NATIONAL INSTITUTE OF GENETICS

JAPAN

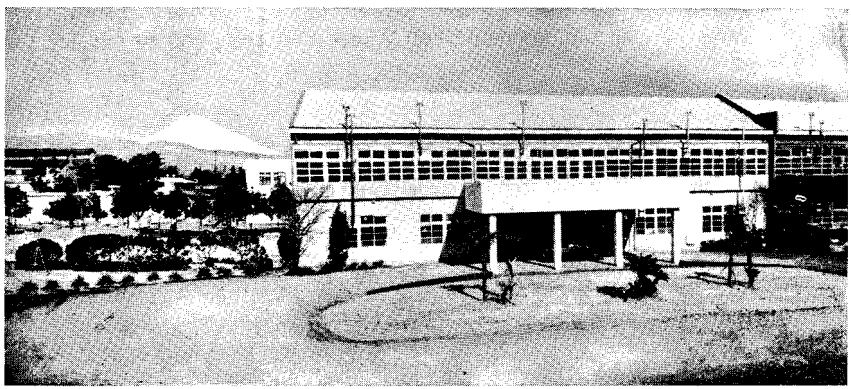
ANNUAL REPORT

No. 11 1960

Published by
THE NATIONAL INSTITUTE OF GENETICS
Misima, Sizuoka-ken, Japan
1961

Annual Report
of the
National Institute of Genetics

No. 11, 1960



Published by
The National Institute of Genetics, Japan
1961

CONTENTS

GENERAL STATEMENT

In this year of 1960, a new department for human genetics was established. Dr. E. Matsunaga became the head of the department. He was formerly professor of legal medicine at Sapporo Medical College, Hokkaido. With the establishment of the new department, our Institute has now seven departments. The new department will be devoted to the studies on blood groups in relation to natural selection as well as the studies of cytogenetics in man. Dr. Y. Hiraizumi came back from U.S.A. after 4 years study in Wisconsin and joined the staff of the department.

On November 7–8, we held a symposium on the effects of radiation in Misima. 72 persons attended the meeting.

The fourth summer seminar on genetics for the senior high school teachers was well attended this year.

ABSTRACT OF DIARY FOR 1960

Feb. 19. 81st meeting of Misima Geneticists' Club.
March 18. 82nd meeting of Misima Geneticists' Club.
April 15. 83rd meeting of Misima Geneticists' Club.
May 21. 31st Biological Symposium.
84th meeting of Misima Geneticists' Club.
29. 32nd Biological Symposium.
June 9. Meeting of all Japan Poultry Breeding Associations.
15. Meeting of Tobacco Research Workers.
July 17. 85th meeting of Misima Geneticists' Club.
20–23. 4th Summer Seminar on Genetics.
Aug. 8. 33rd Biological Symposium.
Sep. 12. 34th Biological Symposium.
13. 35th Biological Symposium.
17. Memorial Lecture and Movies (in Sizuoka).
30. 86th meeting of Misima Geneticists' Club.
Oct. 5. 36th Biological Symposium.
7. 37th Biological Symposium.
21. 87th meeting of Misima Geneticists' Club.
Nov. 7–8. Symposium on genetic effects of radiation.
25. 88th meeting of Misima Geneticists' Club.
Dec. 12. 38th Biological Symposium.
28. 89th meeting of Misima Geneticists' Club.

STAFF

Director

Hitoshi KIHARA, D. Sc., M.J.A.

Members

1. Department of Morphological Genetics:
   Yatarô TAZIMA, D. Ag., Head of Department
   Motoo KIMURA, Ph. D., D. Sc.
   Toshihiko SADO and Kimiharu ONIMARU
2. Department of Cytogenetics:
   Yô TAKENAKA, D. Sc., Head of Department
   Tosihide H. YOSIDA, D. Sc.,

Kazuo MORIWAKI, D. Sc., Tsuguo TATEOKA, D. Sc. (in Canada) and Yoshiaki YONEDA.

3. Department of Physiological Genetics:
   Chozo ÔSHIMA, D. Sc. Head of Department
   Hitoshi KIHARA, D. Sc., M.J.A., Toshifumi TAIRA, Koichiro TSUNEWAKI and Sadao SAKAMOTO (in U.S.A.)
4. Department of Biochemical Genetics:
   Mitsuo TSUZITA, D. Ag., Head of Department.
   Yoshito OGAWA, M. D., Saburô NAWA, D. Sc., Tôru ENDÔ, Bungo SAKAGUSHI (in U.S.A.), Tetsuo INO, Ph. D. and Seizô TSUDA, D. Ag. (in U.S.A.)
5. Department of Applied Genetics:
   Kan-Ichi SAKAI, D. Ag., Head of Department
   Hikoichi OKA, D. Ag.,
   Yukio YAMADA, D. Ag., Akira MIYAZAWA, Takatada KAWAHARA and Shinya IYAMA, D. Ag.
6. Department of Induced Mutation:
   Seizi MATSUMURA, D. Ag., Head of Department
   Sôhei KONDO, D. Sc., Kiyoshi TUTIKAWA, Tarô FUJII and Terumi MUKAI Ph. D.
7. Department of Human Genetics:
   Hitoshi KIHARA, D. Sc. Head of Department
   Yuichirô HIRAIZUME, D. Sc.

Part-time Staff and Research Associates

Yoshinari KUWADA, D. Sc., M.J.A., Emeritus Prof. of Kyoto University
Kan OGUMA, D. Ag., Ex-Director, Emeritus Professor of Hokkaido University
Yoshimaro TANAKA, D. Ag., D. Sc., M.J.A. Emeritus Prof. of Kyushu University
Taku KOMAI, D. Sc., M.J.A. Emeritus Prof. of Kyoto University
Sazirô MAKINO, D. Sc., Professor of Hokkaido University
Kazuo FURUSATO.

Research members under the Grant from the Rockefeller Foundation

Rice Research Group:
Hiroko MORISHIMA, Keizô KATSUYA, Yukio DOIDA, Tadao KATAYAMA, Kokichi HINATA and Masayuki SHINOHARA.
Animal Genetics Group:
Hiromi ISHIWA, Yoshikazu MATANO and Kazuhiko UTSUMI.

Department of Administration

Kunio SHIMIZU, Head of Department
Katsutarô ANDÔ, Chief of General Affairs Section
Tôichi YOSHIKAWA, Chief of Finance Section
Hiroko NAKANO, Naomi MATSUBARA and Keiichi KAWASHIMA
Clerks, Typists, Chauffer, Field Laborers and Janitors 8

Misima Branch of Hatano Tobacco Experiment Station

Masao TANAKA, Head Assistant 5

Association for Propagation of the Knowledge of Genetics

Hitoshi KIHARA, President
Yô TAKENAKA, Managing Director
Yatarô TAZIMA, Managing Director

COUNCIL

Yô K. OKADA, Director of National Science Museum, Chairman
Kan OGUMA, Ex-Director, Emeritus Professor of Hokkaido University
Toshinobu ASAI, Professor of Tokyo University
Tanemoto FURUHATA, Director of Scientific Research Institute of Police
Hideo MATSUKUMA, President of Japan Monopoly Corporation
Toshitarô MORINAGA, Director of National Institute of Agricultural Sciences
Toshio SAITÔ, Governor of Sizuoka Prefecture
Takeo SAKATA, President of T. Sakata Company
Bungo WADA, Professor of Tokyo University
Yoshichi ASAMI, Emeritus Professor of Tokyo University
Seizô KATSUNUMA, President of Nagoya University
Riichi KAWAKAMI, Head of Department of Biometry, National Institute of Public Health
Daigorô MORIWAKI, Professor of Tokyo Metropolitan University
Yakichi NOGUCHI, Professor of Tokyo University
Kenpo TSUKAMOTO, Director of National Institute of Radiological Sciences

PROJECTS OF RESEARCH FOR 1960

Department of Morphological Genetics

Genetics of the silkworm (TAZIMA)
Studies on food preference of the silkworm (TAZIMA)

Radiation mutagenesis in the silkworm (TAZIMA and ONIMARU)
Cytological study of silkworm germ cells (SADO)
Genetic effects of chronic γ-irradiation upon the silkworm (TAZIMA and ONIMARU)
Theoretical studies of population genetics (KIMURA)

Department of Cytogenetics

Cytology and genetics of tumors (YOSIDA)
Experimental breeding and genetics of mice and rats (YOSIDA and KURITA)
Biochemical study on genetical abnormalities of mice (MORIWAKI)
Determination and differentiation of sex in higher plants (TAKENAKA)
Induction of abnormal mitosis and inhibition of growth by substances extracted from certain plants (TAKENAKA)
Interspecific hybridization in Nicotiana (TAKENAKA and FURUSATO)
Genetics of *Pharbitis nil* (TAKENAKA)
Origin of *Prunus yedoensis* (TAKENAKA)
Cytological studies on the yeast cell (YONEDA)

Department of Physiological Genetics

Genetical studies on insecticide-resistance in Drosophila (ÔSHIMA)
Physiological studies on eye-pigment formation in Drosophila (TAIRA and (ÔSHIMA)
Population genetics of deleterious genes in natural populations of Drosophila (ÔSHIMA)
Studies on the origin of rice (KIHARA)
Studies on nucleus substitution in wheat and related species (KIHARA)
Studies on stoneless pomegranates (KIHARA)
Monosomic analysis of 6x wheat (TSUNEWAKI)

Department of Biochemical Genetics

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

Studies on fine gene structure (TSUJITA)
Genetics of virus (TSUJITA)
Immunogenetics of salmonella (IINO)

Department of Applied Genetics

Studies on breeding and genetics in poultry (YAMADA and KAWAHARA)
Quantitative genetics in Drosophila (YAMADA)
Theoretical studies on plant breeding techniques (SAKAI)
Studies on competition and migration in plants and animals (SAKAI, IYAMA and NARISE)
Population-genetic studies in ' Red-Rice ' growing among upland rice (IYAMA)
Genetic studies of alkaloid content in tobacco plants (SAKAI and IYAMA)
Biometrical study of sytoplasmic inheritance (SAKAI, IYAMA and NARISE)
Polyploidy and sterility in fruiting plants (FURUSATO and MIYAZAWA)
Genetic studies of some physiological and agronomic characters of rice (OKA)

Department of Induced Mutation

Radiation genetics of mice (SUGAHARA, TUTIKAWA and MURAMATSU)
Radiation population-genetics of Drosophila (MUKAI)
Triploidy breeding of sugar beets (MATSUMURA)
Relation between the quality of radiations and mutations (MATSUMURA and KONDÔ)
Radiation genetics of cereals (MATSUMURA, FUJII, NEZU and KATSUYA)
Radiation genetics and its practical application (MATSUMURA, FUJII and MABUCHI)
Radiosensitivity in plants (FUJII)
Biophysical consideration of radiation genetics (KONDÔ)
Radiation dosimetry (KONDÔ and ISHIWA)

Department of Human Genetics

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

JOINT RESEARCHES SUPPORTED BY A GRANT FROM THE ROCKEFELLER FOUNDATION

I. *Origin of Rice*

Section I. Collection and preservation of *Oryza* species (H. KIHARA)

a. Strains of 25 species so far collected amount to more than 4,300.
b. Collection-tour was made this year by Dr. H. I. OKA to Latin America.

Section 2. Morphology and physiology of *Oryza* species (S. MATSUMURA)

a. Comparison of radio-sensitivity of *Oryza* species (T. FUJII)
b. Genome-analysis of *Oryze* species (H. KIHARA, S. MATSUMURA, M. NEZU, T. KATAYAMA and T. MABUCHI)
c. Susceptibility of wild and cultivated rice strains to blast fungus (K. KATSUYA)
d. Surface structure of lemma, palea and leaves of *Oryza* species (H. KIHARA and T. KATAYAMA)
e. Investigation of photoperiodic responses of *Oryza* species (T. KATAYAMA)
f. Floating habit of wild and cultivated rice (H. KIHARA, T. KATAYAMA, K. TSUNEWAKI)

Section 3. Population-genetics in wild and cultivated rice (K. I. SAKAI)

a. Estimation of genetic variability among and within populations of wild rice (K. I. SAKAI, S. IYAMA and T. NARISE)
b. Estimation of the percentage of out-crossing by a biometrical method (K. I. SAKAI, S. IYAMA and T. NARISE)
c. Competition between wild and cultivated rice strains (K. I. SAKAI and T. NARISE)
d. Comparative studies of seedling characters of wild and cultivated rice (K. I. SAKAI, T. NARISE and S. IYAMA)
e. Genetic relationship between wild and cultivated rices in Ceylon and India (T. NARISE and K. I. SAKAI)
f. Variation studies of blast disease resistance in wild rice populations (T. NARISE and K. I. SAKAI)

Section 4. Genetic studies in wild and cultivated rice (H. I. OKA)

a. Statistical-systematic studies of wild and cultivated rice strains (H. MORISHIMA and H. I. OKA)
b. Survey of variations between *O. perennis* and *O. sativa* f. *spontanea* (H. MORISHIMA, W. T. CHANG and H. I. OKA)

c. Crossing-experiments and sterility of hybrids between wild and cultivated rice strains (K. HINATA and H. I. OKA)
d. Survey of variations between and within *O. breviligulata* and *O. glaberrima* (H. MORISHIMA and H. I. OKA)
e. Comparative studies in *O. sativa* and *O. glaberrima* (K. HINATA, H. MORISHIMA and H. I. OKA)
f. Hybrid swarms between wild and cultivated rice populations (H. I. OKA and W. T. CHANG)

Section 5. Cytogenetics of *Oryza* species (Y. TAKENAKA)

a. Karyotype analysis of *Oryza* species (Y. YONEDA and M. SHINOHARA)
b. Comparative observations of chromosomes in haploid plants of *Oryza* species (C. H. HU)
c. Embryological studies in *Oryza* species (Y. DOIDA)

II. *Studies on genetic radiation effects on animals*

Section 1. Radiation mutagenesis in the silkworm (Y. TAZIMA)

a. Two types of dose-rate dependence of radiation-induced mutation rate observed in spermatogonia and oögonia of the silkworm (Y. TAZIMA, S. KONDÔ and T. SADO)
b. Cytological basis of radiation-induced sterility in the male silkworm (T. SADO)
c. A theory for the frequency distribution of cluster mutants (S. KONDÔ)

Section 2. Mutation in mammals (T. SUGAHARA)

a. Recessive lethal mutations induced by chronic irradiation given through the whole development of mice during three successive generations (T. SUGAHARA, K. TUTIKAWA, S. MURAMATSU and Y. TAKADA)
b. Shift of secondary sex ratio among the progeny of chronically irradiated male mice (F_1 generation) (K. TUTIKAWA)

