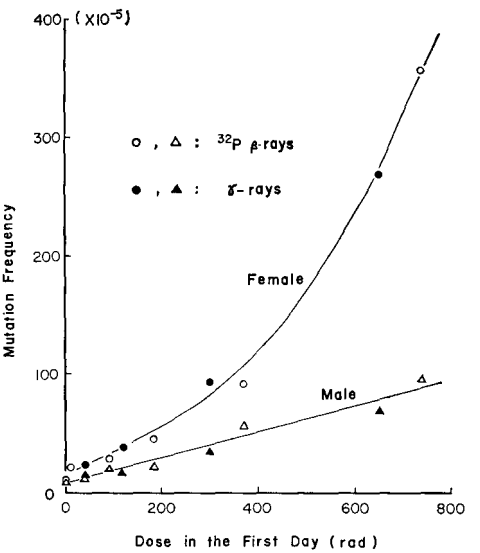


Fig. 1. Mutation frequency *versus* dose curve for ^{32}P treatment and γ-irradiation. The ^{32}P dose in rad units was determined experimentally.

1) This work was supported by grant No. 93001 for Intramural Cooperative Scientific Research from the Ministry of Education.

showing no excretion of ^{32}P. The results are shown in Fig. 1. The graph clearly shows that there is excellent agreement in induced-mutation frequency at the same absorbed dose between the ^{32}P-injected and the γ-rayed groups for both sexes.

From these experimental data and those of the previous experiment for the male sex, our conclusion is that mutations induced by ^{32}P-transmutation could not be detected in the silkworm under conditions such as ^{32}P ingestion or injection.

101. Radiosensitivity of muscle tissue formation in regenerating tissue of Triturus

Yoshito OGAWA

Actin formation in regenerating tissue of adult *Triturus* with respect to X-irradiation at various point of the regenerating progress was dealt with in a previous report (OGAWA, Annual Report No. 11, 1961). The effect of X-ray irradiation on myosin formation in the regenerating hind limb tissue of *Triturus* was examined and it was concluded that it is due to radiosensitivity of chemo-differentiation of muscle tissue in the regenerating granula.

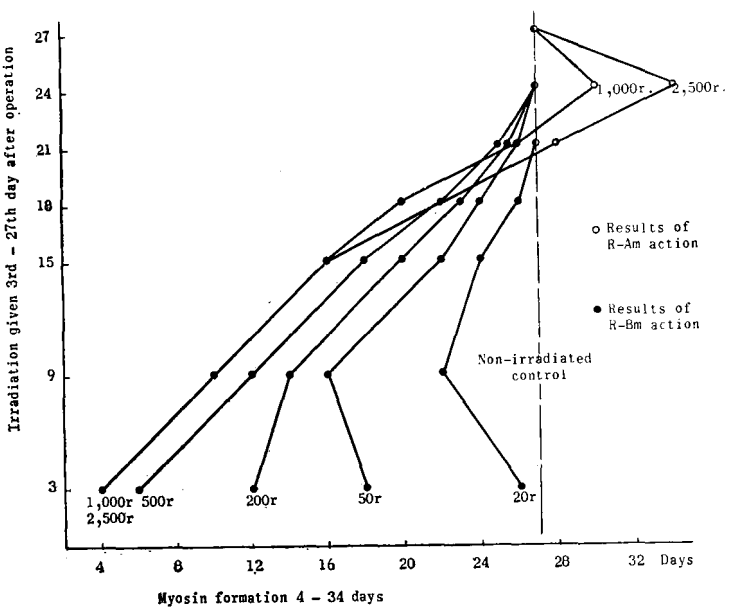


Fig. 1. Synthesis of myosin in regenerating hind limb tissue of *Triturus* irradiated by X-rays with doses from 20 r to 2,500 r.

About 3,500 animals, at various stages of the recovery progress in the limb tissue after amputation at the knee, were exposed to single doses of X-rays of 20 r, 50 r, 200 r, 500 r, 1,000 r and 2,500 r and the examination for the first detectable trace of myosin in the regenerating tissue was carried out 3, 9, 15, 18, 21, 24 and 27 days after the operation. The antigen for the preparation of anti-myosin serum by injecting into rabbits was isolated from the skeletal muscle of adult *Triturus*. The titer of anti-serum was adjusted to 1:512 before using the precipitation with saline extracts of the granulated tissue.

In the non-irradiated control group, myosin first becomes detectable in the regenerating tissue 27 days after operation (OGAWA, 1958). The time of myosin formation in the irradiated group is shown in Fig. 1. A dose of 20 r given 3 days after amputation results in one day earlier myosin formation. When a heavy dose of 2,500 r was given 24 days after operation, the synthesis of myosin was remarkably inhibited and its formation

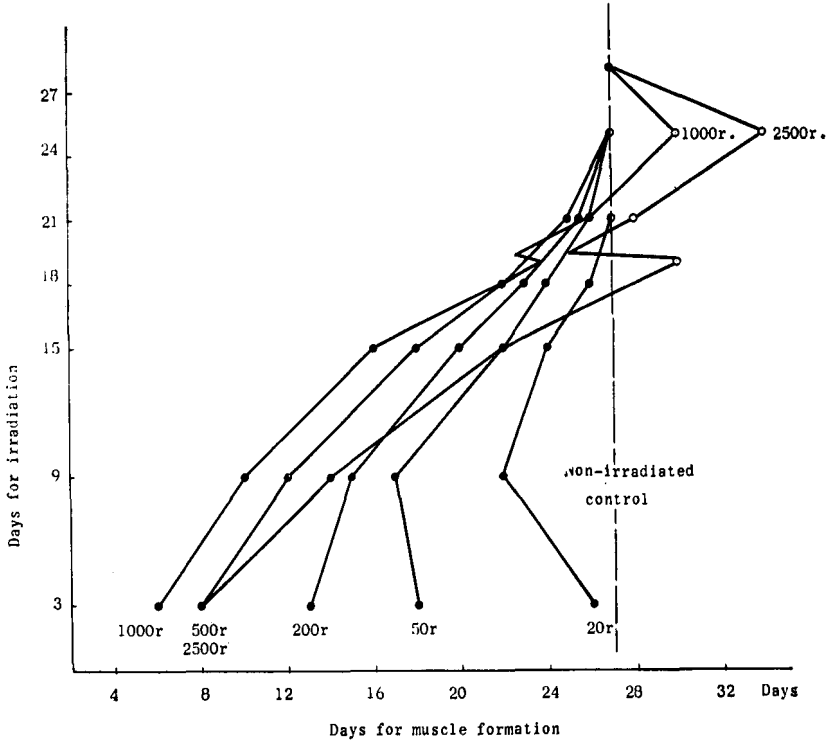


Fig. 2. Chemo-differentiation of muscle tissue in regenerating limb tissue of *Triturus* when X-irradiated with 20 r to 2,500 r.

was first recognized on the 34th day after amputation.

The curves in Fig. 1 show a similar tendency as was found for myosin formation in early embryonal stages. These results suggest the presence of two factors sensitive to X-rays in myosin formation during tissue regeneration as well as in early development. One (R-Am) suppresses the synthesis of myosin and the other (R-Bm) markedly promotes it after X-irradiation. The inhibiting factor R-Am does not react to X-rays until doses over 1,000 r are applied, but factor R-Bm is sensitive to X-rays and is detectable at the dose of only 20 r. The most effectful irradiation time is the 24th day after amputation for factor R-Am and the 9th day for factor R-Bm.

The time of complete actin and myosin synthesis in regenerating muscle tissue of X-irradiated *Triturus* is graphically represented in Fig. 2.

F. RADIATION GENETICS OF PLANTS

102. Differences in effects of γ-rays and fast neutrons from Po-Be source on einkorn wheat and paddy rice

Seiji MATSUMURA

Fast neutrons were obtained from a Po-Be source. The nuclear reaction is ${}^{9}_{4}\mathrm{Be}(\alpha, \mathrm{n}){}^{12}_{6}\mathrm{C}$, and ${}^{210}_{84}\mathrm{Po}$, whose half life is 138 days, is used as an α-emitter. The resultant neutrons are emitted as discrete spectrum with the maximum energy of 11 MeV and a mean energy of approximately 7.2 MeV. Dry seeds were attached to the surface of a stainless steel cylinder of 10 cm diameter, which contained the Po-Be neutron source (^{210}Po 10 c) in the center.

The irradiation dose, preliminarily applied to einkorn wheat seeds, ranged from 24 to 125 rad for 3～15 days. At the same time γ-radiations with ^{137}Cs 6,000 c were used for comparison at 5～15 kr and 20 kr/hr. Fast neutrons at 24～125 rad were not markedly effective in inhibiting seed germination and seedling growth and decreasing survival in the field or seed fertility in X_1, but were for chlorophyll mutation effective even at 75～125 rad. It is assumed that these neutrons are more effective than 14 MeV neutrons, and the RBE value for chlorophyll mutation frequency is about 40 against γ-rays.