Section 3. Cytology and cancer problem (T. H. YOSIDA)

a. Modification of frequency of X-ray induced chromatid breaks in Ehrlich tumor cells by pretreatment with DNP (T. H. YOSIDA, Y. MATANO and K. MORIWAKI)
b. Effect of sodium azide (NaN_3) on X-ray induced chromatid breaks (T. H. YOSIDA and K. UTSUMI)
c. Change of ATP content in Ehrlich tumor cells after treatment with DNP and NaN_3 (K. MORIWAKI)
d. Cytological observations of ascites tumors treated with chronic γ-radiation (T. H. YOSIDA, T. TAKAHASHI and Y. KURITA)

e. Induction of mouse leukemias by X-irradiation (T. H. YOSIDA and Y. KURITA)
f. Chromosomes of leukemias developed spontaneously and induced by X-rays (T. H. YOSIDA and Y. KURITA)

Section 4. Biochemistry of radiation mutagenesis (M. TSUJITA)

a. The composition of ribonucleic acid in yeast irradiated with ultraviolet light (S. NAWA)
b. The base requirement of UV-induced mutations (S. NAKAI)

Section 5. Mutations in populations (C. ÔSHIMA)

a. Analysis of persistence of X-ray induced lethal chromosomes in experimental populations (C. ÔSHIMA and O. KITAGAWA)
b. Polygenic mutation rates of chaeta number in *D. melanogaster* (Y. YAMADA)
c. Some calculations on the mutational load (M. KIMURA)

FOREIGN VISITORS IN 1960

March	10.	Mr. L. F. CHAO (The Botanical Institute, Academica Sinica)
	15.	Mr. R. SHARMA (Assam Raw Silk Bureau, India)
	28.	Dr. E. E. PARCHWITZ (Atomic Energy Commission, West Germany)
	29.	Dr. J. BREWAKER (Brookhaven National Laboratory, U.S.A.)
	31.	Dr. H. KUCKUCK(Institute for Horticultural Plant Breeding, Hanover Technical College, Germany)
April	5.	Mr. T. W. LIU (Joint Commission of Rural Reconstruction, China)
	11.	Dr. & Mrs. J. M. WILKS (Summerland B. C., Canada)
	21.	Dr. R. E. CLELAND (Indiana University, U.S.A.)
	28.	Dr. A. H. MOSEMAN (The Rockefeller Foundation)
May	29.	Dr. R. A. FISHER (University of Cambridge, England)
June	7.	Dr. & Mrs. H. K. NANDI (Agricultural Bureau, West Bengal, India)
	11.	Dr. R. AUECHAGIT (Chulalongkorn University, Thailand)
	20.	Mr. G.KEMMLER, (Potash Research Association, West Germany)
July	1.	Mr. J. W. FOSTER (Texas University, U.S.A.)
	22.	Dr. H. H. HUBBELL Jr. (Oak Ridge National Laboratory, U.S.A.)
Aug.	8.	Dr. H. H. CURTIS (Brookhaven National Laboratory, U.S.A.)
	10.	Dr. H. LEA (Sydney Agriculture Experiment Station)
Sep.	12.	Dr. T. MAKINODAN (Oak Ridge National Laboratory, U.S.A.)
	13.	Dr. L. C. STRONG (Roswell Park Memorial Institute, U.S.A.)

Sep. 17. Mr. S. WORTMAN (International Rice Research Institute, Manila, Philippines)
Mr. P. R. JENNINGS (The Rockefeller Fundation)
26. Dr. & Mrs. F. F. HILL (The Ford Fundation, U.S.A.)
29. Mr. C. S. K. HYUN (College of Agriculture, Seoul National University, Korea)
Mr. PHAM HUY LAN (National Agricultural Institute, Vietnam)
Mr. NGUYEN VAN CHI (National Agricultural Institute, Sough Vietnam)

Oct. 5. Dr. S. OHNO (Hope Medical Center, U.S.A.)
7. Mr. S. SAMPATH (Central Rice Research Institute, India)
26. Dr. O. MUHLBOCK & Dr. A. DUX (Holland Cancer Institute, Holland)

Nov. 7. Dr. H. LANGENDORFF & Dr. H. J. MELCHING (Freiburg University, Germany)
28. Dr. & Mrs. E. J. WELLHAUSEN (The Rockefeller Fundation)

Dec. 12. Prof. G. S. STENT (California University, U.S.A.)
16. Dr. F. W. SANDERS (Oak Ridge National Laboratory, U.S.A.)

RESEARCHES CARRIED OUT IN 1960

A. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF ANIMALS

1. *The persistence of induced lethal genes in experimental populations*[1)]

(By Chozo OSHIMA and Osamu KITAGAWA)

The method of maintaining experimental populations has been described by BUZZATI-TRAVERSO (1947). Following this method, an experimental population was started by introducing ten pairs of flies, heterozygous for an induced recessive lethal gene, into the genetic background consisting of inbred Samarkand chromosomes. The population has been continuously cultured in six vials. Four populations were replicated for each kind of lethal heterozygotes and the generations were counted in fifteen day

Table 1. Zygotic frequencies of lethal genes in experimental populations

Generation / Lethal genes	X	XIX-XXI	Q_{21}	XXVI, XXVII	Q_{27}
7	0.1788	0.1702**	(0.0902)	0.0454	(0.1127)
8	0.0466**	0.0208*	(0.0371)	0.0000**	(0.0196)
10	0.0168**	0.1234	(0.0154)	0.0216	(0.0901)
16	0.1482	0.0834	(0.0816)	0.0440	(0.0667)
	mean 0.0976*	0.0995	(0.0635)	0.0278	(0.0766)
18	0.2482	0.1380	(0.1049)	0.0202*	(0.0976)
26	0.2666*	0.0444	(0.1081)	0.0000**	(0.0392)
	mean 0.2574*	0.0912	(0.1066)	0.0101*	(0.0716)
Total mean	0.1509	0.0967	(0.0825)	0.0217	(0.0750)
Theoretical frequency (s=0)	0.1818±0.0386	0.0909±0.0287		0.0714±0.0258	

Q_{21}: Expected frequency based upon the observed frequency in generation 10.
Q_{27}: 〃 〃 〃 〃 〃 〃 21.
** : Significant at 1% level. * : Significant at 5% level.

1) This work was supported by Grant RF 57178 from the Rockefeller Foundation.

periods. The number of flies in the population increased as the time passed and reached about one thousand flies. At an arbitrarily chosen generation, fifty male flies were sampled from two populations and they were examined by the test-cross to the original lethal heterozygotes in order to find out whether one of their second chromosomes had the same lethal gene or not. By means of three such sampling tests in the course of twenty-seven generations, we could evaluate the decreasing frequencies of the lethal gene in the experimental populations. The individual persistence of six (L 7, 8, 10, 16, 18, 26) among twenty-seven induced lethal genes was actually followed up respectively. The zygotic frequencies of these lethals decreased as shown in Table 1.

The total mean zygotic frequencies of these lethals were observed to decreased from 1.000 to 0.1509, 0.0967 and 0.0219 at the initial, tenth, twentieth, and twenty-seventh generation, respectively. Four lethal heterozygotes (L 7, 8, 10, 16) out of six had relatively high coefficients of selection (mean $\bar{s}=0.18281$) and the other two (L 18, 26) had negative coefficients of selection (mean $\bar{s}=-0.04055$) in the homozygous genetic background. From the results shown in Table 1, the persistence of the latter two lethal genes during ten early generations was found to be higher than that of the former four lethal genes. However, in the later generations all lethal genes were presumed to be similarly selected out. As long as the genetic background of flies in the population has been kept homozygous, lethal genes having an advantageous effect on the viability of heterozygotes could be allowed to persist in the population. When the genetic background became heterozygous by spontaneous mutations in the later generations, their superiority would be lost. The total mean zygotic frequencies decreased showing non-significant differences from the theoretical frequencies, when s was assumed to be zero, although their mean $\bar{s}$ was 0.10836.

2. *The persistence of deleterious genes in natural populations of Drosophila melanogaster*

(By Chozo OSHIMA and Osamu KITAGAWA)

The second chromosomes of *D. melanogaster* were isolated from several Japanese wild populations by using the method of completely marked inversion. The relative frequencies of chromosomes carrying lethal, semilethal, subvital and normal genes were estimated. The results obtained in 1959 had been published in the previous annual report (No. 10). A similar sampling of second chromosomes from the same populations was

carried out also in 1960 and the results were compared with those obtained in 1959, as shown in Table 1.

Table 1. Frequencies of deleterious and normal second chromosomes isolated from natural populations

Population	Percentage of wild type flies					No. of chromosomes tested
	0(1)*	0-16.7 (sl)	16.8-25.0 (sv)	25.1-33.3 (n)	33.4-41.6 (n)	
Suyama (1959)	9	6	11	43	9	78
Suyama (1960)	17	2	12	84	18	133
	26	8	23	127	27	211
Total (%)	12.32 ± 2.26	3.79 ± 1.32	10.90 ± 2.08	60.19 ± 3.37	12.80 ± 2.30	
Juriki (1959)	7	2	9	30	8	56
Juriki (1960)	6	1	3	34	7	51
	13	3	12	64	15	107
Total (%)	12.15 ± 3.16	2.80 ± 1.59	11.21 ± 3.05	59.81 ± 4.74	14.02 ± 3.36	

* 1: lethal. sl: semi-lethal. sv: subvital. n: normal chromosome.

Table 2. The results of allelism test between natural lethals

Population	No. of lethals	No. of crosses	No. of allelic crosses	Allelic rate
Within Suyama (1959)	9	36	1	2.78
Within Juriki (1959)	6	15	0	0.00
Between Suyama & Juriki (1959)	15	54	1	1.85
Total		105	2	1.90
Within Suyama (1960)	17	136	6	4.41
Within Juriki (1960)	6	15	1	6.67
Between Suyama & Juriki (1960)	23	102	1	0.98
Total		253	8	3.16
Between Suyama & Juriki (1959) and Suyama & Juriki (1960)	38	345	3	0.87

The different classes of deleterious chromosomes were similar in relative frequencies in Suyama and Juriki populations and there was apparently no fluctuation between samples collected in 1959 and 1960.

The lethal chromosomes were maintained in the *Cy* balanced system in the successive generations. Diallel crosses were performed with all lethal strains to determine the allelic rate within and between populations. After maintaining the lethal chromosomes during the year 1959, they were subjected to cross-testing with new lethal chromosomes isolated in 1960 from the same populations. The results are shown in Table 2.

The allelic rates in Suyama and Juriki populations underwent scarcely any change during one year, but they seemed to have increased slightly during 1960. Most interesting was that the two lethals isolated in 1959 were found again in 1960. This finding shows that the two lethals have been maintained in the same population at least for a year. According to PROUT's formula (1954), the effective size of population was deduced from the data given in the table. The effective size of Suyama and Juriki populations was estimated to be about 1700.

3. *Heterozygous effects of natural lethal and semi-lethal chromosomes on pre-adult viability*

(By Chozo OSHIMA)

The second chromosomes of *Drosophila melanogaster* carrying lethal, semi-lethal, subvital and normal genes were isolated individually from several natural populations and their relative frequencies were determined.

The viability of heterozygotes for lethal or semi-lethal genes (or chromosomes) has been estimated by STERN et al. (1952) and several other investigators. The findings of all these authors agree in that the heterozygotes are about 3–5 per cent less viable than lethal-free zygotes.

The present author has determined the viability of thirty-five lethal heterozygotes and thirty-two semi-lethal heterozygotes as compared with that of normal heterozygotes carrying chromosomes free of deleterious genes. The mean selection coefficient ($\bar{s}$) of lethal heterozygotes was estimated to be 0.01243 from counts of 85909 flies and the mean selection coefficient ($\bar{s}$) of semi-lethal heterozygotes was estimated to be 0.00678 from counts of 75482 flies.

The selection coefficients of the lethal heterozygotes were distributed from +0.1837 to −0.1186; two of them were highly significantly less viable than normal heterozygotes, one was significantly less viable and one was significantly more viable, all others were not significantly differ-

ent by the results of χ^2-test. The analysis of variance concerning the results of viability test was performed and on the whole no significant differences between viabilities of lethal, semi-lethal and normal heterozygotes, were observed. The mean selection coefficient for lethal genes in an unirradiated large population was estimated by PROUT (1954) at 0.014. This figure is closer to ours than to those given by other investigators. The mean selection coefficient of semi-lethal heterozygotes was about one half of that of lethal heterozygotes. This result is different from that obtained by HIRAIZUMI and CROW (1960).

4. *The persistence of some natural lethal chromosomes in experimental populations*

(By Chozo OSHIMA)

The several natural lethal chromosomes isolated in 1959 from the large Kofu and Katunuma populations and also from the small Suyama and Juriki populations were introduced into individually experimental populations which have been cultured continuously in a constant-temperature room at 25°C. The method of maintaining these populations was the same as described in a joint paper with induced lethal genes (Oshima and Kitagawa, this Ann. Rep).

The decrease from the initial zygotic frequency 1.0000 has been followed up in the course of fifteen generations. To determine the frequency at an arbitrarily chosen generation, one hundred chromosomes sampled from the population were examined, to find out whether they have contained

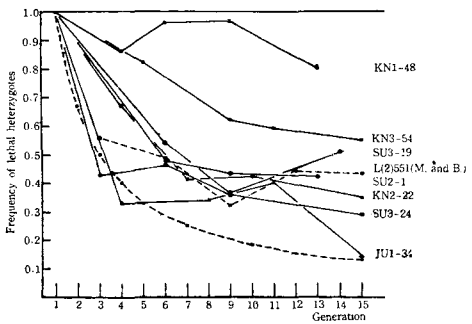


Fig. 1. Persistence of lethal genes in experimental populations.
Dotted line: theoretical curve ($s=0$)
L(2)55i: lethal gene found by MUKAI and BURDICK

lethal genes or not, by the test-cross to the original lethal strain. The results are shown in Figure 1.

These declining curves were compared to the theoretical curve which was given by the no-selection coefficient ($s=0$). Two lethal genes, KN 1-48, KN 3-54, isolated from the Katunuma population have retained a remarkably high frequency and most of the others have decreased at a similar rate as that of the lethal gene which showed a single gene heterosis reported by MUKAI and BURDICK (1959).

Although these data have not been completed, they indicate that there could have been some lethal genes involved showing heterosis in the heterozygous state in natural populations. From the results of allelism test, lethal genes SU 3-19 and SU 2-1 were found to be identical. Those and SU 3-24 lethal gene were demonstrated to have been maintained, at least for one year, in the Suyama population as described in my other paper.

5. *Selection experiment on migrating activity of Drosophila melanogaster*

(By Takashi NARISE and Kan-Ichi SAKAI)

The effect of selection on migrating activity was investigated with wild flies of *D. melanogaster* captured in Te-sima and Igro-sima populations, which formed the TS and IG strains, respectively. One hundred and twenty flies (60 males and 60 females) were introduced to the "original tube" and were kept within it for one day, then nine tubes containing food media were connected radially to the original tube in such way that the flies could migrate from the original to the connected tubes through the connecting narrow paths. All migrants to the connected tubes were collected for breeding the next generation. The over-all migrating activity, involving mass- and random-migrating activity, was examined in every three generations with 30 replications. At the 21st generation of selection, however, test was made for the random- and mass-migrating activity separately with five replications for each. All the experiments were conducted in a dark room at 25°C.

Variance analysis of migrating activity showed that there was a significant difference in the migrating activity among the selected lines.

Investigation of the mass- and random-migrating activity was made with selected lines of the 21st generation of selection, some of which being derived from TB and others from IG strain. In the two groups of lines, the selection for high mass-migrating activity as well as for high random- migrating activity was found to be quite effective. The differ-

ence between the reginal unselected strains and the selected line groups was statistically significant for both migrating activities.

Based on these results, it is concluded that migrating activity, either random or mass, of Drosophila flies is a genetic character, and natural populations of the fly involve a great deal of genetic diversity for these characters.

6. *The effect of inbreeding on migrating activity of Drosophila melanogaster*

(By Takashi NARISE and Kan-Ichi SAKAI)

In the preceding issue of this Annual Report, the result was described and discussed of an experiment dealing with the mass-migrating activity of sixty-five inbred lines of *Drosophila melanogaster*, inbred for 20 or 30 generations. In the present report, the investigation of the same lines with regard to the random-migrating activity is reported. Those sixty five inbred lines were derived from four wild populations collected in 1956. Details of the method of the experiment have repeatedly been given in earlier papers. The experiment was replicated five times.

After 20 or 30 generations of inbreeding, those lines provided with either very high or very low random-migrating activity were established. It is of interest to find no apparent depression due to inbreeding in any of the lines. That the above mentioned migrating activity might be genetically controlled could be demonstrated by further inbreeding experiments.

Analysis between random- and mass-migrating activity showed that they were independent from each other, the correlation coefficient being so low as $r=0.172$.

7. *Estimation of genetic parameters in dairy cattle*

(By Yukio YAMADA)

Genetic parameters in the Advanced Registry Holstein cattle population in Japan were estimated by regression method. A total of 3503 dam-daughter pairs tested during the 1948~58 period were used for the analysis. Among the total, 2401 dams were sired by 399 bulls of domestic and 652 dams 114 bulls of imported origin. Analyses were performed within these two groups.

The average milk production in the group of domestic origin was 406 kg less than that of foreign origin. This difference is, however, not attribu-

table to the excellency of imported bulls, because imported dam's production was 457 kg higher than that of the domestic dams. This should be attributable to the dams' superiority owing to the breeder's choice. The same tendency was observed in fat percentage and fat production. So, it is safe to say that the average genetic potentiality of milk production of the bulls of domestic origin is already on the same level as that of the imported bulls.

With respect to the variance and covariance in production traits no appreciable difference was observed between the two group, and hence only pooled estimates of genetic parameters are given here.

Heritabilities are estimated to be 0.434 ± 0.021 for milk yield, 0.423 ± 0.041 for fat percentage, and 0.512 ± 0.042 for fat production.

Genetic correlations are -0.135 ± 0.045 between milk yield and fat percentage, 0.901 ± 0.008 between milk yield and fat production, and 0.273 ± 0.058 between fat percentage and fat production.

8. *Variance increase of chaeta numbers in heterozygotes of Drosophila melanogaster*

(By Yukio YAMADA)

In order to eliminate any chance of cross-over between irradiated and normal or marked chromosomes during isogenisation processes leading to the extraction of homozygous lines, the following simple and reliable technique for estimating within- and between-line- variance was contrived: In the P_1 generation, a number of samples of 15 adult males from an inbred Oshyoro line were irradiated with certain doses of γ-rays of ^{137}Cs at the dose rate of 306 r/min. The irradiated 15 males of each sample were mated immediately after the treatment to each batch of 15 virgin females of an inbred H-80 line, whose genotype was +/+; Cy/Pm; Ubx/Sb. Resulting F_1 males of genotype +/→; Pm/+; Sb/+ were selected and individually mated to each batch of 3 virgin females of the Oshyoro line. Scorings were made with respect to abdominal and sternopleural bristles for 10 wild type F_2 individuals for each male line. The flies were heterozygous for second and third chromosomes with respect to radiation.

Experimental results are summarized in Table 1.

Increases in between-line-variance by radiation were observed and some of them reached to the significance level, while no differences among the mean values were observed. The variance observed on the basis of heterozygotes had to be doubled for comparison with random mating condition. Thus, the increments of genetic variance give rise to the value of 30.94×10^{-5}/r and 10.08×10^{-5}/r, respectively, for abdominals and

Table 1. Means and variances in bristle numbers of heterozygotes

Treatment	Number of genotypes samples	Abdominals			Sternopleurals		
		Mean	Variance		Mean	Variance	
			Within	Between		Within	Between
0 r	89	29.76	5.2016	0.6118	14.68	2.7203	0.4764
250 r	88	29.75	5.1227	0.6778	14.73	2.6278	0.5472
500 r	96	30.00	5.0597	0.4820	14.32	2.7170	0.3683
750 r	100	29.45	5.4507	0.9191**	14.99	2.7878	0.5181
1000 r	102	30.24	5.3769	0.8549*	14.87	2.9926	0.5242
1500 r	84	30.15	5.9114	0.6919	14.48	2.5208	0.4788
2000 r	84	29.70	5.4987	0.9633**	14.65	2.6005	0.6215*
Variance increment per rad			15.47×10^{-5}			5.54×10^{-5}	

* $.05<P<.10$ ** $P<.05$

sternopleurals. These are considerably larger than the values obtained on the homozygote basis as previously reported. Details will be discussed elsewhere (Proc. Jap. Jour. Gent. 36).

9. *Heterosis and reciprocal cross differences in egg production in some breed matings*

(By Takatada KAWAHARA)

During four years from 1956 to 1959, intra- and inter-breed matings, involving White Leghorns (WL), Barred Plymouth Rocks (BPR), Nagoyas (NG) and Rhode Island Reds (RIR), were conducted. Data were collected from 916 purebred and 1132 reciprocal F_1 hybrid pullets. Mating systems were such that the purebreds and crossbreds were half-sisters. Number of eggs laid up to 120 days after the first egg was recorded for each pullet to find hen-housed, hen-day and survivor egg production. The means and their standard errors for the three traits obtained from pooled four year observations, are presented in Table 1. An effect of heterosis appeared in all traits in every crossbred. The potence ratios (MATHER 1949) of various crossbreds were 3.61, 0.93, 6.81, 10.35, 1.16, 1.62 in hen-housed production, 11.68, 2.78, 43.90, 73.32, 1.03, 1.47 in hen-day production rate and 22.09, 5.00, 12.25, 21.72, 1.05, 1.41 in survivors production rate, respectively, for WL♀×BPR♂, BPR♀×WL♂, WL♀×NG♂, NG♀×WL♂, WL♀×RIR♂ and their reciprocals. The finding of differences between reciprocal F_1 hybrids in egg production performance is noteworthy. The statistical analysis shows that the F_1 hybrids between WL sires and NG or

Table 1. Means of measurements for the three traits up to 120 days after the first egg in purebred and crossbred pullets

Breed or cross	No. of pullets started	Hen-housed egg production (No.)	Hen-day egg production rate (%)	Survivors egg production rate (%)
WL	475	76.82±1.04	66.16±0.77	66.92±0.84
BPR	365	71.49±1.58	64.66±1.12	66.11±1.26
NG	53	79.02±2.62	65.85±2.20	65.85±2.20
RIR	23	50.35±4.91	41.96±4.18	41.96±4.18
WL♀×BPR♂	470	83.79±1.21	74.17±0.80	75.46±0.83
BPR♀×WL♂	442	76.62±1.11	67.50±0.79	68.54±1.06
WL♀×NG♂	37	85.41±4.03	72.81±1.91	72.94±1.94
NG♀×WL♂	76	89.30±2.41	77.37±1.61	78.00±1.35
WL♀×RIR♂	59	78.97±2.46	66.53±1.88	67.50±1.83
RIR♀×WL♂	48	85.06±2.34	71.88±1.66	72.00±1.82

RIR dams produce more eggs than reciprocals. However, BPR♀×WL♂ was consistently poorer in egg production performance than the reciprocal hybrid. From these evidences either sex-linkage (WL crosses with NG or RIR), or cytoplasm-genotype interaction and/or interaction between autosome sand sex-chromosomes (WL cross BPR), may be factors determining the mode of inheritance for egg production.

The crossbreds appear to be less variable than the purebreds, particularly in terms of coefficients of variability. Heritability estimates for hen-housed production were 0.217, 0.429, 0.350 and 0.361 in WL, BPR, WL♀×BPR♂ and its reciprocal, respectively.

10. *Influence of heterosis on hatchability of F_1 hybrids between two breeds of domestic fowl*

(By Takatada KAWAHARA)

Within- and between-breed matings were performed using 11 sires and 84 dams of White Leghorns (WL), and 11 sires and 58 dams of Barred Plymouth Rocks (BPR). Average coefficients of inbreeding of parental purebreds were 2.3%, 4.6%, 5.1% and 3.9%, for WL sire, WL dam, BPR sire and BPR dam, respectively. Mating systems were such that these purebreds and the F_1 hybrids were maternal half-sibs, thus comparisons were possible between groups of pure and crsssbred types. Data were collected from 1934 purebred and 2316 crossbred fertile eggs. The average hatchability and embryonic mortality at different incubation periods

and the significance tests based on the Chi-square method are given in Tables 1 and 2.

The results of statistical analysis of the data are summarized as follows;

1) Average hatchability of fertile F_1 eggs was 7.47% higher than that of purebreds (WL=88.16%, BPR=81.47%, WL♀×BPR♂=92.59% and BPR♀×WL♂=91.97%), and WL was for 6.69% higher than for BPR; the differences were statistically significant at the 1% level. Hatchability of

Table 1. Hatchability of fertile eggs from identical dams mated alternately with sires of different breeds

Breed or cross	No. of sires	No. of dams	No. of fertile eggs	Embryonic mortality (%) (incubation periods) 1-7 (days)	8-18 (days)	19-22 (days)	Hatchability of fertile eggs in %
WL	9	84	1335	3.75	1.05	7.04	88.16±2.26
WL♀×BPR♂	9	84	1183	4.40	0.42	2.62	92.59±2.02
BPR	11	58	599	7.68	2.17	8.68	81.47±4.05
BPR♀×WL♂	11	58	1133	4.59	0.97	2.47	91.97±1.95

Table 2. Chi-square value of significance test for the differences between breeds or crosses

Comparison	Embryonic mortality (incubation periods) 1-7 (days)	8-18 (days)	19-22 (days)	Fertile egg hatched
WL vs. BPR	13.57**	3.78	1.60	15.49**
WL♀×BPR vs. BPR♀×WL♂	0.49	2.53	0.06	0.29
WL vs. WL♀×BPR♂	0.68	3.29	25.99**	13.78**
BPR vs. BPR♀×WL♂	7.00**	4.13*	34.29**	41.93**
WL vs. BPR♀×WL♂	1.11	0.04	27.22**	9.77**
BPR vs. WL♀×BPR♂	8.24**	12.15**	32.89**	49.33**

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

WL♀×BPR♂ was 0.62% higher than that of its reciprocal, but this difference was not significant statistically.

2) Mortality of embryos after the 19th day was for F_1 hybrids less than for purebreds (WL=7.04%, BPR=8.68% vs. WL♀×BPR♂=2.62%, BPR♀×WL♂=2.47%), and the differences were statistically significant at the 1% level. It is obvious that the difference in average hatchability

between the purebreds and the crossbreds was mainly due to mortality of embryos in the late incubation period. The percentage of dead embryos at various incubation periods were for BPR markedly higher than for the other groups.

3) Frequencies of non-genetic abnormalities observed were 0.075%, 0.334%, 0.254%, 0.618%, in WL, BPR, WL♀×BPR♂ and the reciprocal cross, respectively. The occurrence of abnormalities was higher among BPR-maternal embryos than WL-maternal ones (BPR-maternal groups= 0.52% vs. WL-maternal groups=0.16%) and this difference was close to the significance level ($\chi^2=3.34$, $.10>P>.05$). The difference of 0.27% in the occurrence of abnormalities between the purebreds and the F_1 hybrids (purebreds=0.15% vs. crossbreds=0.42%) was not statistically significant.

11. *Negative correlation between rate of development and female fertility in Drosophila melanogaster*

(By Yuichiro HIRAIZUMI)

The relation between two major components of fitness, rate of development and female fertility, was studied for chromosomes II and III of *Drosophila melanogaster*. The developmental rate is a major component of pre-adult fitness and fertility is the important part of adult fitness. The principal conclusions were as follows:

1. There was no detectable maternal effect on the components of fitness.
2. To a first approximation chromosomes II and III contributed to each component in a simple multiplicative fashion although significant, but slight, deviations from this rule were observed.
3. The rate of development was negatively correlated with female fertility when it rose above a certain level but correlation was positive when the rate did not reach this level.
4. Some possible significance of this negative correlation in natural populations, and the relative importance of the developmental rate in determining the total fitness, measured in terms of Fisher's Malthusian parameter, m, were studied.

12. *Some properties of the "sex-ratio" agent in Drosophila*

(By Bungo SAKAGUCHI and Donald F. POULSON*)

It was demonstrated by MALOGOLOWKIN, POULSON and WRIGHT (Genetics

* Osborn Zoological Laboratory, Yale University, New Haven, Connecticut.
(Supported by grant 6017 from N. S. F.)

44: 59–74, 1959) that the maternally transmitted condition known as "Sex-Ratio" (SR) in *D. willistoni* can be transferred to previously normal strains of this species. The present authors (Ann. Rep. Nat. Inst. Genetics, Japan, 10: 27–28, 1959, Anat. Rec. 138: 381, 1960) found that the "SR" agent is present at high concentrations in the hemolymph of "SR" adults of *D. willistoni* and that the agent can be transferred into *D. melanogaster* where the "SR" condition has been maintained for many generations.

The nature of the "SR" agent of *D. willistoni* was experimentally examined from the biophysical and cytological view points.

(1) Estimation of size of the "SR" agent

In order to estimate the size of the "SR" agent, filtration experiments were carried out by using Millipore filters with 0.3 μ and 0.1 μ pore size. The supernatant obtained by centrifuging a homogenate of "SR" female adults of *D. willistoni* was filtered through Millipore filters and aliquots of the filtrates were injected into *D. melanogaster* hosts. The results of these experiments showed no reduction in infections at 0.3 μ pore size and 50% reduction at 0.1 μ pore size in comparison with the control in which only supernatant is injected.

The experimental determination of target size of these particles by means of X-ray inactivation has begun. Dosages greater than 1×10^5 r result in inactivation of the "SR" properties of the particles.

(2) Assay of the "SR" agent by dilution

Inocula of differing titers were prepared by diluting hemolymph of "SR" female adults of *D. willistoni* with Drosophila Ringers solution. An inbred line of *D. melanogaster*, Oregon R, provided hosts for the titration. Dilutions of 1:1, 1:10, 1:100, 1:1,000, 1:10,000 were used. The incubation period was then measured at each dilution, as were the percentage of infections.