Further, dry seeds of paddy rice were subjected to fast neutrons from the same Po-Be source at 158～440 rad for 2～10 weeks. For comparison γ-rays were used at 5～25 kr and 10 kr/hr. In this experiment also no clear inhibiting effects were noticed on seed germination,

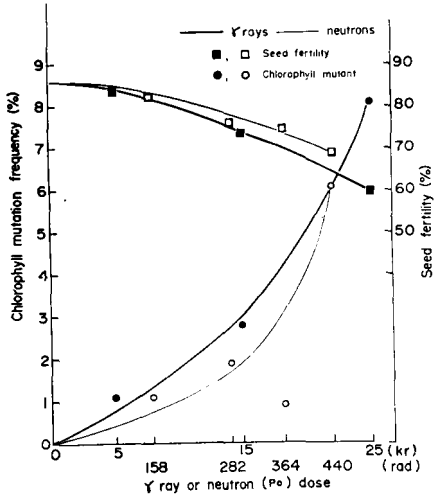


Fig. 1. Effects of neutrons (Po-Be) and γ-rays on seed fertility (1963) and chlorophyll mutation rate (1964) in rice.

seedling growth and survival in the field in the neutron-treated lots. Fig. 1 shows the relation between doses of γ-rays (kr) and fast neutrons (rad) and seed fertility or chlorophyll mutation frequency. From the figure RBE of the fast neutron from Po–Be source against γ-rays becomes near 20 for seed fertility and mutation frequency. This value is also higher than that of 14 MeV neutrons.

103. Differences in effects of γ-rays and fission neutrons in the polyploid wheat series

Seiji MATSUMURA

Dry seeds of *Triticum monococcum flavescens*, *T. durum Reichenbachii* and *T. vulgare erythrospermum* were subjected on Oct. 21, 1963, to fission neutrons from the ORNL Health Physics Research Reactor, and γ-irradiation from ^{60}Co source was also carried out simultaneously in Oak Ridge National Laboratory by courtesy of Dr. J. A. AUXER. Intensity of γ-rays in this series was 175 kr/hr. Neutrons were given for 804 seconds to the seeds at various distances from the center of the neutron reactor. The seeds of *T. monococcum* received doses of 103～1,027 rad and those of *T. durum* and *T. vulgare* doses of 257～2,057 rad. There was approximately 10 per cent additional exposure due to γ-rays associated with the neutron field.

The data for germination, seedling growth and seed fertility are given for fission neutrons and γ-radiation in Table 1. In June (harvest season) of 1963 we had unusually abundant rainfall. Therefore the seeds were not good and germination was generally low. The higher was the dose of fission neutrons and γ-rays, the more delayed were germination and growth of seedlings, and the more reduced was seed fertility. In general, *T. monococcum* was the most sensitive to fission neutrons and γ-rays. There was as expected no significant difference between *T. durum* and

Table 1. Effects of fission neutrons and γ-rays on germination, seedling growth, fertility and mutation in wheat.

Species	Dose	Germination rate (%)	Seedling length (cm)	(Index)	Seed fertility (%)	Chlorophyll mutation rate (%)
T. monococcum flavescens ($n = 7$)	Control	63	9.26	(100.0)	92.24	0.00
	N-103 rad	58	9.93	(107.2)	80.04	3.04
	N-257	59	6.81	(73.5)	74.00	6.52
	N-514	63	4.98	(53.8)	56.38	11.07
	N-770	39	5.97	(64.5)	43.51	9.60
	N-1,027	40	3.50	(37.8)	41.71	17.19
	γ-5 kr	59	6.71	(72.5)	58.99	5.22
	γ-10	52	5.18	(55.9)	34.51	8.38
	γ-15	42	2.96	(32.0)	34.33	7.58
T. durum Reichenbachii ($n = 14$)	Control	72	7.67	(100.0)	90.97	0.00
	N-257 rad	54	7.61	(99.2)	81.67	2.54
	N-514	70	8.37	(109.1)	76.91	3.25
	N-1,027	24	6.41	(83.6)	64.95	10.87
	N-1,543	30	5.13	(66.9)	55.57	7.14
	N-2,057	32	4.73	(61.7)	30.17	23.81
	γ-10 kr	36	6.70	(87.4)	85.77	0.00
	γ-17.5	28	5.14	(67.0)	82.76	0.00
	γ-25	24	4.82	(62.8)	74.96	14.29
T. vulgare erythrospermum (n=21)	Control	96	15.24	(100.0)	73.71	0.00
	N-257 rad	88	14.46	(94.9)	69.17	0.00
	N-514	88	13.30	(87.3)	60.10	0.00
	N-1,027	86	10.51	(67.0)	47.79	0.24
	N-1,543	92	9.86	(64.7)	26.22	0.00
	N-2,057	80	5.96	(39.1)	14.08	0.00
	γ-10 kr	86	13.73	(90.1)	70.46	0.00
	γ-17.5	88	11.09	(72.8)	69.53	0.00
	γ-25	86	6.08	(39.9)	63.10	0.00

T. vulgare. The frequency of spike progenies with chlorophyll mutations in X_2 was also investigated, as shown in the last column of Table 1. As expected, the higher was ploidy, the lower was mutation frequency. Only one *albina* mutation was found in hexaploids.

The RBE values of different characters were calculated roughly from Table 1. For seedling height the RBE value of fission neutrons to that or γ-rays was found to be 10~15. It was calculated for seed fertility as about 10 for diploids and as about 50 for tetra- and hexaploids and also for chlorophyll mutations as 25 or more in di- and tetraploids. In general, the RBE value was lower for the characters observed in earlier stages than at maturity, especially in polyploids.

104. Differences in effects of γ-rays and 14 MeV neutrons on einkorn wheat

Seiji MATSUMURA

Dry seeds of *Triticum monococcum* were exposed in August, 1963, to monoenergetic 14 MeV neutrons from T(d, n) reaction neutron generator in Hiroshima University by courtesy of Dr. H. YOSHINAGA. The neutron intensity was 9×10^9 neutrons/sec on average for 94~281 min, excluding γ-ray contamination of less than 10 per cent. For comparison γ-ray exposure was carried out simultaneously with 3.1 kr/hr for 102~510 min with the 6,000 c ^{137}Cs source in our institute.

Germination, survival in the field, seed fertility and chlorophyll mutation were investigated. The results of the present experiment are roughly in good accord with those of our earlier works (MATSUMURA, 1961, 1964) with 14 MeV neutrons obtained from ^{3}H(d, n)^{4}He reaction produced in the 250 kV Cockcroft-Walton accelerator in the Biology Division, Oak Ridge National Laboratory. The data summed up from both experiments are given in Table 1. The RBE of 14 MeV neutrons to that of γ- and X-rays was calculated from the table. It was found to be about 10 for germination rate showing a 10% depression as compared with the control and about 13 for seedling height showing a 25% depression. Also the RBE value was calculated as about 15 for seed fertility showing a 30% depression and for chlorophyll mutation rate amounting to 5%. It was generally lower for characters observed in earlier than in later stages, at maturity.

According to the above mentioned experiments with fission and fast neutrons from Po-Be, it is assumed that they are more effective than 14 MeV neutrons, and also that fast neutrons (Po-Be) are clearly the most effective. In general, the RBE of fast neutrons is clearly larger

Table 1. Effects of 14 MeV neutrons and γ- or X-rays on germination, seedling growth, fertility and mutation in einkorn wheat.

Dose	Germination (Index)	Seedling length (Index)	Survival (Index)	Seed fertility (Index)	Chlorophyll mutation rate (%)
Control	100.0	100.0	100.0	100.0	0.00
N-0.5 krad	99.1	87.6	87.3	63.0	4.68
N-1.0	95.0	63.1	80.8	56.0	14.36
N-1.5	87.3	42.7	61.3	26.4	5.48
N-2.0	74.2	24.7	2.1	8.6	—
N-2.5	74.0	6.6	0.0	—	—
γ-5 kr	108.1	90.6	89.1	76.2	4.00
γ- or X-10	92.3	76.6	82.4	61.8	6.98
γ-15	83.8	53.9	86.7	56.8	4.81
γ- or X-20	64.8	28.4	34.9	64.2	9.30

for higher plants than for animals and microorganisms, and it increases with increasing LET (linear energy transfer). The extraordinary large RBE values for mutations in higher plants strongly indicate, as also other evidences do (STADLER, 1932, 1954; MATSUMURA *et al.*, 1963; KONDO, 1964), that the majority of ionizing-radiation induced mutations are due to chromosome aberrations or gross deletions.

105. Effects of 14 MeV neutrons in T. monococcum

Taro FUJII

F_1 seeds from the cross between the normal type and a *chlorina* mutant were subjected to γ-rays and 14 MeV neutrons, as in the previous experiment (FUJII, Annual Report No. 14, 1963). 8,600 rad of γ-rays and up to 900 rad of neutrons had no apparent effect on germination rate. Survival rate in the γ-ray lot was slightly lower and was in the three neutron lots slowly decreasing with increasing dosage. A similar tendency was also observed in the average number of tillers, but it was not marked.

Somatic mutation rate was calculated on spike basis. As shown in Table 1, the frequency of tillers with *chlorina* stripes increased with increasing neutron dosage. The frequency of mutated tillers in the highest dosage lot was 0.79%; it was twice as large as that of the lowest dosage lot, and that of 8,600 rad of γ-rays was about one half as large as that of the 324 rad neutron lot. An almost linear relation was observed between mutation frequency and neutron dosage, but the mutation rate at higher dosages was too high for linear relation in the

Table 1. Frequency of somatic mutations.

Treatment (krad)	No. of seeds	Germination rate (%)	Survival rate (%)	Total no. of spikes	Average no. of spikes per plant	*Chlorina* stripes	
						No. of plants	No. of spikes (%)
Control	239	44.8	87.9	3,228	34.3	0	
γ-8,600	400	50.2	83.2	6,101	35.3	6	11 (0.18)
n-324	399	50.6	77.2	5,617	36.0	13	22 (0.39)
n-661	399	50.1	76.2	4,806	31.6	11	25 (0.52)
n-900	399	43.6	73.0	3,902	30.7	11	31 (0.79)

γ-irradiation experiments. From the result, we may say that somatic mutation is caused, for the most part, by chromosome aberrations.