When a whole set of flies similarly injected is put through this procedure, some variation is observed in the individual incubation time, but the mean is a function of the titer, being linearly related to the logarithm of the dilution. Thus multiplication of the "SR" agent follows an exponential curve similar to those for bacterial or viral multiplication.

(3) Effects of osmotic pressure on the "SR" agent

The authors (Anat. Rec. 138: 376–377, 1960) have shown by phase contrast microscopy that the blood of "SR" females of *D. melanogaster*, *D. willistoni*, and *D. nebulosa* contains many minute motile granules and enormous numbers of very fine motile filaments (0.1–0.2 μ by 4–8 μ) which possess the properties associated with small spirochetes. That these are causally related to the "SR" condition in these species is established (Science, in press, 1961).

The effects of osmotic pressure on the fine motile filaments, in the blood of "SR" females of *D. melanogaster* and *D. willistoni* were examined by phase contrast microscopy using various concentrations of sucrose in solution and other media. When put into distilled water, the filaments swelled within several minutes and became spherical. Such swelling occurs only after about 15 hours in Drosophila RINGER's or in 0.1 M sucrose solution. When the filaments are mixed with either 0.25 M sucrose solution or hemolymph of normal *D. melanogaster*, the intact form is maintained for a long time, at least for 200 hours. On the other hand, if sucrose concentration is increased, viz. 0.4 M, 0.6 M, 0.8 M, the filaments show shrinkage after about 70 to 100 hours.

In blood treated with penicillin the filaments become rapidly abnormal by swelling and after one hour of treatment such blood loses the capacity to produce "SR" infections when injected into normal hosts.

These observations support the interpretation that the "SR" agent is spirochetal rather than viral in nature in these species of *Drosophila*.

13. *A new case of spontaneous "post-axial polydactylism" in the house mouse*

(By Tosihide H. YOSIDA and Hitoshi SAKAMOTO)

Post-axial polydactylism of the front feet of the house mouse was found by A. Nakamura in Hamamatsu Kita High School and the morphological and genetical characters of the abnormality were studied by the present authors. The abnormality is characterized by the appearance of an extra appendage on the ulnar side of one or both front feet. When only one foot is affected it is more frequently the right one. The degree of manifestation is different by individuals, some of them having only a rudiment of a claw, but others having a claw rudiment and an abortive phalange. Genetical analysis of polydactilism showed that this character depends on a major dominant gene and possibly some minor recessive genes which may modify the manifestation of the character. In total of 430 mice obtained from matings between polydactyl animals for 5 generations 369 (85%) were polydactyl in both front feet, 35 (8.1%) in the right foot only, and 5 (1.1%) in the left foot only. The remaining 21 mice (5.8%) had normal feet. In outcrossing to various inbred strains (C57BR, DBA/Ma, dba and C3H) polydactilism was found in about one half of the F_1. In F_1 the frequency of polydactyl females amounted to 60.3% while in the reciprocal cross it was only 42%. Female mice seem to be somewhat more liable to the manifestation of polydactylism than the males.

F_2 offspring from crosses between F_1 and normal mice obtained from

C57BR (♀)×polydactyl (♂) developed the charactor in 10.4%, while those from polydactyl (♀)×C57BR (♂) were observed to show polydactylism in 33%. Based on the above investigation it is assumed that the manifestation of polydactylism may be affected by the cytoplasm of the mother.

14. *Invasion of tetraploid cells from a common strain of Yoshida sarcoma*

(By Tosihide H. YOSIDA)

Last year, the invasive ability of diploid and tetraploid tumor cells was studied with a subline of YOSHIDA sarcoma in which tetraploid cells occurred at a high percentage (YOSIDA 1960). Intraperitoneal transplantation of lung tissue of the tumor bearing rat resulted in a remarkable increase of tetraploid cells. It was concluded that tetraploid tumor cells invaded the organs easier than diploid cells.

In order to reinvestigate the infiltration ability of tetraploid tumor cells, a common strain of YOSHIDA sarcoma was used. This tumor contained tetraploid cells at 7.3%. Their number was reduced to 7.0% by intraperitoneal transplantation of ascites tumor, while it was increased to 12.0 and 33.3% by intraperitoneal inoculation of spleen tissue of the same tumor bearing animal. When kidney tissue was inoculated the frequency of tetraploid cells increased to 19.0%, while it decreased to 2.5 and 5.3% in two rats inoculated with thymus tissue of the same rat. By serial transplantation of spleen of two rats in which tetraploid cells occurred at 33.3%, the frequency of tetraploid cells increased markedly to 50.6% in one and 68.0% in the other rat. The results indicate that the infiltration ability of tetraploid YOSHIDA sarcoma cells into lung, spleen and kidney was much stronger than that of the diploid stem cells, while the latter showed a predominant proliferation in the thymus gland.

15. *Invasiveness of hypertetraploid tumor cells from a hyperdiploid strain of Ehrlich ascites carcinoma*

(By Tosihide H. YOSIDA)

As shown in the preceding report, the tetraploid cells of YOSHIDA rat sarcoma had stronger infiltration ability into some organs than diploid cells. By double inoculation with hypotetraploid (ELT) and hyperdiploid cell strains (ELD) of Ehrlich ascites carcinoma, YOSIDA and ITOH (1960) found that the ELD tumor cells were much stronger in competition than the ELD cells in the case of ascites tumor inoculation, but the latter

showed a higher ability than the former to invade a few organs. In order to investigate in detail the invasive ability of tetraploid cells, the ELD strain was used. This tumor contained hypertetraploid cells at 13.0%. After the usual transplantation of the ascites tumor the frequency of hypertetraploid cells decreased slightly to 11.0% at the next transplant generation, while it increased to 71.0 and 30.9% in two mice which were inoculated with lung tissue of the same tumor bearing animal. By intraperitoneal transplantation to two mice of the ascites tumor of the former mouse which had developed by lung tissue inoculation, the frequency of hypertetraploid cells decreased to 67.0% in one and 46.2% in the other.

Frequency of hypertetraploid and hyperdiploid cells in the metastatic tumors of ELD strain was investigated by intravenous injection. This strain was characterized by containing only 0.2% hypertetraploid cells. By intravenous injection of the tumor cells into tail veins two mice developed tumors. One of them developed a tumor under the skin in the upper part of the ventral side and the other developed two tumors on the dorsal side and in the kidney. These three tumors were separately injected into peritoneal cavities of normal mice. Four mice inoculated with the ventral tumor pulps developed ascites tumors. They contained tetraploid cells at 85.0, 47.3, 57.0 and 57.8%, respectively. One ascites tumor developed by inoculation of the dorsal tumor was characterized by tetraploid cells at 40.4%, while the transfer of tumor pulps developed in the kidney resulted in the development of two ascites tumors. They contained tetraploid cells at 22.6 and 11.6%, respectively.

Based on the above investigations the conclusion may be drawn that the hypertetraploid tumor cells which were contained in the ELD strain had a higher ability for invasion or metastatis.

16. *Chromosome condition of hypertriploid Hirosaki sarcome*

(By Kazuei TANAKA and Tosihide H. YOSIDA)

USUBUCHI and ABE (1960) established a tetraploid subline of the Hirosaki sarcoma and reported that the modal occurrence of the chromosome numbers in its cells was 70-72. The present authors reinvestigated the karyotypes of 68 cells of the subline. According to our investigation, the number of chromosomes varied from 54 to 109 with the mode at 67 (29.4%) The number of rod-, V- and J-shaped elements was also variable, and the mean frequency of their occurrence was 25.66 ± 1.05, 18.44 ± 0.92 and 23.04 ± 0.94, respectively. Numerical variation of the characteristic large V-elements from 2 to 6 was observed, showing the model occurrence at 4. However, there was no correlation between the total chromosome

number and the number of V-shaped elements.

Based on the above results it is assumed that the cells with hypertriploid chromosome constitution may be in the process of transformation of the tumor stem line.

17. *Alteration of cell populations in serial transplantations of hypotetraploid Ehrlich carcinoma*

(By Yoshikazu MATANO)

The hypotetraploid Ehrlich carcinoma was obtained from the Institute for Infectious Diseases, Tokyo, through the Zoological Institute, University of Tokyo, in March, 1960. Since then this carcinoma has undergone 41 transplantations in Swiss albino mice inbred in our institute. In the early samplings the carcinoma was characterized by hypotetraploid 75 chromosome stem cells with two large metacentric marker chromosomes.

Recent samplings (December, 1960) from the 31st transfer generation revealed, however, that the stem-cells of this carcinoma showed the addition of one outstandingly large telocentric chromosome. In order to find its origin, the preparations from the 11th transfer generation were reexamined with the result that the cells with the outstandingly large telocentric chromosome already existed in the tumor population though in very low frequency.

Previously, it was reported by YOSIDA (1959) that in a new subline of Yosida sarcoma two types of stem-cells existed together for about 20 transfer generations. A similar observation was made in MTK-sarcoma II by the present author (MATANO 1960). In the present case found in the Ehrlich carcinoma a gradual alteration of stemline chromosomes occurred in the course of transfer generation.

18. *Proliferation of Ehrlich ascites carcinoma cells in vitro*

(By Kazuhiko R. UTSUMI and Tosihide H. YOSIDA)

Hyperdiploid Ehrlich ascited carcinoma cells were withdrawn by a syringe on the seventh day after transplantation from peritoneal cavities, washed with a sufficient volume of Hanks' solution, then centrifuged for 10 minutes at 1,000 rpm. The cellular sediment was resuspended in a partly synthetic culture medium of the same volume. The cells in the suspension amounted to several ten millions per milliliter. This suspension was placed in flat test tubes or TD-flasks and incubated at 37–38°C. The medium was renewed every other day during two weeks and thereafter every 3–4 days.

Observation was made *in situ* every day with an inverted phase-contrast microscope. After a few hours, the cells became attached in a mass to the bottom of flasks and almost all died within a week. They were not removed until subcultures were made, since a few surviving cells were among them which could be selected for further subcultures. After two or three weeks, clonal divisions appeared here and there and later, the glass surface was replaced by a living cellular monolayer. Subcultures from the primary culture were made weekly, and after three or four weeks the cells began to show a striking growth, and assuming spindle or polygonal shapes, they migrated to the glass surface. The growing cells were flat or round; the former were living sound cells which contained large nucleoli and appeared to have cellular active metabolism, while most of the latter were considered as dead. In the flasks, small amounts of cells or cell-clumps were usually detached from the monolayer and were suspended in the medium. When they were placed in new flasks, they could proliferate on the glass surface as before. This indicates that some of the round cells were alive.

Thereafter the subcultures were made successively every week or every ten days. Two separate attempts at adaptation *in vitro* of the ascites cells gave similar results, and it may be assumed that the adaptations begins after a month, or in other words the cell population which can be subcultured needs at least one month to establish itself, and thereafter subcultures may be successful. In both cases, cells were maintained for 4-5 months, with three transplant-generations in one case and ten in another case. The results indicate that Ehrlich ascites carcinoma cells require merely a long term nourishment for adaptation to *in vitro* conditions.

19. *Studies on mouse leukemia I. Incidence of leukemia in AKR/Jax strain mice bred in Misima*

(By Tosihide H. YOSIDA, Yoshinori KURITA and Hitoshi SAKAMOTO)

AKR/Jax strain mice were obtained from the Jackson Memorial Laboratory by the senior author in 1958, and were later bred in his laboratory. The incidence of spontaneous leukemia in this strain was 89.39%. In males it amounted to 87.9% and in females to 90.6%, with no significant difference between the two sexes. The analysis of the leukemias showed that the percentage of generalized lymphatic leukemias was in females 27.6%, and in males 24.1%, and that of thymic lymphosarcoma was in the former 25.9% and in the latter 22.41%.

A comparison of blood counts of leukemic and non-leukemic mice showed

that the ratio of red blood cell counts for these two groups was (381.9 $\pm$17.6)$\times 10^4$: (940$\pm$72.0)$\times 10^4$ and that of white blood cell counts (38.19 $\pm$32.77)$\times 10^3$: (10.82$\pm$1.44)$\times 10^3$. The ratio of young forms of neutrophiles was for leukemic and non-leukemic mice 3.33: 0.06; of segment forms of neutrophiles 5.67: 34.25; of eosinophiles 0: 2.38; of monocytes 1.6: 2.38; and of lymphocytes 89.43: 61.44.

Regression of age on the incidence of leukemia was linear. Regression line is shown as $Y=12.11X-69.92$, where Y stands for the leukemia incidence and X for the age in months beginning with the sixth month. The t-test of the regression coefficient showed that it was highly significant ($p<0.001$).

Though the incidence in females did not differ significantly from that in the males, the regression coefficients were significantly different from each other. In females the incidence was $Y=12.41X-73.43$, and in males, $Y=7.73X-26.31$.

Based on the above investigation it is assumed that males of the AKR/JaxMs strain have slightly stronger resistance to the development of spontaneous leukemia than the females. It seems that sex hormones affect the incidence of leukemia in this strain. Usually, leukemias in male mice of AKR/JaxMs strain developed at a considerably older age than in the females; the average age of females developing leukemia was 10.25 months but 12.25 months in males.

20. *Studies on mouse leukemia II. Susceptibility to radiation induced leukemias in various substrains of mice bred in Misima*

(By Yosinori KURITA and Tosihide H. YOSIDA)

The total number of 238 mice covering 8 substrains (C3H/HeMs, C57BL/6Ms, C58/Ms, A/HeMs, DM/Ms, C57L/Ms, RF/Ms and SL/Ms) were used for induction of leukemias by X-irradiation. X-rays were given to the total body at the average age of 2.0 months. The conditions were as follows; 250 kVp; 1.5 mA; 0.3 mm Cu+0.5 mm Al filter; TDS 50 cm; 60.8 r/min. Total 600 roentgen were given in four 150 r fractions at one week interval. The mice were kept about 2 years.

Leukemia incidence in C57BL/6Ms strain was 21.43 percent, in RF/Ms strain 71.5 percent and in C58/Ms strain 10.0 percent. The average age before leukemia development was 10.5 months in C57BL/6 strain, 4.5 months in RF/Ms strain and 12.0 months in C58/Ms strain. In RF strain 0.03 mg of 20-methycholanthrene was injected subcutaneously after total 300 r irradiation.

Although C58 strain mice are known as a high leukemic strain, no

mice of this strain in our colony had developed spontaneous leukemia in the past 8 years, but in the above experiments a relatively high susceptibility to radiation induced leukemia was found in this strain. In RF strain mice leukemia induction was accelerated if they were irradiated in combination with application 20-methylchrolanthrene.

Leukemias developed in C57BL strain had the character of thymic lymphosarcoma, while in RF strain they had the character of generalized lymphatic leukemia; myeloid leukemias were never seen in this strain.