Decrease of fertility was marked with increasing dosage. When the fertility was compared between mutated and non-mutated tillers within each individual, average fertility of the former was lower than that of the latter. These facts also support the view that most of somatic mutation arises from chromosome aberrations.

Average number of mutated tillers per individual roughly increased with increasing dosage, as the result of repeated occurrence of the same kind of mutation within the same individual.

106. Lethal effects of heavy ionizing particles in Arabidopsis

Taro FUJII

Heavy-ion linear accelerator (HILAC) is especially important in radiation biology because accelerating beams of He-, C-, N- or Ar-ions, *etc.*, can be obtained from this machine. As the space age rapidly approaches, information on the application of these beams to plants could be interesting to space biologists, such ions being abundant in the cosmos. Dry seeds were subjected to 1~5 krad of He- and C-ions respectively, and 3 and 5 krad of Ar-ions from the HILAC machine with the energies of 10.4 ± 0.2 MeV per nucleon by courtesy of Dr. J. LYMAN, University of California. Survival rate decreased markedly with the increase of dosage, especially in the treatment with He-ions; in 5 krad lot it was reduced to about one half of that of the control lot. Decrease of survival rates in C-ion lots was not so severe, amounting to 66 per cent in the 5 krad lot. On the other hand, 3 and 5 krad treatments with Ar-ions showed a slight decrease of survival rate while their LET was strikingly higher than with the other ions. RBE values increased with the increase of LET and the peak of RBE was observed at about 70~100 keV/μ of LET.

The RBE decrease with increasing LET may be interpreted as follows. The number of ions in the tissue increases with increasing LET. But a certain number of ions in the given tissue is sufficient for producing biological damage, and those produced in excess at a very high LET range become ineffective and therefore the RBE values slow down. This may have caused a low effect in the Ar-ion lot. From the results, RBE values for the killing effect seemed to be about 20 for He-ions, about 15 for C-ions and less than 10 for Ar-ions, when γ-rays were used as standard. These results are in good accord with those reported concerning RBE *versus* LET relationship.

Growth in the lots irradiated by those heavy ions was very bad, many plants became dwarf and often had deformed leaves and branching which occur seldom in γ-irradiations. The facts suggest that the accelerated heavy ions act not only on the nucleus or chromosomes but also on the tissues. It is known that the RBE values of neutrons were around 15 when they were examined for chromosome aberrations or somatic mutations, and their LET's were almost similar to those of He- and C-ions.

In my previous experiment, dosage up to about 5 krad of neutrons had no effect on survival rate and caused no severe alteration of growth habit (FUJII, Annual Report No. 14, 1963). A decrease in survival rate should have been expected in the neutron irradiations because similar dosages were applied both in experiments with neutrons and heavy ions. This discrepancy awaits further investigation.

107. Effects of dose rate and fractionation of γ-rays on mutation in maize pollen grains

Tomoo MABUCHI

Mature pollen grains of a dominant line (*Su*) of maize were irradiated by ^{137}Cs γ-rays and dusted on the ears of a recessive line (*su*). Mutations (*Su*→*su*) were detected in the kernels developed from the fertilization by the treated pollen. The results are shown in Tables 1 and 2.

The same dose was given to pollen grains at 6 different dose rates of 4～917 r/min for 1.2～275 min (Table 1). There was no significant difference in the frequency of partial endosperm mutation between different dose rates. But whole mutation was considerably higher at higher dose rate (more than 36 r/min) than at the lower rate (less than 15 r/min). A dose of 1,100 r at 917 r/min produced twice as many whole mutations as the same dose at 15 r/min. Total mutation frequency decreased exponentially with decreasing dose rate and reached constant level. The data clearly show that there must be a threshold for a dose

Table 1. Effects of dose rate on mutation. (dose: 1,100 r)

Dose rate (r/min)	Duration of exposure (min)	No. of seeds set	Mutation frequency (%)		
			Whole	Partial	Total
–	–	2,869	0.03	0.06	0.10±0.06
917	1.2	1,502	2.26	0.73	3.00±0.44
250	4.06	1,655	2.48	0.48	2.96±0.42
125	8.12	2,454	2.36	0.81	3.18±0.35
36	30.66	1,861	1.67	0.70	2.36±0.35
15	73.31	2,010	1.19	0.65	1.84±0.30
4	275.0	2,314	1.30	0.52	1.82±0.28

Table 2. Effects of storage and radiation fractionation on mutation. (dose: 1,100 r, dose rate: 917 r/min)

	Duration of storage or interval (min)	No. of seeds set	Mutation frequency (%)		
			Whole	Partial	Total
	–	2,869	0.03	0.06	0.10±0.06
Single irrad.	10	4,464	2.37	0.58	2.96±0.25
	30	4,254	2.33	0.63	2.96±0.26
	60	4,170	2.45	0.50	2.95±0.26
Fractionation	10	3,782	1.82	0.42	2.25±0.24
	30	4,944	1.76	0.40	2.16±0.21
	60	3,133	1.56	0.41	1.98±0.25

required to raise the mutation rate.

A dose of 1,100 r at acute irradiation was applied to the pollen grains. After irradiation they were stored in the γ-room (23°C) for 10, 30 and 60 minutes and then dusted on the ears. A mutation rate of nearly 3% was found in these 3 lots (Table 2). It is clear that there was no storage effect on mature pollen grains.

Fractionated doses (each 550 r) were given twice with intervals of 10, 30 and 60 minutes. Partial mutation frequency was not significantly different among 3 irradiated lots, but whole mutation slightly decreased with increasing interval (Table 2). Therefore, the decrease in mutation frequency was not due to storage effect but to restitution of chromosome breakages.

108. *Photoreactivation of ultraviolet-induced mutation in maize endosperm due to pollen irradiation*

Mituo IKENAGA and Tomoo MABUCHI

The reversal of ultraviolet-induced damages by subsequent exposure to visible light has been observed in various organisms over a number of years. The phenomenon of photoreactivation is of great importance to radiobiology, for it might be a specific process revealing the mechanism of ultraviolet (UV) effects. So far as photoreactivation of UV-induced mutation is concerned, investigations have been scarecely carried out in higher plants. In the present study an endosperm maize mutant, *su*, was used. The pollen was 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. Immediately after UV exposure, the irradiated pollen was divided into two halves, one was re-spread on a Petri dish and exposed to visible light from 20 W Toshiba fluorescent lamp for 30 min at the distance of 10 cm (PR treatment), and the other half was held for 30 min in darkness (dark treatment). After PR or dark treatment, the pollen was dusted on the silks of the recessive line, and the pollinated silks of the dark treatment group were wrapped in aluminum foil as soon as possible in order to shut out the sun light.

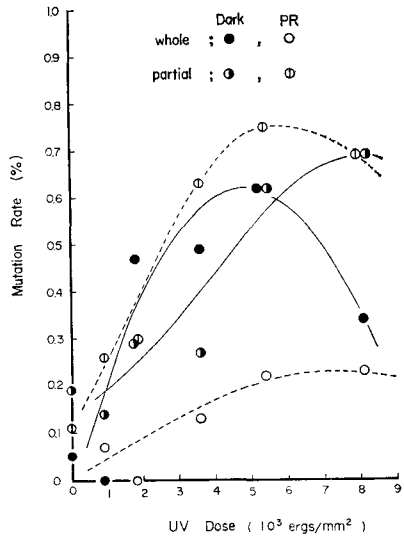


Fig. 1. Curves of whole and partial mutation rates *versus* UV dose with or without photoreactivation.

Induced whole and partial mutants (chimeras) were scored separately, and the obtained mutation rates are given in Fig. 1. As seen from the graph, mutation rates of whole mutants were considerably decreased by exposure to visible light. On the other hand, partial mutation rates were slightly increased by PR treatment. In a parallel experiment with pollen irradiated with 1,000 r of γ-rays, the same PR treatment was completely ineffective. From the obtained data and considering Ito's case with yeast (ITO *et al.*, 1964) it may be assumed that partial mutation may be caused by an UV-induced alteration in one chromatid at *Su* locus and whole mutation by the alteration in two chromatids, since meiosis occurs about ten days before

UV irradiation. The fact that whole mutation is reversible by visible light more frequently than the partial one suggests that photoreactivation may proceed in some way from whole to partial mutation and from there to normal endosperm. Finally, we may at least conclude that photoreactivation of mutation in higher plants really exists, though it is not certain which source of visible light, fluorescent or natural daylight, are effective for photoreactivation.

109. Effects of temperature on post-irradiation storage in einkorn wheat

Tomoo MABUCHI

It is well known that post-irradiation storage increases radiation damage in X- or γ-irradiated seeds, especially in super dry seeds (moisture: 4~8%). Recently, MATSUMURA *et al.* (1961) reported that chronic irradiation was more effective in producing radiation damage than acute treatment in dry seeds of upland rice. However, they could not find such a relationship in seeds of einkorn wheat. An experiment was carried out to test whether or not a storage effect exists during chronic irradiation of seeds of einkorn wheat.

Dry seeds of einkorn wheat were irradiated with 10 and 15 kr γ-rays at acute dose rate of 10 kr/hr and stored in constant temperatures of 0°C, 13°C and 30°C for 436 hours. Furthermore, some of the treated seeds with 15 kr were also preserved at 13°C for 138 and 700 hours. All treated seeds were sown almost simultaneously, just after storage. The

Table 1. Relation between post-irradiation temperature and radiation damage.