21. *Studies on mouse leukemia III. Chromosomes in spontaneous and induced mouse leukemias*

(By Yoshinori KURITA and Tosihide H. YOSIDA)

Chromosomes in nine spontaneously developed leukemias in AKR strain were analysed. Among them five (AKLA, AKLD, AKLE, AKLF and AKLJ) were characterized by the model chromosome number 40 and two (AKLC and ALKH) had the mode at 41 chromosomes. Of the remaining two one (AKLI) had bimodal distribution with one mode at 40 and the other at 41 chromosomes and the other (ALKG) had the mode at 40 and hypotetraploid numbers (70–80). All chromosomes were characterized by rod shape like those of normal somatic cells. Among leukemias induced by X-irradiation 3 strains (BLXLA, RFXLB and RFXLC) were observed. The mode in BLXLA tumor cells was 40 and they were characterized by the normal rod shape. It is noteworthy that 21% of cells had 41 chromosomes in 1–3 transplant generations, which were reduced to 3.0% in the 26th transplant generation, whereas the number of 40 chromosome cells increased to 63%. No chromosomal differences were found between spontaneous and induced leukemias. Among two leukemias developed in RF-strain, one (RFXLC) was characterized by the modal chromosome number at 41, but the other (RFXLB) was characterized by bimodal occurrence of 41 and 42 chromosomes.

22. *Susceptibility of rats to tumor induction by treatment with methylcholanthren*

(By Tosihide H. YOSIDA and Yoshinori KURITA)

Wistar/Ms and Long Evans/Ms rat strains were used for the test of susceptibility to 20-methylcholanthren-induced tumors. Total number of animals used in the present study was 45 (WISTAR, 26, and LONG EVANS, 19). All of them were about 2.5 months old. 0.5 mg of 20-methyl-

colanthren suspended in propylen glycol was injected under the skin on the ventral side of each animal.

The incidence of tumors developed after injection of the chemical was in Wistar rats 71.4% and 85.7% in Long-Evans rats. Animals in both strains developed tumors 4.5 months on an average after treatment with the chemical. There was no difference between the strains in the latent period preceding the appearance of tumors. Neither there was a significant difference in the incidence of tumors between both strains ($\chi^2=0.664$, $0.30<p<0.50$) or both sexes ($\chi^2=0.701$, $0.3<p<0.5$ for Wistar strain rats, and $\chi^2=1.296$, $0.20<p<0.30$ for Long Evans strain).

Transplantation experiments with these tumors were all negative; the tumor pulps were injected subcutaneously to 30 young animals of each strain, but no growth of grafted tumors resulted. A cytological study of these tumorsris now in progress.

23. *Maternal effect of* $+^{lem}$ *gene on pterine reductase*

(By Mitsuo TSUJITA)

Recently, the structure of the yellow pigment in the silkworm was studied by NAWA who ascertained that this pigment is 2 amino-4 hydroxy-6 lactyl-7·8 dihydropteridin. This revealed that the yellow silkworm pigment is the same as sepiapterin of *Drosophila melanogaster*. A study on pterine reductase and on the maternal effect of $+^{lem}$ gene on this enzyme was carried out. The outline of the experimental results is as follows:

The activity of pterine reductase was observed in the integument, adipose tissues, mid-gut and silk-gland of larvae, but not in their body fluid. In the pupal and adult stage, the enzyme activity was detected not only in the integument, adipose tissue, and male and female genital organs including testes and ovarian tubules but also in the body fluid. Among various tissues adipose tissue showed the strongest enzyme action.

On the contrary, enzyme activity could not be detected in several tissues of larva, pupa and adult lemon. However, as adipose tissue and integument exhibited a weak or very weak enzyme activity, it seems that a small amount of the enzyme can be produced in some tissue cells of the lemon individuals. But in the integument and in several tissues of the lethal lemon larvae immediately after the first moulting no enzyme activity could be detected. Therefore, it appears that in this mutant it is almost completely lacking.

From these experimental results it may be safely concluded that the accumulation of the yellow pigment in the hypodermis of lemon and lethal lemon larvae is due to low activity or lack of enzyme action controlled

by $+^{lem}$ gene.

Eggs immediately after having been laid by normal moths show enzyme action although it is weaker than in the maternal body before egg laying. The enzyme activity of the eggs becomes stronger one day after they were laid, and this condition continues for several days; further stronger activity was observed with approaching hatching. On the contrary, in the lemon eggs (lem/lem, lem/lem^1) enzyme activity was scarcely recognized from the stage immediately after they were laid until the stage of hatching.

In the eggs of the cross $+\times$ lem, almost the same enzyme activity as in a normal strain can be observed. In the eggs of the cross lem $\times+$, enzyme activity is scarcely observed for 30~40 hours after they were laid at the room temperature of 23~25C, but it appears thereafter. Although the activity increases with incubation, it is always weaker than in the eggs of the cross $+\times$ lem. The same condition was observed even at the stage of young larvae immediately after hatching.

Almost the same maternal effect of $+^{lem}$ on pterine reductase can be observed in the reciprocal crosses between $+$/lem^1 and lem/lem^1.

Thus, a clear-cut maternal effect of $+^{lem}$ on pterine reductase has been proved.

In another experiment, mutual transplantation of ovaries between normal and lemon larvae was carried out and the enzyme activity of the ovarian tubules which developed in the body of the female host was measured. It may be concluded from the result of this experiment that the enzyme activity of the eggs is not determined by their genotype but by that of the maternal body in which the eggs develop. It is likely that the enzyme or it's components may be transmitted from mother's tissues to the developing egg cells in the transplanted ovaries through body fluids.

24. *Genetical and biochemical studies on the metabolism of pteridines in silkworm*
—The structure of the yellow pigment—

(By Saburo NAWA)

It was established that the yellow pigment occurring in the epidermis of the mutant *lemon* has the structure 2-amino-4-hydroxy-7, 8-dihydro-6-lactylpteridine, which is identical with the structure of Sepiapterin found in Drosophila. The experiments were carried out in a similar way as those in Drosophila (see following papers: This Annual Report, **10** 104-105 (1959) and Bull. Chem. Soc. Japan, **33** (10), 1555-1560 (1960)).

25. *Biochemical studies on drosophila pterine reductase*

(By Toshifumi TAIRA and Saburo NAWA)

It is a well-known fact that the red and yellow eye-pigments, namely drosopterin and sepiapterin, found in *D. melanogaster* are the products of pteridine metabolism. For analysis of the mechanisms of the eye-pigment formation *in vivo*, therefore, it is highly important to clarify in detail the pteridine metabolism.

Pterine reductase extracted from *Drosophila* converted sepiapterin into tetrahydro-form in the presence of reduced triphosphopyridine nucleotide (TPNH), as TAIRA (1960) has reported. The reductase has been further purified and its properties have been examined. An enzymatic reduction of sepiapterin could be detected spectrophotometrically by disappearance of the yellow color and fluorescence characteristic of sepiapterin. The curve of product converted enzymatically was similar to those of tetrahydropteridines reported by several authors. Stoichiometrical analysis showed that one mole of TPNH was necessary for one mole of sepiapterin reduced. These findings suggest that the tetrahydro-form converted enzymatically from sepiapterin is tetrahydro-sepiapterin and that the present enzyme is a true reductase.

In the absence of oxygen, the tetrahydrosepiapterin converted enzymatically from sepiapterin was gradually converted further products by non-enzymatic oxidation. In this case, the main product was indistinguishable from biopterin in various R_f values and chemical natures examined.

In the presence of oxygen, the tetrahydrosepiapterin was oxidized into two kinds of products; one of them was in a trace of original sepiapterin, and the other was in a great amount of blue fluorescent pteridine. The latter consisted of further two kinds of pteridines; one was 2-amino-4-hydroxypteridine and other 2-amino-4-hydroxypteridine-6-carboxylic acid.

On the basis of these experimental results, the metabolic relationships among drosophila pteridine derivatives could be presented as follows:

$$\text{Sepiapterin} \rightleftarrows \text{Tetrahydrosepiapterin} \rightleftarrows [\text{Dihydrobiopterin}] \rightleftarrows \text{Biopterin}$$
$$\downarrow$$
$$\text{2-amino-4-hydroxypteridine}$$

It remains elucidate, however, what is the immediate precursor of drosopterin and what mechanism underlies the process of its formation. According to our previous reports, it is assumed on the basis of genetical analyses that drosopterin might be converted from sepiapterin. The involved enzymatic system is controlled by the mutant gene Hn^{r3}. The enzyme activity of a Hn^{r3} male was much lower than that of a normal male. This may be due to a high concentration of an inhibitor, which is a deriva-

tive of pteridines. The detailed results of the continued investigation will be published elsewhere.

26. *Phosphatase activities in the skin of hairless mice mutants*

(By Kazuo MORIWAKI)

As shown in FRASER's experiments (1946), the hairless character of the rhino mouse is fixed in the skin immediately after birth and becomes apparent 2 weeks later. As a preliminary step to a study of the developmental process leading to hairlessness, the biochemical changes accompanying the manifestation of the character were examined.

In rhino mice (hr^{rh}), acid phosphatase (Pase) activity in the skin of the homozygotes was markedly higher than that of the heterozygotes as previously described (MORIWAKI, 1959). This result suggested a comparison between homo- and heterozygotes in the activities of inorganic pyrophosphatase (PPase) and apyrase. A considerable increase in PPase activity could be observed in the homozygotes in parallel with depilation, whereas no notable change in apyrase activity occurred. After depilation, the glycogen content was markedly increased in the homozygotes, in which an appreciable increase in lipid phosphate content was also found. Effects of metals on acid Pase activity both of homo- and heterozygotes could be hardly recognized using Mg^{++}, Ca^{++}, Fe^{++}, Zn^{++}, Sn^{++} and EDTA at concentrations from 10^{-2}M to 10^{-4}M. To confirm the relationship between hairlessness and the above chemical changes other hairless mutant mice were employed.

Similar results as those obtained with rhino mice have been obtained with alopecia mice (*ap*) which are characterized by cyclic monthly depilation. It is noteworthy that in this case reproduction of hair in the skin of homozygotes was also accompanied by a considerable decrease in acid Pase activity.

Furthermore, in another hairless mutant strain, furless (*fs*), the relationship between hairless and acid Pase activity increase was nearly the same; i.e. acid Pase activity in the homozygotes was increased by about 40 per cent over that in the heterozygotes.

27. *Comparison of the DNA content of diploid and tetraploid Ehrlich ascites carcinoma cells according to microspectrophotometer measurements*

(By Kazuhiko R. UTSUMI and Tosihide H. YOSIDA)

The relation between DNA-content and chromosome number was studied in the diploid (ELD) and the tetraploid strain (ELT) of the Ehrlich ascites carcinoma cells. The DNA measurements were made with a microspec-

trophotometer (Olympus) adopting the two-wavelength method.

On the seventh day after transplantation of the Ehrlich ascites tumor, a high rate of growth and abundant mitoses were observed. The ascites exudates were withdrawn with a glass capillary from the peritoneal cavity of a tumor bearing mouse. One part of the sample was used for DNA measurements and the other part for the analysis of the chromosome numbers.

Chromosome numbers: From squash preparations of both strains of the carcinoma, stained by acetic dahlia, a hundred well spread metaphases were examined. The mean chromosome number was 44.51 for the diploid cells and 73.49 for the tetraploid cells. Thus, the former strain was hyperdiploid and the latter hypo-tetraploid.

DNA measurements: The tumor exudates were smeared on slides, fixed with Carnoy's solution for 20 minutes. The smear preparations were hydrolysed for 10 minutes with 1N-HCl at 60 ± 0.5°C, then stained for 1.5 hours with Schiff's reagent at room temperature, and later bleached with glycine-buffered SO_2-water for 15 minutes. The relative amounts of DNA were measured by a microspectrophotometer according to Patau's formula using the wavelengths of 482 mμ and 561 mμ. One hundred interphase nuclei of ELD, and 200 interphase nuclei of ELT cells were used for DNA measurements. The mean amount of DNA was 0.093 for ELD and 0.136 for ELT. Further, the DNA-contents of resting nuclei of normal somatic cells of mouse, monocytes from ascites fluid and splenic cells were also measured. The mean DNA-content of somatic nuclei whose chromosome number was reported as 40 by MAKINO was 0.080. In comparing the observed mean values of DNA-content with the expected values computed from the mean chromosome numbers, no significant difference was found between them.

From those results it is concluded that DNA measurements could be used for the determination of ploidy of resting nuclei.

28. *DNA-content of Ehrlich ascites carcinoma cells with special reference to its variation during mitosis*

(By Kazuhiko R. UTSUMI)

The DNA measurements were made with a microspectrophotometer (Olympus) adopting the two wavelength method. The relative amounts of DNA were computated according to Patau's formula. The two wavelengths employed were 482 mμ and 561 mμ.

The hyper-diploid strain (ELD) and the hypo-tetraploid strain (ELT) of the Ehrlich ascites carcinoma cells were employed. On the seventh day

after transplantation when the tumors showed a high rate of growth and abundant mitoses, ascites exudates were withdrawn with a glass capillary from the peritoneal cavity of a tumor bearing mouse. The tumor samples were smeared on slides, then fixed with Carnoy's solution. Hydrolysis with 1N-HCl was applied for 10 minutes at 60±0.5°C. After hydrolysis, the smear preparations were stained with Schiff's reagent for 1.5 hours at room temperature. Thereafter, they were bleached with glycinebuffered SO_2-water for 15 minutes.

DNA-content of nuclei at various mitotic phases: The amounts of DNA of nuclei were measured at interphase, prophase, metaphase, anaphase and telophase of both cell strains, hyper-diploid and hypo-tetraploid. In both the change in DNA-content of nuclei during mitosis took a parallel course. The amounts of DNA at metaphase, were twice as much as those at anaphase and telophase. On the other hand, the amounts of DNA at prophase were approximately 45% of those at metaphase. In interphase nuclei of ELT the distribution of DNA amount from nucleus to nucleus was bimodal. Two groups could be established: nuclei containing lower amounts (lower groups) and those containing higher amounts of DNA (higher group). The amounts of DNA of the lower group were consistent with those of ana- or telophase nuclei, while the amounts of DNA of the higher group were consistent with those of prophase nuclei. The same analysis was made of ELD cells, which showed a unimodal distribution, and the same results were obtained.

Nucleus size and DNA-content: Further, the correlation between the DNA-content and size of nuclei was investigated. The diameter of nuclei at interphase and prophase was measured with an ocular sliding-micrometer and the data were used for computing their area. Since the nuclei were flattened by being dried on the slide and had a discoidal shape, their area served for comparing their relative size. There was a close correlation between the DNA-content of nuclei and their size. In general, the larger the size, the higher was the amount of DNA. The dispersions of prophase nuclei corresponded with those of the higher group in both strains of carcinoma cells.

These results support the view that doubling of DNA content occurs during interphase in agreement with the data of SWIFT (1950), FOWARD and PELC (1952), KIMBALL and BARKA (1959).