Dose (kr)	Storage (hrs)	Temperature (°C)	Seedling height (cm)	(Index)	Seed fertility (%)	(Index)
0	—	13	9.4	(100.0)	72.1	(100.0)
10	436	0	9.3	(98.9)	66.5	(92.2)
〃	〃	13	8.8	(93.6)	65.6	(90.9)
〃	〃	30	8.6	(91.4)	60.1	(83.3)
〃	〃	13	9.2	(97.8)	66.3	(91.9)
15	700	13	6.7	(71.2)	51.5	(71.4)
〃	436	0	8.4	(89.3)	58.5	(81.1)
〃	〃	13	7.1	(75.5)	49.1	(68.0)
〃	〃	30	7.0	(74.4)	48.6	(67.4)
〃	138	13	7.7	(81.9)	57.9	(80.3)
〃	0	13	7.8	(82.9)	63.1	(87.5)

data for seedling height and seed fertility in the X_1-generation are shown in Table 1.

There was found a slight radiation damage in acute 10 kr irradiation. The seedling height and seed fertility were significantly reduced with prolongation of storage, when the irradiated seeds were stored at 13°C after 15 kr irradiation. The results show that there was a clear storage effect.

There was also a clear storage effect on seedling growth and seed fertility of γ-irradiated seeds, stored at 13°C and 30°C for 436 hours after 15 kr irradiation but no clear storage effect was found in seeds, stored at 0°C for 436 hours.

The results of this experiment show that storage effect was greatly influenced by irradiation dose, temperature and storage duration. It is therefore considered that storage effect is noticeable if seeds are irradiated chronically, but the effect may be often masked due to modifying factors, *i.e.* temperature or water content.

110. Oxygen and storage effects on radiation damage in rice and einkorn wheat seeds

Tomoo MABUCHI and Seiji MATSUMURA

1. Rice

Dry seeds of paddy rice with 12.0 % moisture were sealed in ampules filled with oxygen, nitrogen and air, and were 120 hours after sealing exposed to γ-rays from ^{137}Cs 6,000 c at 10 and 20 kr with dose rate 10 kr/hr. Irradiated and unirradiated seeds were sown immediately after irradiation, and growth of seedling was investigated. Seeds in oxygen showed slightly lower growth of seedlings at 0, 10 and 20 kr than those in air. On the other hand, seeds in nitrogen had similar growth of seedlings at 10 kr and showed a slight protection effect for inhibition of seedling growth at 20 kr, as compared to those in air.

Further, seeds similarly sealed and irradiated were stored in underground-room temperature (19～22°C) for 576 and 1,056 hours. Other pretreated seeds were stored after similar sealing and irradiated in similar conditions. The data are shown in Fig. 1. Pre-irradiation storage showed a slightly lower effect in inhibiting seedling growth in oxygen and air treatments than post-irradiation storage, especially at 20 kr. On the other hand, nitrogen treatments were generally more protective for radiation damage as expected, than air and oxygen ones. Further, the relation between pre- and post-irradiation storage in nitrogen seemed to be in the reverse, compared to the results of oxygen and air treatments.

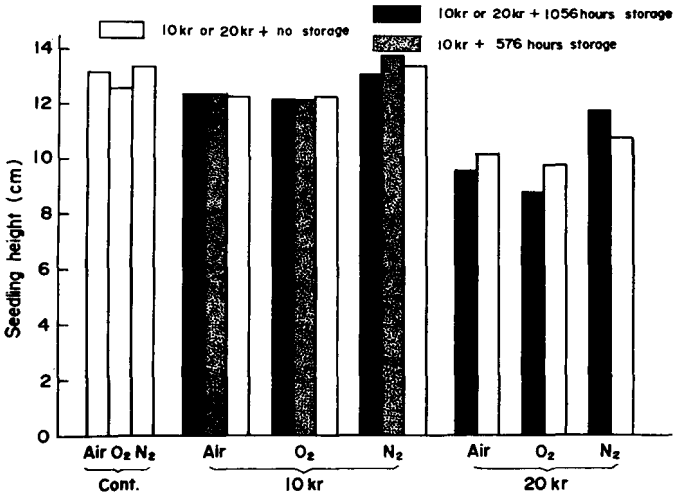


Fig. 1. Oxygen and storage effects on radiation damage in rice seeds (1964).

2. Einkorn wheat

Dry seeds of *Triticum monococcum flavescens* containing 13% water were sealed in ampules filled with oxygen, nitrogen and air, and 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.

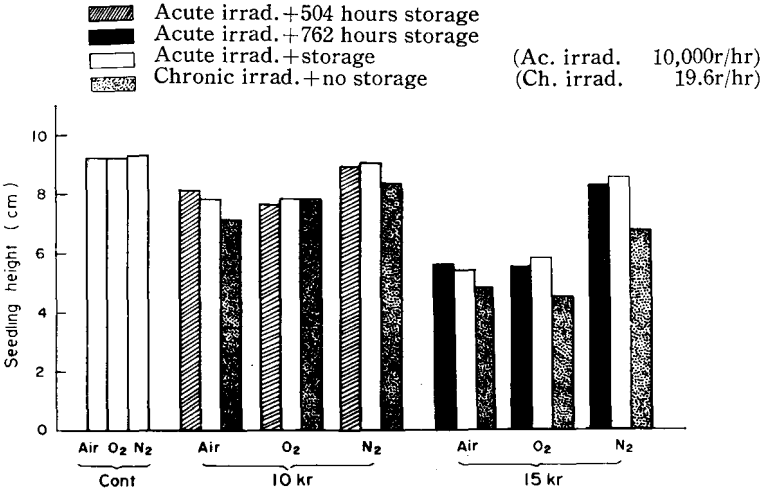


Fig. 2. Oxygen and storage effects on radiation damage in einkorn wheat seeds (1964).

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 date for inhibition of seedling growth are shown in Fig. 2. In general, chronic irradiations were found to be more effective than acute ones, especially at higher irradiation with 15 kr. 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 the inhibition of seedling growth, especially in acute irradiations. Unexpectedly, post-irradiation storage had no effect on the inhibition, also in acute irradiations.

These results might be explained by the relationship between the production and decay of free radicals and radiation damage. It is assumed that nitrogen treatment decreases the radical yield, and the production and decay of the radicals are more gradual and slower in chronic than in acute irradiation.

111. Radio-sensitivity in pollen grains of Triticum *and* Aegilops

Tomoo MABUCHI and Seiji MATSUMURA

Relation between radio-sensitivity and ploidy or genome constitution was investigated in several species of *Triticum* and *Aegilops*. Anthers before dehiscence were irradiated by X-rays (173 kVp, 25 mA, 1 mm Al filter) at 1, 2 and 3 kr with dose rate of 200 r/min. Pollen grains were dusted on the emasculated florets of the same species. Seed set, mean grain weight and germination were recorded.

Seed setting was noticeably impaired even at 1 kr and reduced to 24~75 % and 3.1~25 % of control at 2 and 3 kr, respectively. Germination rate also decreased to 35~88 % of control at 1 kr and was markedly affected (3.3~22.5 %) at 2 kr. Percentage of viable seedlings (per cent success of crossing) was calculated from seed setting×germination rate. It was for diploid *Triticum* and *Aegilops* species about 25 % of control at 1 kr, and for several species of tetra- and hexaploids it was ranging from 30 to 55 %. It is, therefore, concluded that diploid species are most sensitive to radiation, *T. monococcum* as well as *Ae. squarrosa*, but there is no clear difference between tetra- and hexaploid *Triticum* species.

112. Radio-sensitivity of aged wheat seeds

Seiji MATSUMURA and Taro FUJII

Difference in radiation susceptibility between aged and fresh seeds was examined. 7.5 and 15 kr were applied to seeds of diploid wheat. No germination was observed in the control lot of 2 year old seeds, and we had no seedlings for irradiation. Non-irradiated 1 year old seeds showed very low germination rate; a few seeds germinated in the 7.5 kr lot and no germination was observed in the 15 kr lot. Unfortunately germination rate of fresh seeds was as low as 60%; 7.5 kr of γ-rays did not much reduce it (54 per cent), and a few seeds germinated in the 15 kr lot. Thus, the decrease in germinability by irradiation of 1 year old seeds was in the whole series markedly lower than that of fresh seeds. A similar tendency was also observed in seedling length which was measured 14 days after sowing, and the decrease of survival rate in April was also marked in the 1 year old series as compared with that of fresh seeds.

10 and 20 kr of γ-rays were given to tetra- and hexaploid seeds taking into consideration their higher resistance to radiation than that of diploid seeds. Germinability of 2 year old seeds was very low in both, tetra- and hexaploid lots, and no germination was observed at 20 kr irradiation. But germination rate of control lots was better for younger than for older seeds. This trend was marked in irradiated lots. For instance, germination rate in 20 kr lots of 1 year old tetraploid seeds was 11.4% amounting to index 55 when the index of 100 is given to the germination rate in the control lot. On the other hand, 68% germinated in the 20 kr lot of fresh seeds, the index being 73 against that of the control. Seedling length and survival rates also showed a similar tendency. In the hexaploid species, a similar decrease of germinability was observed both in irradiated fresh and in 1 year old seeds. Namely, sensitivity was almost the same in fresh and in 1 year old seeds of this species whereas 2 year old seeds had higher sensitivity. From the results, it can be said in general that older seeds have higher radiation susceptibility than younger ones.

The decrease in germinability due to age was largest in diploid wheat and smallest in hexaploid wheat. We may assume from the results that at a higher ploidy level, we may expect a higher stability not only in respect to radiations but also to the physiological changes caused by aging.

113. Killing effect of UV-rays in Arabidopsis

Taro FUJII

Effects of ultraviolet-rays of 253.7 mμ on *Arabidopsis* seedling were examined. Seeds were sown on wet filter paper in Petri dishes. 200 seeds were used for each lot. Experiments were carried out under continuous artificial light of about 1,000 lux in an air-conditioned room at 25±2°C. About 5 mm long seedlings were exposed to 1~100 min of 90.3 ergs/mm²/sec intensity and were kept in a dark room for 24 hours for the elimination of photoreactivation effect or UV effect fixation. Killing effectiveness of UV-rays was very high; about half of the treated seedlings died at 10 min treatment and almost all died out at 100 min treatment as shown in Table 1. Later, irradiated seedlings were placed

Table 1. Killing effectiveness of UV-rays.

Exposure time in min	Per cent of survivals	
	With dark treatment	Without dark treatment
0	94.5	100.0
1	42.1	98.4
3	17.8	40.8
5	13.6	15.9
7	25.1	—
10	11.1	30.8
20	15.5	25.7
30	23.5	38.1
50	8.7	34.3
100	5.5	33.2

in the experimental room in light just after UV irradiation for examination of the photoreactivation effect. Survival rate became in all lots better than in dark treatment; about 30% of seedlings survived even in the highest dosage lot. By this procedure a photoreactivation effect was recognized.

I do not know whether or not the severe killing effect coincides with the induction of gene mutation, because the stem of young seedlings is very soft and transparent and it seems that UV-rays act on the stem rather than on the growing point. These questions are now examined in experiments with heterozygotic material.

114. Effect of EMS on germination of einkorn wheat

Taro FUJII

Very high mutagenic effects of ethyl methane sulphonate (EMS) were already reported by many researchers. Normal seeds, and heterozygotic seeds from the cross between *chlorina* and normal green in *Triticum monococcum flavescens* were used in this experiment. The aim of this experiment was to determine the differences in mutagenic effect for a specific gene and the difference in the appearance of mass mutation between γ-ray and EMS treatments. For two hours steeped seeds were treated with 0.1, 0.3 and 0.5 % EMS solutions for 22 hours. For comparison, for 24 hours steeped seeds were subjected to 0.5, 1.0 and 1.5 kr of γ-rays. Moreover, EMS and γ-ray treatments were combined to examine the synergistic effect of both treatments. The treatments were so combined that 2 hours steeped seeds were placed in EMS solution for 22 hours and γ-ray exposure was done after 5 min washing with tap water. Steeping and EMS treatments were done under room condition at 20°C.

γ-rays had almost no effect on germination rate of the normal strain, but the F_1 hybrids were affected. In EMS treatments, germination rate in both strains decreased with increasing concentration of EMS, more markedly in the F_1 lot, as shown in Table 1. (Of course, germination

Table 1. Germination rates of γ-ray and EMS treatments of normal and F_1 seeds.

Treatment	Normal	F_1
γ-ray 0.5 kr	86	54
1.0	86	50
1.5	88	46
EMS 0.1 %	91	50
0.3	80	48
0.5	70	32
EMS 0.3 %+γ-ray 1.0 kr	78	38
EMS 0.5 %+γ-ray 1.0 kr	52	14
—	90	78

rate of F_1 seeds was a little inferior to that of the normals because they are usually smaller than normal seeds.) Moreover, combination of EMS and γ-ray treatments showed severer killing effect than each single treatment; about 50 % and only 14 % seeds germinated in the normal and the F_1 batch, respectively in the EMS 0.5 % and γ-ray 1 kr treatment lot. Almost all seedlings died out in these lots. Additive or synergistic effects of both treatments are assumed. The appearance of *chlorina* stripes due to somatic mutation is now under examination.

115. Developmental-genetic study on culm internodes, leaf sheaths and leaves in irradiated progeny of barley

Genkichi TAKEDA and Kan-Ichi SAKAI

Three groups of short-culm mutant lines derived from X- and γ-rayed seeds of three barley varieties were investigated for six internodes, two upper leaves and their leaf sheaths. Examined was the main culm of each plant. The lines were in generation X_5 or X_6 and the number of lines was 10 to 16 in each group.

Analysis of variance showed that variation among lines were found to be statistically significant in all characters. It was also found in case of internode length that interactions between internode position and line were statistically significant.

Genetic correlations among internodes *inter se*, internodes and leaf sheaths, and also leaf blades and leaf sheaths were investigated. Although internodes were more or less mutually correlated genetically, genetic correlations between two adjoining internodes tended to be higher than those between two distant ones, and particularly genetic correlations between second and third, and third and fourth internode length were highest in all line groups. Genetic correlation between leaf sheath length and the corresponding internode length was high, about 0.7~0.9, whereas leaf blade was found to behave in another way: the genetic correlation between leaf blade length and either leaf sheath or internode length was so low as 0.4~−0.4. It is concluded that genes responsible for internode and leaf sheath formation should be to a certain extent the same, whereas those responsible for leaf blade formation are different from them.

Developmental instability in the length of internodes was investigated on the basis of intraline interplant variation. It was observed that the instability was different among lines in every group, and that the interactions between internode position and line were also statistically significant in two groups. Of special interest was that the third and fourth internodes were highly stable while the upper as well as the lower ones were unstable.

Findings from this study are summarized as follows:

(1) The same genes are likely to govern formation of culm and leaf sheath, but they are different from those controlling leaf blade formation.

(2) Internodes in central position of a culm are developmentally highly stable while upper and lower ones are unstable. It means that, in some sense or other, the central parts of a culm are developmentally highly buffered, which deserves a further investigation.

116. Effect of gamma-ray irradiation and maleic hydrazide treatment on the pH 7.5 esterase of corn seedling

Toru ENDO

One of the first detectable biochemical changes in germinating seedlings as a result of irradiation of dormant seeds is an increase in activity of a number of enzymes. This condition holds for the acidic esterases in maize but not for the pH 7.5 esterase. In this study dry corn seeds were irradiated with 50 kr gamma-ray using a cobalt-60 source, germinated in the dark at 30°C, and changes in activity of the various esterases in crude seedling extracts during growth were determined by comparison of zymograms developed following starch gel electrophoresis. Analysis was made of extracts from the first internode and the coleoptilar region (which included the young leaves) of 4 through 9 day-old seedlings.

Growth inhibition in irradiated material became strikingly apparent 5 days after germination. The esterase levels in 4 day-old irradiated seedlings were not different from the control. By 5 days the acidic esterases increased greatly in activity in the first internode and the increase presented through the 9 day material. The pH 7.5 esterase did not show this increase and its level fell off during growth paralleling the decrease in the control seedlings. On the other hand, in the coleoptilar region, the acidic esterases of the irradiated material remained at about the same level as the control but the pH 7.5 esterase showed a marked decrease in activity with age relative to the control. Thus the levels of the acidic and the pH 7.5 esterases are effected differently by irradiation in the two plant parts and in no case did the irradiation cause an increase in the pH 7.5 esterase. This is an interesting finding since the pH 7.5 esterase in maize is associated with active growth.

Soaking of dry seeds in a 0.01 *M* solution of maleic hydrazide for 24 hours decreases growth of the seedlings to about the same extent as 50 kr gamma-rays. However, the esterase levels in the seedlings from the two treatments were very different. The acidic esterases were not increased in the first internode in maleic hydrazide treated material and the pH 7.5 esterase level remained high in both tissues even in the older seedlings

117. Dose-rate distribution in the ^{137}Cs gamma-greenhouse

Takesi KATO[1] and Mituo IKENAGA

The facility for continuous and extremely low dose rate γ-ray irradiation with ^{137}Cs 40 c, the so-called gamma-greenhouse, roughly air conditioned, was built in March, 1964. The dosimetry of the γ-room was carried out by Victoreen condenser chambers, air saturated Fricke dosimeter and glass dosimeter. The measured value of the effective source intensity was 34.6 c (Sept., 1964) for the Victoreen 2.5 r (γ) condenser chambers. The relation between the dose rate and distance from the source is shown in Fig. 1 for the Victoreen condenser chamber readings.

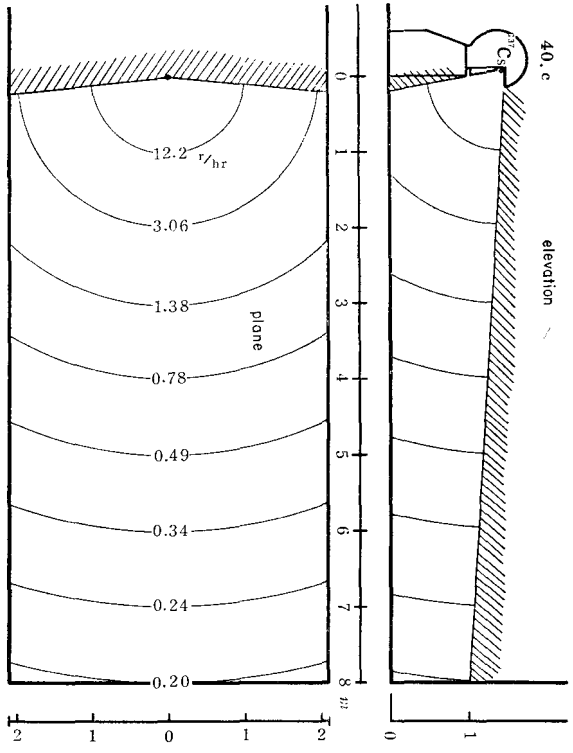


Fig. 1. Dose rate distribution in γ-greenhouse.
(Measured in Sept., 1964)

[1] Visiting researcher from Department of Fundamental Radiology, Faculty of Medicine, Osaka University.