29. *Two factors influencing sensitivity to x-rays in the synthesis of actin during early embryonal stages*

(By Yoshito OGAWA)

In a previous note (Y. OGAWA, Nature. 186, 77. 1960) it was shown that the synthesis of muscle proteins takes place in early *Triturus* embryos irradiated by x-rays (50 r, 200 r and 500 r) 108 hrs. after fertilization. On the basis of this finding an investigation of the mechanism of the synthesis of muscle proteins in early developmental stages seemed to be promising. Therefore, actin formation with respect to age sensitivity in relation to x-rays as found in the embryos of *Triturus pyrrhogaster*, Boie has been studied in detail and the results are here reported.

Approximately 7,500 embryos at different developmental stages were exposed to x-rays at single treatments with 5 r, 10 r, 30 r, 50 r, 200 r, 500 r, 1,000 r and 2,500 r. Examination for the first detectable trace of actin after fertilization was carried out in periods of 24 hrs. after irradiation. The technique employed was essentially the same as before (Y. OGAWA, Nature. 182, 1312. 1958). G-actin, prepared from adult *Triturus*, was injected into rabbits and the obtained anti-serum was raised to their serological specificity by resorption test with saline extracts of liver, spleen and skin tissue of *Triturus*. The titre of antiserum was adjusted to 1 : 512 before carrying out the precipitin reaction with saline extracts of x-irradiated embryos.

In normal embryos, actin first became detectable 132 hrs. after fertilization. The time of actin formation after fertilization in the treated embryos is shown in Fig. 1. A dose of 5 r given 36 hrs. after fertilization shows

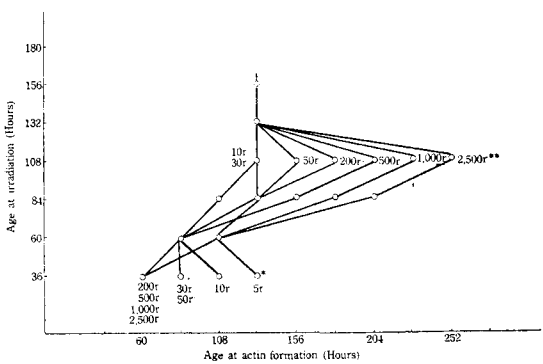


Fig. 1. Synthesis of actin in early *Triturus* embryos irradiated by x-rays applying doses from 5 r to 2,500 r.

no effect on actin formation (Fig. 1*), but in the case of irradiation 108 hrs. after fertilization with a heavy dose of 2,500 r, first actin formation was recognized 252 hrs. after fertilization (Fig. 1**).

The curves shown in Fig. 1 indicate the presence of two factors influencing sensitivity to x-rays in the synthesis of actin during early embryonal stages. One (A) promotes the synthesis of actin and the other (B) markedly suppresses it after x-irradiation. Factor A is very sensitive to x-rays and is easily detectable with only 5 r doses. On the contrary, factor B does not react to x-rays until doses over 50 r are applied. Factor A is clearly detectable by irradiations between 36 and 84 hrs. and the most effective time of irradiation in the above experimental conditions is 60 hrs. after fertilization. Exposures between 84 and 108 hrs. after fertilization show the reaction of factor B to x-rays. The most effective time after fertilization of x-irradiation for factor B is 108 hrs.

The differentiation of muscle tissue is not asccomplished before both actin and myosin formation coincide. Studies on myosin formation in x-irradiated *Triturus* embryos are on the way.

30. *Biochemical relation between the syntheses of actin and myosin in the regenerating limb tissue of Triturus*

(By Yoshito OGAWA)

In the skeletal mascle the formation of actin precedes that of myosin in the early developmental stages as well as in regenerating tissues. The following questions therefore arose: Does the formation of actin induce that of myosin? According to my previous note, it had been already proved by the investigation of the synthesis of both those proteins in the X-irradiated embryo that during the skeletal muscle differentiation in the early developmental stage the formation of actin does not induce that of myosin. The present note deals, from the same view point, with the synthesis of muscle protein in the regenerating hind-limb tissue after amputation at the knee of adult *Triturus pyrrhogaster*. The same serological technique was used as that employed in the experiment with early embryonal stages.

G-actin and myosin, prepared from the skeletal muscle of adult *Triturus pyrrhogaster* by the method of Straub and Szent-Györgyi, were injected into rabbits and the obtained anti-sera were raised to their serological specificity by a resorption test with saline extracts of liver, spleen and skin tissue. The titres of both sera were adjusted to 1 : 512 before carrying out the precipitation reaction with salin extracts of granulated tissue of the amputated limbs. Total body x-irradiation with 200 r, 500 r,

1,000 r and 2,500 r was made 18 day after the operation.

In the non-irradiated control, actin and myosin first became detectable in the regenerating limb tissue 20 and 27 days after the operation, respectively. (Y. OGAWA, Nature. 182, 1312. 1958).

In the case of irradiation, the time of myosin formation after operation was changed to 24 days with 200 r, 22 days with 500 r and 20 days with 1,000 r, though no significant influence was found in actin formation with the above mentioned x-ray doses. (Fig. 1). A heavy dose of 2,500 r changes

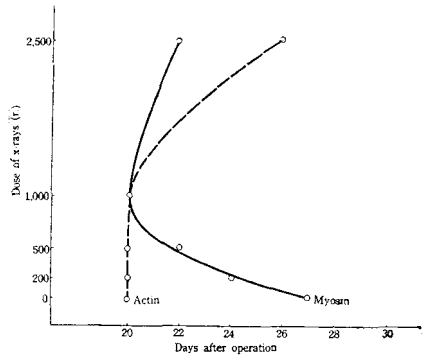


Fig. 1. Synthesis of contractile proteins in regenerating limb tissue of *Triturus pyrrhogaster* irradiated by x-rays 18 days after operation.

the time of actin and myosin formation to 26 and 22 days after operation, respectively. Thus, x-irradiation 18 days after the operation, markedly promoted the synthesis of myosin and suppresed the synthesis of actin. The order of actin and myosin formation in the regenerating limbs tissue of irradiated *Triturus* at the heavy dose of 2,500 r was reversed to that found in the non-irradiated control. This finding is in full agreement with the results of the experiment with early embryonal stages.

Accordingly, it may be concluded that in the skeletal muscle the processes of synthesis of those two proteins are independent of each other in the regenerating tissue as well as in the early developmental stages of the embryo.

31. *Effects of glucuronic acid and related compounds on the synthesis of skeletal muscle proteins in early Triturus embryo*[1]

(By Yoshito OGAWA and Noriko KARUBE)

This note deals with the effects of Na-Glucuronate, glucosamine and glucuronolactone on the synthesis of skeletal muscle proteins in early *Triturus* embryos.

Immediately after fertilization, the embryos were raised in 0.01% solution of the above three chemicals at 20°C and actin and myosin formation in the developing embryos was investigated. The identification of muscle proteins was carried out by means of a serological technique (Y. OGAWA, Nature. 182, 1312. 1958).

In the non-treated control group, actin and myosin first became detectable in early embryos 132 and 176 hrs. after fertilization, respectively. Na-Glucuronate had no remarkable influence on the formation of muscle proteins, but glucosamine promoted the synthesis of myosin only and both proteins were detected in embryos treated with glucosamine 132 hrs. after fertilization. In the embryos treated with glucuronolacton, actin and myosin were first detected after 156 and 108 hrs. respectively. Thus, glucuronolactone suppressed markedly the synthesis of actin and promoted that of myosin. The order of actin and myosin formation in embryos treated with glucuronolacton was therefore reversed to that found in the non-treated control.

The three compounds have the same main chemical structure and are different only in the free radical. The above results may be useful to researchers of the mechanism of muscle protein synthesis during early embryonal stages.

B. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF PLANTS

32. *Genome analysis in the genus Oryza II*[2]

(By Hitoshi KIHARA, Mitsuya NEZU, Tadao C. KATAYAMA, Seiji MATSUMURA and Tomoo MABUCHI)

Genome analysis of *Oryza* species was continued this year. 25 interspecific hybrids were newly produced, among which 8 hybrids were cytologically investigated. The results of the investigation are summarized in Table 1.

1) This work was supported by Grant from Tokyo Seikagaku Kenkyūkai.
2) This work was supported by Grant RF 57080 from The Rockefeller Foundation.

Table 1. Chromosome pairing in 8 interspecific *Oryza* hybrids

F_1 hybrids (♀×♂)	No. of cells analyzed	Chromosome conjugation	
		Mode	Range of number of bivalents
O. sativa indica×*O. stapfii* and reciprocals	90	12_{II}	12
O. glaberrima×*O. perennis*	50	12_{II}	12
O. australiensis×*O. officinalis*	100	24_{I}	0～3
O. breviligulata×*O. eichingeri*	14	$4_{II}+28_{I}$	0～9
O. alta×*O. officinalis*	12	$12_{II}+12_{I}$	12～14
O. stapfii×*O. latifolia*	13	36_{I}	0
O. latifolia×*O. alta*	12	24_{II}	24
O. malabarensis×*O. latifolia*	14	$12_{II}+24_{I}$	7～12

According to the table the genome constitutions of *O. stapfii* and *O. alta* are AA and CCDD, respectively. The genome formulae given by MORINAGA and KURIYAMA (1960) to *O. sativa*, *O. perennis*, *O. glaberrima*, *O. breviligulata*, *O. officinalis*, *O. latifolia* and *O. eichingeri* have been confirmed by our results. The genome of *O. australiensis* can not be either C or A, because F_1 hybrids between this species and *O. sativa* had 24 univalents in the majority of PMC's as previously reported. Partial homology between A and B genomes, as reported by KIHARA and NEZU (1959), has been confirmed by the cross, *O. breviligulata*×*O. eichingeri*. Concerning the genome constitution of *O. malabarensis*, the following possibilities are considered; (1) one of *malabarensis*' genomes is homologous to either C or D genome, or (2) each of its two genomes is partially homologous to C and D. The first possibility is most likely, because 12 bivalents have been observed in the majority of PMC's in the F_1 hybrids between this species and *O. latifolia*.

33. *Floating habit of 10 strains of wild and cultivated rice*[1)]

(By Hitoshi KIHARA, Tadao C. KATAYAMA and Koichiro TSUNEWAKI)

Resistance to flood is an important economic character of rice in tropical and subtropical countries. One of the factors determining it is the floating habit. Using the floating rice samples collected by the members of the National Institute of Genetics, the authors have carried out the following experiment in order to elucidate the nature of this character.

10 strains belonging to *Oryza sativa*, *O. perennis* or *O. glaberrima* were

1) This work was supported by Grant RF 57080 from The Rockefeller Foundation.

examined for their floating habit by sinking them up to 3m deep at 6 different rates, namely, 0 (control), 1.5, 3.1, 4.6, 6.2 and 9.3 cm per day. Number of days of floating, plant height, leaf length, total internode length, number of internodes and average internode length were observed.

The following results were obtained.

(1) The 10 strains employed can be classified into 3 groups regarding floating habit. Strains *1* and *2* of *O. sativa* and strain *5* of *O. perennis* are pronouncedly floating, strains *6* and *7* of *O. perennis* and strain *8* of *O. glaberrima* are slightly floating, and strains *3* and *4* of *O. sativa* and strains *9* and *10* of *O. glaberrima* are non-floating.

(2) In the pronouncedly floating rice an increase of plant height under inundated condition is almost entirely dependent upon the increase of the total internode length and very little upon leaf length. Elongation of the latter is, however, rather important in the slightly floating rice.

(3) The increase of total internode length is almost equally dependent upon the increase of internode number and the elongation of internodes.

(4) Rate of internode elongation increases, within a limit, proportionally to the sinking rate. The floating habit of individual strains can be characterized by 3 factors, *a*, *b* and *c* which determine a regression of the rate of internode elongation on the sinking rate. Factor *a* corresponds to a point where the regression line crosses the vertical axis and indicates the rate of internode elongation achieved under normal conditions. It can be used as an index of resistance against a sudden flood. Factor *b* is a slope of the regression line and indicates the capacity of a strain to elongate the internodes in correspondence to rising water. The third factor *c* is the maximum rate of elongation.

(5) Analysis of those 3 factors is a useful means for characterizing the floating habit of individual strains. Among the 3 pronouncedly floating strains, strain *1* has an extremely large *a* and the smallest *b*, being most resistant against a sudden flood. Strain *2* is characterized by the smallest *a* and *c*. Strain *5* has the largest *b* and *c*, being most resistant for chronic inundation. All 3 slightly floating strains have only small *a* and *c* but their *b*'s are comparable to those of the pronouncedly floating strains.

34. *Production of polyploid wheat by nitrous oxide*

(By Hitoshi KIHARA and Koichiro TSUNEWAKI)

In 1954. Östergren reported that N_2O treatment produced polyploids in *Crepis capillaris*. The same treatment was applied by a few other Swedish workers. The present authors tested Östergren's method in a tetraploid

wheat species, *Triticum dicoccum* Khapli (2n=28).

20 florets per spike were emasculated before flowering and artificially pollinated two days after the emasculation. The spike bearing culms were cut off at the third internode counting from the top 24 hours after the pollination and treated with N_2O gas for 5, 10 or 15 hours, each treatment at pressures of 3 and 6 atmospheres. The treated spikes were kept in flasks containing a culture solution prepared after White's formula to which glucose was added as a carbohydrate source in a concentration of one per cent.

The seeds obtained from the treated spikes were placed on moist filter paper in petri-dishes and allowed to germinate. Roots were collected from each germinating seed, pretreated at 0°C for 24 hours and fixed with 3:1 acetic alcohol. The root tips were stained with Feulgen's leucobasic fuchsin after hydrolysis with N-HCl for six minutes.

Effects of N_2O treatment on seed-setting, germination and chromosome number are summarized in Table 1.

Table 1. Effects of N_2O treatment on seed-setting, germination and chromosome number of *Triticum dicoccum* Khapli

Treatments	(a) Seeds set (%)	(b) Germination	Success of pollination (%) (a)×(b)	No. of plants cytologically examined	Proportion of		
					diploid	tetraploid	aneuploid
Control	79.2	98.9	78.3	60	100.0	0.0	0.0
3 atm's for 5 hrs.	74.4	76.5	56.9	56	100.0	0.0	0.0
〃 〃 10 〃	71.9	66.1	47.5	59	30.5	45.8	23.7
〃 〃 15 〃	63.1	83.2	52.5	55	16.4	72.7	10.9
6 atm's for 5 hrs.	80.7	70.8	57.1	50	100.0	0.0	0.0
〃 〃 10 〃	78.6	86.3	67.8	62	19.4	37.1	43.5
〃 〃 15 〃	69.3	72.2	50.0	47	2.1	46.8	51.1

Treatments for 5 hours did not induce any aberrant chromosome numbers. Treatment at both 3 and 6 atm. pressures for 10 or 15 hours produced tetraploids and aneuploids in 70 per cent of plants or more, obtained from treated flowers. The best success was obtained from the treatment at 6 atm. pressure for 15 hours, by which 98 per cent of treated florets gave polyploids and aneuploids.

35. *F_1 monosomic analysis of Triticum macha*

(By Koichiro TSUNEWAKI and Hitoshi KIHARA)

Gene analysis of *Triticum macha* is important, because this species seems to be the most probable progenitor of hexaploid wheats. It is also known to have a normal allele of the third necrosis gene that has not yet been identified.

The authors studied this species by crossing it to 21 monosomic lines of Chinese Spring. F_1 plants were grown in the greenhouse and their chromosome numbers were determined from root-tip mitosis.

Due to semi-lethality of the hybrids, only a few F_2 seeds were produced which did not allow to carry out the monosomic analysis to the F_2 generation. The results obtained in the F_1 generation are briefly reported.

Necrosis: All the F_1 disomics and 20 monosomics were necrotic with the exception of mono-XVIs, which were all normal. Among the 20 necrotic monosomics, the F_1 mono-IIIs performed better as to plant height and, especially seed setting than any other necrotic di- and monosomics. These findings indicate that chromosomes XVI and III of Chinese Spring carry the third complementary necrosis gene and a modifier that conditions severe necrosis, respectively.

Growth habit: Monosomic F_1 plants for either chromosome IX or XVIII of Chinese Spring had winter growth habit, indicating that those chromosomes of *T. macha* carry recessive genes controlling this habit. They seem to be the same as those found in other hexaploid wheats.

Awnedness: Monosomic F_1 plants for chromosome VIII or X were awnletted, whereas all the other hybrids were awnless. This result indicates that *T. macha* carries *hd* and b_2 on chromosomes VIII and X, respectively.

In comparison with Chinese Spring, *T. macha* seems to have the following genes for the three characters studied so far:

	Character (Responsible chromosome)						
	Necrosis			Growth habit		Awnedness	
	V	XIII	XVI	XVIII	IX	VIII	X
Chinese Spring	ne_1	ne_2	Ne_3	$Sg_1{}^c$	$Sg_2{}^c$	*Hd*	B_2
T. macha	Ne_1	Ne_2	ne_3	sg_1	sg_2	*hd*	b_2

36. *Monosomic and conventional analyses of glume hairiness and ear density in 8 varieties of common wheat*

(By Koichiro TSUNEWAKI)

Inheritance of glume hairiness and ear density of 8 agronomic varieties of common wheat, *Triticum aestivum* was studied by monosomic and conventional analysis. Among them, Elgin and Red Egyptian belong to subspecies *compactum* and Chinese Spring, Prelude, Red Bobs, S-615, Jones Fife and Kharkov to *vulgare*. Concerning the hairiness of glumes, Prelude and Jones Fife are pubescent and the other 6 varieties are non-pubescent.

The results obtained by the two methods were in complete agreement with each other. They are summarized as follows:

(1) Glume hairiness is controlled by a single dominant gene, *Hg*, located on chromosome XIV. Genotypes of Jones Fife and Prelude and the other 6 varieties are *Hg Hg* and *hg hg*, respectively.

(2) Spike density characterizing the subspecies *compactum* is determined by spike length but not by the number of spikelets per ear. Short ear length of *compactum* is controlled by a single dominant gene, *C*, located on chromosome XX. Genotypes of Elgin and Red Egyptian and the other six varieties are *CC* and *cc*, respectively. The different spike lengths found among *vulgare* varieties are associated with the number of spikelets per ear and are controlled by polygenes.

37. *Monosomic analysis of synthesized hexaploid wheats*

(By Koichiro TSUNEWAKI)

Polyploidy is a prevailing phenomenon in higher plants. For a study of the evolutionary significance of polyploidy, a comparative gene analysis of amphidiploids and their ancestral species is a useful approach. Furthermore, this type of work will bear informations on the origin of genes now present in the amphidiploids.

A comparative gene analysis of common wheats and their ancestors, Emmer wheats and *Aegilops squarrosa*, is now possible, because (1) a number of hexaploid wheats have been synthesized from various species of Emmer wheats and various types of *Ae. squarrosa* and (2) the monosomic series of a common wheat established by Sears has made gene analysis of hexaploid wheats very easy.

A large amount of informations have been accumulated on the chromosome locations of genes which control morphological and physiological

characters of common wheats. In order to get comparable informations for Emmer wheats and *Ae. squarrosa*, the author crossed 21 monosomic lines of Chinese Spring (common wheat) to 5 synthetic hexaploid wheats listed in the following:

Synthetic 6x	Source	Synthetic components	
		Emmer wheat	Ae. squarrosa
ABD-1	Kihara's No. 1	*T. dicoccoides spontaneo-nigrum*	*Ae. squarrosa* No. 2
〃 2	〃 No. 3	*T. turgidum*	〃
〃 3	Sears'	*T. dicoccum* Vernal	*Ae. squarrosa* (Sears')
〃 4	〃	*T. durum melanopus* Golden Ball	〃
〃 5	〃	*T. durum hordeiforme* Carleton	〃

The results obtained from the F_1 generation of those crosses suggest the following possibilities for the origin of genes controlling growth habit and awnedness of common wheats.

Growth habit. sg_1 and sg_2 genes controlling winter growth habit of common wheats have been derived, respectively, from winter *Ae. squarrosa* and winter Emmer species such as *T. dicoccoides*. Sg_2 gene known to be present in early spring varieties of common wheats has been originated from early species of Emmer wheats, such as *T. turgidum* and *T. dicoccum*. Sg_2^e gene present in late spring varieties of common wheats has been originated from late spring varieties of Emmer such as *T. durum* varieties. Sg_2 and Sg_2^e genes of Emmer wheats appeared to be epistatic to sg_1 gene of *Ae. squarrosa*.

Awnedness. 2 awn-promoting genes, a_1 and a_2 of common wheats, have been originated from Emmer wheats and *Ae. squarrosa*, respectively. 3 inhibitors, *Hd*, B_2 and A_4 present in Chinese Spring are likely to have arisen at the hexaploid level.

38. *Marker lines in Triticum aestivum var. S-615*

(By Koichiro TSUNEWAKI)

In order to establish isogenic marker lines of individual chromosomes in common wheat, the author is carrying out a project to isolate major genes from known sources and to bring them into the same genetic background by successive backcrosses. An awned, spring *vulgare* variety, S-615, has been chosen as the recipient variety. The present stage of

project is represented in the following table:

In order to mark the other 9 chromosomes, on which no major genes have been found, artificial production to translocations between *Hp* gene of rye and wheat chromosomes has been attempted by irradiating a wheat-rye addition line provided by Dr. L. E. Evans.

Chromosomes marked	Symbols	Characters controlled	Donor varieties	No. of backcrosses made
III	*v*	virescent	Chinese Spring [1]	1
IV	*Hp*	hairy peduncle	Rye [2]	1
V	Ne_1	necrosis	Prelude	1
VIII	*Hd*	hooded awn	Chinese Spring	2
IX	B_1	awn suppression	Jones Fife	2
X	B_2	〃	Chinese Spring	2
XI	*Re*	red coleoptile	Hope [1]	1
XIII	Ne_2	necrosis	Kharkov	2
XIV	*Hg*	hairy glume	Jones Fife	2
XVI	*sp*	sphaerococcum characters	*T. sphaerococcum*	2
XVIII	sg_1	winter growth	Kharkov	2
XX	*C*	compact ear	Elgin	2

[1] Obtained from Dr. E. R. Sears

[2] Originated from Cornell University

39. *An early ecotype of Agropyron tsukushiense var. transiens Ohwi*

(By Sadao SAKAMOTO)

A. tsukushiense var. *transiens*, that grows in fields or along road-sides, is very common and widely distributed in Honshu, Kyushu and Shikoku. It is also known in China, Manchuria and Korea. This species is hexaploid (2n=42) and shows a wide variation in many characters. A strain which differs in several characteristics from the common strain (designated as common type) was found in the vicinity of Misima. After ecological and genetical studies of this strain, it was recognized as an ecotype of *A. tsukushiense* var. *transiens*.

Characteristics of this ecotype which differ from those of the common type are as follows: 1) This ecotype was found as a swarm in the idle lying paddy fields in the valleys of hilly regions. 2) It is strongly waxy and deeply anthocyan-pigmented. 3) The upper side of rosette leaves is pubescent. 4) Plant height, the first internode from the top, flag leaf

and spike are shorter. Number of spikelets is much smaller. 5) Empty glume, lemma and palea are longer, but awn is shorter. 6) Seeds are larger and heavier. 7) This ecotype first flowers at the end of April, about 25 days earlier than the common type.

Hybrids between the ecotype and the common type were easily obtained by artificial pollination and their growth was very vigorous. Out of the 19 characters of the F_1's, 14 were intermediate between those of the parents. At meiosis of F_1 hybrids, a quadrivalent was observed in 14 % of 163 metaphase plates. Pollen-fertility and seed-fertility of the F_1's were 77 % and 62 %, respectively, being lower than those of both parents. The germination and growth of the F_2's were very good, and all plants were fertile. Eight characters, namely, pubescence of rosette leaves, color of anthers, date of flowering, plant height, length of the first internode, flag leaf and spike, and number of spikelets, were observed in the P, F_1, F_2 and the first backcross generations. The mode of inheritance of the first two characters showed a 3:1 segregation in the F_2's, and no linkage was found between them. Segregation of the other six characters was typically continuous, suggesting that those characters were controlled by a larger number of genes. Among these six characters, genetic correlations were observed in the F_2's. Negative correlations were found between the date of flowering and the other five quantitative characters, while the corresponding correlations were positive in the parents.

Among several characteristics which distingush the ecotype from the common type, the following three are the most important:

1) Differentiation in the growing habitat.
2) Genetic differentiation relating to some quantitative characters.
3) Difference in the flowering time.

Since the earliness of flowering was strikingly characteristic, this ecotype was designated as " early ecotype ".

The early ecotype was often found together with Chinese milk-vetch (*Astragalus sinicus* L.) in paddy fields. Its distribution seems to follow that of Chinese milk-vetch.

40. *Photoperiodic responses of Oryza species III*[1]

(By Tadao KATAYAMA)

The present author had already reported his investigation in rice on the influence of plant age on photoperiodic sensitivity. In this year, an experiment was carried out in order to study the following three points; (1) relationship between photoperiodic sensitivities of various *Oryza* species

1) This work was supported by Grant RF 57080 from The Rockefeller Foundation.

and latitude of their habitats, (2) photoperiodic sensitivities of various species which occur in the state of nature in the same latitude, and (3) effect of twilight on photoperiodic responses. The results of the experiments are briefly presented.

(1) Among photoperiodically sensitive strains belonging to various *Oryza* species, whose habitats vary between 10°N and 35°N, a high negative correlation was found between the latitude of the habitats (X) and heading dates expressed by number of days from seeding to heading (Y), when they are grown at Mishima (35°N). The relationship between X and Y can be expressed as follows:

$$Y=185-3.0\,X$$

(2) The cultivated ones become sensitive to short-day treatment at an earlier plant age than the wild ones in the following order:

O. sativa<*O. glaberrima*≒*O. sativa spontanea*<*O. perennis*

(3) Near the critical day length, twilight given after dark periods delays heading in all three species, *O. sativa*, *O. glaberrima* and *O. perennis*. On the other hand, the same twilight given after light periods delays heading of some species but does not affect that of others.

41. *Studies on the intercellular spaces in rice*

(By Tadao KATAYAMA)

On the aerating system of roots of *Oryza sativa* and its physiological significance, several reports have been published.

As a quantitative determination of the total intercellular air space in rice was lacking, the author investigated the variations of its volume in relation to cultivation procedures and developmental stages.

Two kinds of Japanese rice, a paddy rice and an upland variety were used as materials. Two other crop plants, *Zea mays* L. and *Vicia faba* L., were also used for comparison.

Computations were made by the following formulae:

$V=\frac{G_1-G_2}{S}$ .....volume of the materials in mm³

$v=\frac{G_3-G_2}{S}$ .....volume of the intercellular space in mm³

$v\%=\frac{v}{V}\times 100$....volume percentage of the intercellular space, G_1....weight in air, G_2....weight when submerged in water, G_3....weight when infiltrated and submerged in water S....apparent specific weight of water used (= [specific weight of water]−[specific weight of air])

1) The intercellular spaces of root are larger in rice than in corn and broad bean. The paddy varieties develop larger air canals than the upland ones when raised under the same conditions. The root tissues attain the ultimate volume at some distance from the root tip varying in accordance to the variety and culture conditions.
2) The development of air canals in the roots of rice is markedly influenced by cultivation conditions especially with respect to the air content of the soil. The volume of the total air space is three times as large in non-aerated as in aerated soil.
3) The structure of the leaf and the volume of its total air space vary with the ascending position of the leaf. Differences were also observed in various parts of a leaf.
4) The intercellular spaces are larger in the blade than in the sheath of young leaves but such relationship cannot be clearly seen in older leaves.
5) The total intercellular space of the leaf is larger in paddy rice than in upland rice at young seedling stage but the relation is not clear at later stages.
6) The volume per cent of air space in the leaf is also influenced by environmental conditions of the soil. In the paddy it is larger than in the upland field. The differences are especially clear in young seedlings and become later obscure.
7) Such remarkable adaptability of rice to moisture and aeration may explain why this crop can be grown either in paddy or upland fields.

42. *On the mechanism of the appearance of gigas-plants from mullisomic dwarf wheat*

(By Tomoko OHTA and Seiji MATSUMURA)

In the offspring of the pentaploid wheat hybrid between *Triticum Spelta* and *T. polonicum* dwarf plants possessing 40 chromosomes (20_{II}) are found. They are nullisomics, deficient in a chromosome pair or the D-genome. As it is the a~g-chromosome in the D-genome which is missing, they are called a~g-dwarfs. So-called giant plants of normal height and vigor appear unexpectedly in their selfed progeny. These are respectively called a~g-gigas, according to the original seven different dwarfs, in which the missing D-pair is replaced by the homoeologous A- or B-chromosome pair.

Gigas-plants are assumed to appear from nullisomic dwarf wheat as a result of chromosome aberrations in MI. Various chromosome aberrations in MI and unequal distribution in AI of a~g-dwarf lines were observed and chromosome aberration rate, theoretical and observed appearance rates of gigas were compared for seven dwarf lines. Except the g-dwarf

(Dwl. 5), theoretical appearance rates of gigas are too small against the observation, amounting to 5~10 %. There are many factors involved, such as competitive fertilization, elimination of dwarfs and others. Still more important is the fact that the special chromosomes which are homoeologous to the deficient one, become easily aberrant. In the case of b-dwarf, the special chromosome is a Sat-chromosome which becomes very often a univalent.

43. *Cytological and histological observations on male sterility in sugar beets*

(By Tomoko OHTA and Seiji MATSUMURA)

Two cases of male sterility in sugar beets (*Beta vulgaris*) were cytologically and histologically investigated. One case represents physiological male sterility caused by low temperature. In the investigation of this case, each beet of variety GW 359 was longitudinally cut in two pieces. One half was subjected to low temperature treatment (5°C) for 24 hours, two weeks after the first flowering. The other half was untreated and used as control. Young anthers had a normal appearance before meiosis, but some effect was observed during this stage and giant pollen grains were found. On the other hand, various abnormalities were found in the anthers treated in the stage between pollen tetrad formation and mature pollen grain production, especially when the pollen had developed rapidly absorbing nutrients from the tapetal tissue. The pollen sterility increased markedly.