118. Experimental study on depth dose curve on ^{60}Co γ-ray irradiation

Takesi KATO and Sohei KONDO

To confirm the theoretical estimation of the absorbed dose in the surface region of materials exposed to high energy photons presented in The Annual Report No. 11, experimental studies of the depth dose curves for ^{60}Co γ-ray of various materials have been carried out. In order to measure the true depth dose curve it is necessary to eliminate the external secondary electrons originating from the material outside the dosimeter, since they affect depth dose curve as mentioned in the previous report (KONDO *et al.*, Annual Report No. 11, 1960). The parallel plate ionization chamber used in the measurements has a very thin front wall and the back wall is thick enough to give an electronic equilibrium for ^{60}Co γ-rays. The relative depth dose curves were determined by measuring ionization currents for varying front wall thickness while keeping the back wall thick enough to give a maximum reading. The front wall thickness was varied by adding thin disks of appropriate material to the thin front wall. Materials used for the wall were polystyrene, aluminum, copper, silver and lead which have atomic numbers of 5.8, 13, 29, 47 and 82, respectively.

The results obtained for the five wall materials used are compared with the theoretical curves for which a correction was applied to take into account the contribution of the backward emission of secondary electrons produced by γ-rays. It was found that the agreement between the experimental and theoretical curves was satisfactory when Nakai's approximation formula (NAKAI, personal communication) for energy dissipation of electron in matter was used in the calculation of depth dose curve, while when Spencer's theory (SPENCER, 1958) was used a discrepancy

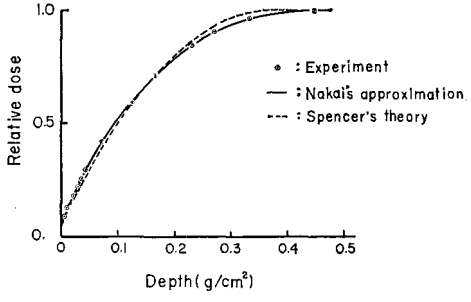


Fig. 1. Comparison of the depth dose curve of polystyrene for ^{60}Co γ-ray with the theoretical curve.

was found for the material of low atomic number. Fig. 1 compares the experimental depth dose curve of polystyrene (circles) with the theoretical curves calculated by using Nakai's formula (solid curve) and Spencer's theory. The discrepancies between experiment and theory may be attributable to neglecting the effects of electron straggling when the theoretical formula is used.

119. Fluorescence quenching and variation with solute concentration of fluorescence efficiency in 2,5-diphenyloxazole-toluene solutions

Takesi KATO[1)]

The variation of fluorescence efficiency with concentration for the 2, 5-diphenyloxazole (DPO) solute and for carbon tetrachloride in the toluene solvent was studied under the aerated condition. The ratio of light yield in absence of the quencher, I_0, to that in its presence, I_Q, at fixed solute concentration and at fixed irradiation intensity for direct excitation of the solute by 302 mμ UV was found to follow the relationship $I_0/I_Q=(1+K_2[Q])$, where [Q] is the quencher concentration and K_2 is the quenching constant by CCl_4 relating to the solute DPO; the K_2 value obtained was 6 liters/mole. Under indirect excitation of DPO via primary excitation of toluene solvent by 260 mμ UV, the I_0/I_Q vs. [Q] curve was found to be described by the Stern-Volmer relationship $I_0/I_Q=(1+K_1[Q])(1+K_2\cdot[Q])$, where K_1 is the quenching constant relating to the solvent. We obtained the following values for zero concentration of DPO; $K_1=640$ liters/mole and $k_q=2.5\times10^{10}$ liters/mole·sec, where k_q is the specific rate of quenching calculated from the relation $k_q=K_1/\tau'$, τ' being the decay time of the fluorescence in the quencher-free pure toluene solution. The specific rate, k_t of excitation transfer from toluene molecules to DPO molecules was determined from the observed relationship between fluorescence efficiency and DPO concentration and combined with other relevant data by other authors; $k_t=8.56\times10^{10}$ liters/mole·sec.

The theoretical curves of fluorescence efficiency vs. DPO concentration based on the energy-exchange processes and the experimental values of rate constants mentioned above were found to fit satisfactorily the experimental values of fluorescence except for the low concentration range (Fig. 1). The disagreement may be explained by the assumption that the solvent emission is absorbed by the solute molecules, giving solute fluorescence with quantum efficiency of about unity. The validity of

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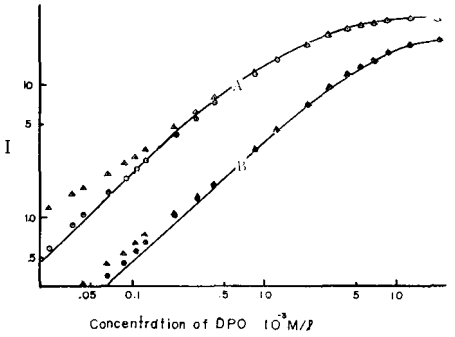

Fig. 1. Comparison of theoretical curves of fluorescence intensity vs. solute concentration with experimental values for the aerated DPO-toluene solutions excited by 260 mμ UV. Curves A and B are calculated ones, (△) and (▲) are the observed values for CCl_4 concentration of zero and 1.7×10^{-3} *M*/l, respectively.

the assumption should, however, be checked experimentally. The quenching constant K_1 under ^{60}Co γ-ray excitation was larger by the factor of 2.2 than that under 260 mμ UV excitation.

G. HUMAN GENETICS

120. Search for selection upon secretor status of ABO blood group substances[1]

Ei MATSUNAGA, Yuichiro HIRAIZUMI, Hisajiro IZUMIYAMA and Toshiyuki FURUSHO[2]

It has been established that the secretion of A, B and H substances into the body fluids is controlled by the autosomal genes *Se* and its recessive allele *se*. From the view-point of population genetics, perhaps the most striking feature of this genetic system is that the relative frequencies of secretor and non-secretor are almost the same among different ethnic groups so far examined, except for small samples of American Indians and Australian aborigines. This fact suggests that the secretion phenomenon might have been subjected to a rather strong natural selection.

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

[2] Tokyo Medical and Dental College.

In order to investigate the selective mechanism, if any, operating upon the secretor status, an extensive field survey was conducted in the summer of 1962 in Ohdate City, located in Akita Prefecture, northern part of Honshu. Approximately 2,500 couples with wife's age ranging from 30 to 40 years and about 6,000 children were tested for ABO blood groups, and saliva samples were taken from each subject, absorbed into a thin paper and kept as a dried stain. Marital histories were taken carefully with the aid of trained midwives concerning the number of pregnancies including induced abortions, pre-natal and post-natal mortality of the children. As to the determination of secretor status, an appropriate serological method was devised by means of absorption experiments with anti-A, anti-B and anti-H using the extracts of *Ulex europaeus*, so that satisfactory results could be obtained with the dried stains as compared with the results given by the raw material of saliva.

Among 11,978 persons tested in all, only four cases of "aberrant excretor" were encountered which showed dissociated secretion of A, B or H substance. The overall frequency of non-secretors was 21.11 per cent, and there were no differences in their distributions among A, B, O and AB groups both in the parental and children's populations. Table 1 summarizes the pooled family data. Among 231 children born to 99

Table 1. Summary of family data.

♂ ♀	No. of couples		Childless couples		No. of children				Unexamined
					Se		se		
	Obs.	Exp.	No.	%	Obs.	Exp.	Obs.	Exp.	
Se×Se	1,461	1,459.4	52	3.6	3,274	3,186.1	370	357.9	152
Se×se	407	384.3	8	2.0	699	699.6	320	319.4	36
se×Se	362	384.3	15	4.1	591	612.4	301	279.6	41
se×se	99	101.1	1	1.0	1	0	230	231.0	6
Total	2,329		76	3.3	4,565		1,221		235

matings of non-secretor×non-secretor, one was found to be secretor. Based on this result, the proportion of illegitimacy in our samples was estimated to be 0.0080 ± 0.0079, which agrees well with the figure of 0.0106 ± 0.0029 estimated from ABO data.

Data concerning reproductive histories were summarized according to mating types in Table 2. Comparisons of the data between secretor and non-secretor wives did not show any significant difference. There were no differences in the proportion of sterile as well as childless

Table 2. Summary of fertility data according to mating types.

♂ ♀	Proportion of sterile couples	Mean no. of pregnancies		Mean no. of living children	Mean interval from the beginning of cohabitation to 1st live-birth	Zygotic loss per pregnany
		Including abortions	Excluding abortions			
					(months)	
Se×Se	0.027	4.231	3.181	2.598	22.91	0.183
Se×se	0.017	4.150	3.202	2.592	22.75	0.190
se×Se	0.030	4.199	3.152	2.577	21.87	0.182
se×se	0.010	3.939	3.030	2.394*	21.75	0.210

* $t=2.165$, $0.05>P>0.02$

couples, or in the proportion of pre-natal and post-natal deaths of the children among the four mating types. The mean number of living children, however, was 2.394 ± 0.089 for non-secretor×non-secretor matings, while it was 2.594 ± 0.023 for the other matings, the difference being significant at the 5 per cent level. This difference is presumably due to a lowered number of pregnancies of non-secretor wives married to non-secretor husbands.

The viabilities of children of secretor and non-secretor types as well as the segregation ratio of the recessive gene *se* from the *Sese* parents were estimated by the maximum likelihood scoring method. Preliminary results obtained by hand computers are represented in Table 3. It is

Table 3. Estimation of some parameters by means of the maximum likelihood scoring method.

Definitions of parameters:

Viability of Se children.....1
Viability of se children.....y
Segregation ratio of *se* gametes in *Sese* parents.....k
Probability that an individual of Se phenotype is *SeSe*....h

Estimated values:

	Initial	Second	Third	Standard deviation
y	1.0000	1.0965	1.1038	0.3419
k	0.5000	0.4940	0.4933	0.0671
h	0.3700	0.3771	0.3767	0.0173

evident from the table that there was no difference in the estimates of viabilities between the two phenotypes, and no distortion in the segregation ratio.

To sum up, our data suggest so far that the gene for the secretor status is apparently neutral with respect to prezygotic and postzygotic

selection upon viability, but there might be some decrease in fertility of non-secretor wives married to non-secretor husbands. The problem how these findings are related to the mechanism by which this polymorphism is maintained, remains to be solved.

121. *Effect of ABO-incompatibility on "waiting time"*

Yuichiro HIRAIZUMI

Recently, attempts have been made to associate "waiting time" to early zygotic wastage due to some genetic mechanisms (H. KRIEGER, personal communication). Accordingly, let the waiting time, x_i be the period between the date when the $(i-1)$-*th* pregnancy of a couple terminated and the date when the next, *i-th* pregnancy started. For the first pregnancy, x_i is the period between the date of marriage and the date when the first pregnancy started. If there are frequent occurrences of unrecognized abortions at the very early stage of pregnancy, they will contribute to increase the waiting time.

A preliminary analysis based upon the data collected at the City of Ohdate, Akita, Japan, showed the following results. The values of x_1 and x_2 were computed in month for each couple, and their average values and proportions are presented in Tables 1 and 2.

Table 1. Average values of x_1 and x_2 (in month).

	Mating Group				
	A-incomp.	B-incomp.	AB-incomp.	Incomp.-pooled	Comp.
$\bar{x}_1$	10.89	11.05	12.40	11.07	10.63
$\bar{x}_2$	21.36	20.65	20.74	20.99	20.31

Table 2. Proportions of couples with $x_1 \leqq 1$ for the first and $x_2 \leqq 10$ for the second pregnancy.

	Mating Group				
Pregnancy	A-incomp.	B-incomp.	AB-incomp.	Incomp.-pooled	Comp.
First	0.207	0.209	0.171	0.205	0.231
Second	0.195	0.209	0.143	0.198	0.236

The distributions of x_1 and x_2 (especially of x_1) among families were much skewed so that it was necessary to find out an adequate transformation for statistical analyses. This transformation has not yet been

found, but taken at face values, x_1 and x_2 were consistently larger in the incompatible mating group. This strongly suggests that unrecognized abortions were occurring more frequently in this group than in the compatible group. This is also supported by the results presented in Table 2. The difference between compatible and pooled incompatible groups was not significant for x_1 ($\chi_1^2=2.24$, $0.10<P<0.20$), but it was significant for x_2 ($\chi_1^2=4.45$, $0.02<P<0.05$). Further analyses are in progress.

122. Studies on the possible genetic consequences of measures affecting population growth

Ei MATSUNAGA

The problem of over-population is growing increasingly serious in many parts of the world, and various methods of family planning are becoming more and more widely used. Although the problems of population numbers and of population quality are separate ones, it has been felt necessary to inquire what genetic consequences may result from measures taken to limit the population growth.

It is well known that Japan was strongly motivated to limit family size after the War. Based on the Eugenic Protection Law passed in 1948, various measures have been actively pursued to slow down a too rapid population increase. These measures include voluntary sterilization and induced abortion for economic reasons as well as from physical considerations for the mother's health, putting emphasis on the encouragement of family planning by means of contraception. As a result, an unprecedented drop in births has been achieved during a short period in this country.

From demographic data showing secular changes in Japan, some aspects can be pointed out which could affect our genetic burden. Reduction in the proportion of births to older mothers and those of high parity should lead to a reduction in the frequencies of certain diseases such as Down's syndrome and Rh-erythroblastosis. Decrease in family size should result in a reduction in the frequencies of consanguineous marriages, and hence reduce the expressed genetic burden. However, these two points are rather of minor importance. Perhaps the major effect of birth control would result from the opportunity to use this measure for eugenic purpose; if the birth of a child with some hereditary disorder could deter the parents from further reproduction, a significant reduction in the frequencies of the disease should be expected. The differential fertility introduced by an uneven distribution of family planning practices among different social strata may bring about some dysgenic effect, but it would

most probably be a transient one. On the other hand, trends towards lower mortality and smaller family size would result in relaxation of natural selection, but the nature and extent of the possible dysgenic effect cannot be predicted at present.

A detailed account on this subject will be presented at the Second World Population Conference to be held in Belgrade in 1965.

123. Human red cell acid phosphatase variants

Tomotaka SHINODA

Variations of red cell acid phosphatase appear to have created a new genetically determined polymorphism in man presumably controlled by three autosomal allelic genes. When hemolysates are examined by starch gel electrophoresis, four zones can be observed of acid phosphatase activity; a faintly active very fast migrating component, a fast one with intermediate activity, a strongly active intermediate component common to all the patterns, and the slowest component with intermediate activity. So far we have been able to distinguish at least four distinct acid phosphatase patterns characteristic of the given human individual. These electrophoretic patterns may be detected as pink zones by incubation of the gel with a solution of phenolphthalein diphosphate in citrate buffer, pH 5.5, followed by addition of ammonium hydroxide to the gel. The zones of enzyme activity can also be observed using p-nitrophenyl phosphate as substrate instead of phenolphthalein phosphate. In this case, however, p-nitrophenol liberated during incubation diffuses much more rapidly than does the phenolphthalein and the zones are therefore much less clearly defined. The attempt was unsuccessful to demonstrate these zones using α- or β-naphthyl phosphate as substrate with an appropriate diazo coupling reagent. This result shows that it is easy to distinguish red cell acid phosphatase from certain other human acid phosphatases which hydrolyze α- or β-naphthyl phosphate at a significant rate. It is of great interest to compare the substrate specificity of acid phosphatases from different origins which may supply informations concerning both structure and function and the genetic factor controlling them.

At the present stage, we have not yet sufficient data for the study of the frequency of the patterns, but the above finding may no doubt add a new problem of polymorphism to human genetics.

124. The nature of molecular heterogeneity between haptoglobin 2-1 and haptoglobin 2-2

Tomotaka SHINODA

The molecular aspect of human serum haptoglobins was investigated with regard to the difference between Hp 2-1 and Hp 2-2. The haptoglobins were prepared from unhemolyzed fresh human plasma by DEAE-cellulose column chromatography and ammonium sulfate precipitation (SHINODA, 1964). The polymers of Hp 2-1 and Hp 2-2 were fractionated according to molecular size by gel filtration on Sephadex G-200. Haptoglobin fraction obtained by gel filtration was analyzed by starch- and urea-gel electrophoresis. As to Hp 2-1 the haptoglobin components eluted first corresponded to those which migrated slowest in the starch gel, whereas those which were eluted later migrated faster in the gel. When gel filtration was made of a sample to which an excess of hemoglobin was added prior to the experiment, the haptoglobin-hemoglobin complex was eluted earlier than free haptoglobin alone. In Hp 2-2, similar results as those with Hp 2-1 were obtained in both gel filtration and starch gel electrophoresis. However, resolution between Hp 2-2—Hb complex and free Hp 2-2 was not sufficiently successful in gel filtration under the condition employed. By using an unfractionated Hp 2-1 it has been demonstrated that Hp 2-1 contains both α^1 and α^2 polypeptide chains in the molecule, as might be expected in the heterozygote. However, this finding does not make it certain whether these two polypeptide chains are derived independently from the respective components of Hp 2-1. The present experiments demonstrated that the early fractions in the gel filtration of Hp 2-1 consist entirely of polymers of slower migrating components and lack the component corresponding to Hp 1-1. However, this fraction is shown, after reductive cleavage followed by urea-starch gel electrophoresis, to contain small amounts of α^1 chain which, therefore, should have been derived from the integral part of the polymers. It may be concluded from these results that the three common haptoglobin types possess in their structure disulfide bonds which are necessary for maintaining the integrity of protein structure and perhaps also for polymer formation, although at present it is not yet certain that the monomeric units are actually linked together by disulfide bonds. Since not all changes in amino acid composition of polypeptides resulted in an altered mobility in electrophoresis as was being demonstrated for some abnormal hemoglobins, it might be premature to conclude that Hp 2-1 contained both the α^1 and α^2 polypeptide chains which are identical to those of Hp 1-1 and Hp 2-2, but it seems that Hp 2-1 contained both chains in the molecule.

125. Clinical conditions of patients with apparently normal chromosomes, I

Hidetsune OISHI and Akira TONOMURA

Chromosome analyses performed on cultured leucocytes of patients having various pathological conditions listed below showed that they all had normal karyotypes of 46 chromosomes.

	Name	Age	Legal sex	Clinical conditions
1	YS	15 years	M	Laurence-Moon-Biedl's syndrome
2	TK	13 years	M	〃
3	SK	8 years	M	〃
4	EE	4 months	M	Harelip; cleft palate
5	HY	2 months	M	Microcephaly; polydactyly
6	KY	2 months	M	Palatorrhaphy
7	MS	4 months	M	Pierre Robin's disease
8	HM	25 years	M	Hypopituitalismus
9	YH	2 months	F	Ehlers Danlos syndrome
10	TN	11 months	M	Chondrodystrophia
11	KO	28 years	F	Marfan's syndrome
12	NS	5 months	F	Pterygium coli
13	MA	14 years	F	Bonne-Viele Ullich syndrome
14	TS	14 years	F	Testicular feminization
15	KM	11 years	F	Adrenogenital syndrome
16	KY	37 years	F	Gonadal disorder
17	AS	25 years	F	Primary amenorrhoea
18	YI	35 years	M	Eunuchoidism
19	SS	21 years	M	〃
20	MK	3 years	M	Male intersex
21	SM	10 years	M	Male pseudohermaphroditism
22	HT	4 years	M	〃
23	KK	17 years	F	〃
24	TY	13 years	M	True hermaphroditism
25	MN	8 years	M	〃

126. In vitro *sensitivity of human lymphocytes to thalidomide and its derivatives*[1)]

Akira TONOMURA and Hidetsune OISHI

From our present knowledge there is every reason to believe that thalidomide exerts a teratogenic effect on foetal tissues of man and other

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

animals. In the present study we have tried 1) to examine the effect of thalidomide on human lymphocytes cultured *in vitro* and 2) to evaluate the usefulness of human leucocyte culture for rapid and sensitive screening of potential drug toxicity.

Human leucocytes obtained from 10 ml of venous blood from healthy donors were cultured according to a modified method of MOORHEAD *et al.* (1960). The drugs used were commercial thalidomide (Isomin), phthalyl glutamic acid, phthalyl glutamine, trinitrophenyl glutamic acid and trinitrophenyl glutamine. The last four compounds were chosen because of their chemical resemblance to thalidomide and occasional questioning of their toxicity under similar experimental circumstances. The drugs were dissolved in 0.02 *M* phosphate buffer solution (pH 7.4) and added to culture media at various concentrations. The cultures were incubated at 37°C for 72 hours. The preparations were made by the use of air-drying technique, and then stained with Giemsa solution. The lymphocytes present were divided into 'small', 'intermediate' and 'blast' types as described in 1962 by ELVES and WILKINSON (Fig. 1). A differential count

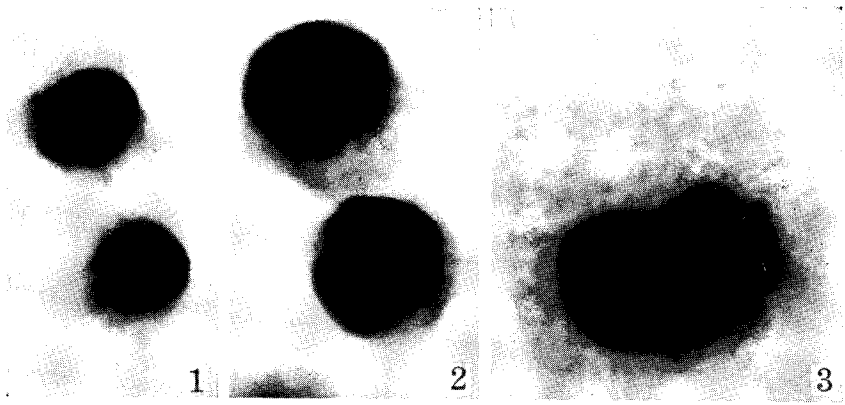

Fig. 1. Photomicrographs of three types of human lymphocytes.
1, small cells; 2, intermediate cells; 3, primitive 'blast' cell.

of 500 cells was made on each slide, and percentages of lymphocytes transformed into intermediate and blast types were determined.

The results obtained for the various groups are summarized briefly in Table 1. In this table, the depression of intermediate and blast types was expressed as a percentage; *e.g.*, if the control culture had 50 % of these cells and the treated 25 %, this would be expressed as a 50 % decrease. The control cultures usually showed 60~70 % of intermediate and blast transformations. Since the appearance of mitotic cells is

coincidental with the appearance of these two types of cells and the increase in DNA synthesis is probably due to the metabolism of these cells, the depression of the two types of lymphocytes may indicate an inhibition of some kind of cellular activity. When the lymphocytes were exposed to thalidomide, a significant effect was obtained at a high degree of confidence ($P<0.01$). Probably significant results ($P<0.05$) were also obtained with phthalyl glutamic acid and trinitrophenyl glutamic acid.

Table 1. The depression of 'intermediate' and 'blast' lymphocyte types in cultures with thalidomide and its derivatives.

Drugs used	Concentration μg per ml	Depression (%)	Significance (P)
dl-Thalidomide (Isomin)	15	38.3	* 0.01~0.001
Phthalyl glutamic acid	28	27.4	0.05~0.02
Phthalyl glutamine	28	19.5	0.1 ~0.05
Trinitrophenyl glutamic acid	17	31.8	0.05~0.02
Trinitrophenyl glutamine	17	16.6	0.3 ~0.2

* Statistically significant.

The results expressed by these figures may indicate that there are some difficulties in proving the usefulness of human leucocyte cultures for studying the potential side action of drugs. Recently, DiPaolo and Wenner (1964), however, demonstrated that thalidomide did not show any effect on the growth of Ehrlich ascites tumor cells *in vitro*. If this is true, it is certain that the phenomenon of the significant sensitivity of human lymphocytes against thalidomide and its derivatives is of great interest, and that much work remains to be carried out to solve this problem.

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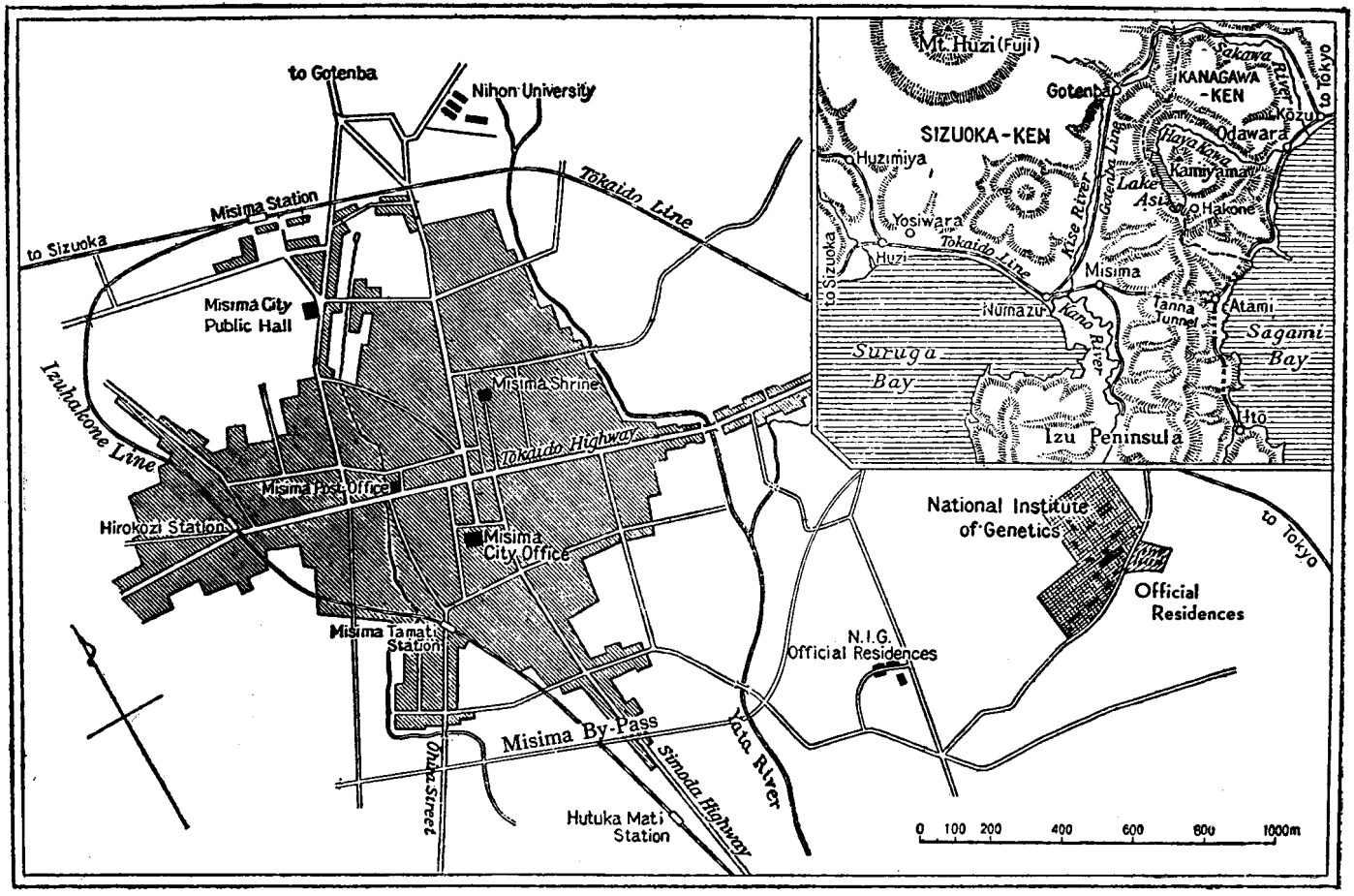
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