The other case represents genetical male sterility. One completely male-sterile plant was found in a commercial hybrid variety, US 216 male sterile × US 226. In its anthers the tapetal cells developed abnormally and remained longer intact than in the normals. The pollen grains, not being able to absorb nutrients from the tapetum, finally degenerated. In this case male sterility seems to be cytoplasmic.

In both cases abnormal development of tapetal cells was most striking. The relation between pollen grains and the tapetal tissue is thought to be the most important cause of male sterility (cf. Seiken Zihô No. 11).

44. *Variation in susceptibility to blast fungus in cultivated and wild rice*[1)]

(By Keizô KATSUYA)

13 strains of wild rice, *Oryza sativa* f. *spontanea*, *O. perennis* and *O.*

1) This work was supported by Grant RF 57080 from the Rockefeller Foundation

breviligulata, and 16 strains of cultivated rice, *O. sativa* and *O. glaberrima*, were tested, concerning variability in susceptibility to blast fungus, *Piricularia oryzae*.

Localities where the seed samples were collected were: Africa, Ceylon,

Table 1. Average value and genetic variability in susceptibility to blast disease in strains of wild and cultivated rice

Species	Locality	Average susceptibility*	Genetic variability (%)
Oryza glaberrima	Africa	0.708	45.28
O. glaberrima	〃	0.936	44.04
O. sativa	Burma	0.598	10.53
O. sativa	〃	0.848	85.36
O. sativa	India	0.608	17.24
O. sativa	〃	0.698	17.56
O. sativa	Malaya	0.592	59.32
O. sativa	〃	0.576	—
O. sativa	Thailand	0.604	0.00
O. sativa	〃	0.578	10.45
O. sativa	Taiwan	0.694	38.65
O. sativa	〃	0.730	85.60
O. sativa	Japan	0.650	17.94
O. sativa	〃	0.616	9.57
O. sativa	Ceylon	1.446	—
O. sativa	〃	1.507	—
O. breviligulata	Africa	0.845	95.54
O. breviligulata	〃	1.087	—
O. sativa f. spontanea	Ceylon	4.498	92.27
O. sativa f. spontanea	India	0.594	17.81
O. sativa f. spontanea	〃	7.178	22.62
O. sativa f. spontanea	Malaya	0.578	25.42
O. sativa f. spontanea	〃	0.823	87.09
O. sativa f. spontanea	Thailand	0.642	32.43
O. sativa f. spontanea	Taiwan	0.622	38.76
O. sativa f. spontanea	〃	1.491	99.79
O. perennis	India	0.648	98.93
O. perennis	Thailand	0.746	32.43

* The estimate for the most resistant plant being 0.5.

India, Burma, Malaya, Thailand, Taiwan and Japan. The plants were grown in pots. Susceptibility to the disease was tested by the leaf-sheath method using the leaf sheath of the second youngest leaf. Testing was done twice for each individual plant. The P-2 strain of blast fungus was used. Average susceptibility degree and genetic variability for each strain are shown in Table 1.

Some strains of *O. sativa* f. *spontanea* collected in India and Ceylon were highly susceptible. *O. breviligulata* collected in Africa, *O. sativa* f. *spontanea* in Taiwan and *O. sativa* in Ceylon were considerably susceptible. Other strains were resistant. It is suggested that in general the strains distributed in Ceylon are susceptible, while those in Malaya and Thailand are resistant.

Table 1 shows that most strains of wild rice and a few strains of cultivated rice were highly heterozygous with respect to blast susceptibility. Those occurring in Africa seem to be considerably heterozygous, while strains from Thailand and Japan were much less heterozygous.

45. *Variability of seed size in wild rice populations of Burma and some African countries*

(By Kan-Ichi SAKAI, Shin-ya IYAMA and Takashi NARISE)

Variability of seed size was measured for several populations of wild rice collected in Burma and a few African countries. The number of populations collected was five for Oryza perennis, seven for *O. sativa* f. *spontanea*, six for *O. breviligulata* and four *O.* barthii. The former two species were from Burma and the latter two from Africa. A number of panicles were collected at random from each population, and ten seeds from each panicle were measured for their length and width. The analysis of variance of data was conducted for each population separately. Estimated values of mean length and width, environmental and genotypic variances, the proportion of genotypic variance to phenotypic variance, and genetic and environmental correlations between seed length and width as well were compared among different species and different strains.

Though a detailed discussion and concluding remarks will have naturally to await the completion of this serial work, a few facts of interest may be pointed out. The first thing to be mentioned is that the wild rice species, *O. breviligulata* and *O.* barthii, growing in Africa have on an average bigger seeds than *O. perennis* or *O. sativa* f. *spontanea* found in Asia. Secondly, wild population in Africa are genetically highly heterogeneous with regard to seed size, the heterogeneity being comparable to that of wild rice strains found in Asia. In this respect, it is of interest to

find that wild populations of *O. perennis* growing in Burma are often unusually homogeneous. Genetic correlation between seed length and seed width is very variable, positive in some strains and negative in others, while environmental correlation between them is very low throughout.

46. *Comparative studies on growth rate of seedlings in wild and cultivated rice*

(By Kan-Ichi SAKAI and Shin-ya IYAMA)

Growth rate of seedlings was compared between cultivated and wild strains of rice. The number of varieties of cultivated rice used in this experiment was ten for *Oryza sativa*, nine for *O. glaberrima* and the number of wild strains was thirteen involving *O. sativa* f. *spontanea*, *O. perennis* and intermediate types between them. Growth rate was measured by seedling length between the second and the fourteenth day after germination.

It was found from this experiment that cultivated as well as wild strains were highly variable in respect of growth rate of young seedlings. The cultivated varieties however had in general a higher growth rate in the seedling stage than the wild strains. Experiments are now under progress for further study.

47. *Seed growth in cultivated and wild rice*

(By Takashi NARISE and Kan-Ichi SAKAI)

The growth of seeds of cultivated and wild rices was investigated. Eight strains of cultivated rice including *Oryza* sativa and *O. glaberrima* and twelve of wild rice including *O. perennis*, *O. sativa* f. *spontanea* and *O. breviligulata* were used in this study. Observation was made in the field and in the greenhouse. One to 29 days old spikelets after anthesis were collected from each strain, and their fresh weight, dry weight and water content per seed were measured.

In cultivated rice, the total fresh weight of seeds generally increased up to the 24th day after fertilization in *O. sativa* and 22nd day in *O. glaberrima*, whlie dry weight increased up to the 22nd day fertilization in *O. sativa* and 20th or 22nd day in *O. glaberrma*, the water content increasing up to the 13th day in both species. In wild rice, an increase in the total fresh weight and dry weight was observed up to the 10th day in *O. sativa* f. *spontanea* and to about the 11th day in total fresh weight and 14 day in dry weight in *O. breviligulata*. The analysis of variance indicated that there was no significant difference in fresh weight between

cultivated rice species, but a significant difference in the dry weight, the sativa strains having heavier seeds than the glaberrima strains.

The growth rate of seeds measured in terms of daily increase in fresh as well as dry weight was compared between wild and cultivated species of rice. The same comparison was also made among strains of either cultivated or wild rices. It was found from this comparison that the growth rate of seed per day was not different among various species, whether wild or cultivated. It is concluded accordingly that the only defference between wild and cultivated rice species in respect of seed growth may be that wild species complete ripening several days earlier than cultivated species, producing smaller kernels than the latter: 23 days were required for *O. sativa* to ripen, 21 or 22 days for *O. glaberrima* and only 10 days for wild rices.

48. *Survey of variation between Oryza breviligulata and O. glaberrima collected from West Africa*

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

An African wild rice species, *O. breviligulata*, may be regarded as the progenitor of *O. glaberrima* which is cultivated in West Africa, since the two species have many common characteristics and fertile F_1 hybrids. With the view to investigating the variation between wild and cultivated forms in these two species, a number of strains belonging to them, collected by Dr. K. FURUSATO of this Institute from West African countries, were grown in a short-day field in Mishima, and data were taken for various characters. By choosing typically wild (*breviligulata*) and cultivated (*glaberrima*) strains, first a discriminant function for classifying wild and cultivated forms was constructed. Measurements of degree of grain shedding, length/width ratio of spikelet, spikelet number per panicle and rachis number per panicle were combined to make up the discriminant formula. Regarding the scores given by this formula, the strains tested (49 in total number) showed a continuous series of intergrades between typically wild and cultivated forms. This pattern of variation from wild to cultivated forms appeared to be similar to that previously observed by the writers in Asian materials including *O. perennis* and *O. sativa* (the so-called *O. sativa* f. *spontanea* is included by us in *O. perennis* as one of its forms).

Secondly, variations of six characters among 37 strains of *O. breviligulata* were studied by factor analysis of correlations. The first factor extracted from the multivariate variation, with a 39 % contribution, was found to indicate the variation in the direction of cultivated type. The second factor made a very small contribution (3.6 %) and did not show a definite

biological significance. This result markedly differs from the variation in Asian populations of *O. perennis*, in which the first factor showed the differentiation into *perennis* and *spontanea* types and the second factor showed the variation in the direction of cultivated type (reported in Annual Report No. 10: 49–51, 1960).

Thirdly, in order to examine how the variation in the direction of cultivated type is related to each character, variation patterns in wild (*breviligulata*) and cultivated (*glaberrima*) forms were compared with each other regarding various characters. Significant differences between the two forms were found in spikelet width, length/width ratio of spikelet, awn length, degree of grain shedding and apiculus hair lenght. Except for the last one, these are the characters differentiating wild from cultivated forms in the group of *O. perennis* and *O. sativa*. In spikelet length, length/width ratio of spikelet, awn length and ligule length, the wild forms (*breviligulata*) had wider range than the cultivated forms (*glaberrima*). In contrast, in apiculus hair length, rachis number per panicle and spikelet number per panicle, cultivated forms a wider range. This pattern of variation does not consist with what is found between *O. perennis* and *O. sativa*.

49. *Parallel variation from wild to cultivated forms in Sativa and Glaberrima Series of Oryza*

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

The wild progenitors of *O. sativa* and *O. glaberrima* are thought to be *O. perennis* (including *O. sativa* f. *spontanea* assumed to be a form of *O. perennis*) and *O. breviligulata*, respectively. The *Sativa* series (including *O. sativa* and *O. perennis*) and the *Glaberrima* series (*O. glaberrima* and *O. breviligulata*) can be discriminated by ligule shape and other characters, and their F_1 hybrids are highly sterile. This study was an attempt to find how the variation from wild to cultivated forms which might have occurred in parallel in the two series of species can be analysed statistically, since parallel variation is regarded to be an obstacle in numerical systematics. Correlation coefficients between strains were calculated from measurements of 20 quantitative characters taken for 20 strains belonging to the above-mentioned four species (five strains for each species). In addition to this, discriminant scores were computed for the 20 strains from a discriminant formula for classifying the *Sativa* and *Glaberrima* series, and a matrix of the discriminant scores was subtracted from the matrix of above-mentioned correlation coefficients. In this process, the discriminant scores were adjusted so that 25% of the total multivariate variation was sub-

tracted. Then the residual matrix was analysed by factor analysis (averoid) to find unknown variation factors. The first factor thus extracted from the residual matrix was found to indicate the differentiation of strains into wild and cultivated forms, spending 38% of the total variation. By scattering the strains tested in the plane defined by the discriminant function and the first factor axis, the parallel variations from wild to cultivated types in the *Sativa* and *Glaberrima* groups were compared. Among the 20 strains tested, the variation in the *Glaberrima* series appeared to be continuous, but in the *Sativa* series the wild and cultivated strains could be separated.

50. *Further studies on hybrid sterility relations among cultivated and wild strains of rice*

(By Kokichi HINATA and Hiko-Ichi OKA)

We have reported last year (Annual Report No. 10: 51-53, 1960) that most Asian wild rice strains of *Oryza perennis* and *O. sativa* f. *spontanea* show a high fertility in F_1 hybrids with different test-strains belonging to Indica or Japonica type of *O. sativa*, and that a clear differentiation into the two types was not found in wild plants. This investigation was continued and more materials were observed. The results were examined by scattering the newly tested strains on a diagram introduced in our last report, in which the axes of abscissa and ordinate represented the first and second component axes extracted from the data for cultivated rice varieties. The diagram after addition of 11 new strains still showed the same features of variation as pointed out last year.

Further, a number of strains belonging to *O. glaberrima* and *O. breviligulata*, obtained from West Africa, were crossed with a set of test-strains (consisting of *O. sativa* and *O. glaberrima*) to examine their hybrid sterility relations. The majority of crosses between *glaberrima* and *sativa*, more than 200 in total number, did not show a pollen fertility higher than 3%. However, in crosses with a few *sativa* strains from India and Viet Nam, it amounted to 5% to 8%. The wild strains of *sativa* type (*O. perennis* and *O. sativa* f. *spontanea*) showed relatively high pollen fertilities in hybrids with *O. glaberrima*, the highest reaching 25%.

51. *Hybrid swarms between wild and cultivated rice species, Oryza perennis and O. sativa*

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

Several wild rice populations supposedly of hybrid origin between wild and cultivated forms were analysed. First, *Patna* population, found in the

suburb of Cuttack, Orissa, India, though it appeared to be a mixture of wild and cultivated plants growing in a paddy field, was shown to be a hybrid swarm. A part of its plants, having many wild characters and a few cultivated ones, were found to be highly heterozygous for genes of cultivated rice. Secondly, *Sampatoon* population, found in a stream running through paddy farms of glutinous rice in the suburb of Chiengmai, Thailand, was observed. It consisted of plants of intermediate *perennis-spontanea* type, and contained many heterozygotes for the glutinous gene. The offspring of the heterozygotes tended to be in various characters intermediate between the wild and the cultivated type. It was also found that, though in northern and north-eastern parts of Thailand, glutinous rice is grown in almost all paddy farms, the majority of wild populations growing in proximity of glutinous rice fields had a low frequency of the glutinous gene, so that a hybrid swarm might be regarded to be rather rare. Thirdly, three small populations found in Tao-Yuan Prefecture, Formosa, which were phenotypically of *perennis* type, were observed. They were found to be highly heterozygous for genes of cultivated rice. Thus, it was pointed out in general that a hybrid swarm could preserve a great amount of genetic variability, which might cover the whole range of variation from the wild to the cultivated type. Based on the results of these observations, emphasis was laid on the significance of hybridization in the process of creation of cultivated rice. (Published in Evolution, in press)

52. *A Survey of drought resistance in wild and cultivated rice strains*

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

In *Oryza sativa*, the so-called Indica varieties are generally more resistant to drought than the Japonica varieties (OKA 1953, 1958). An experiment was made to compare this character between several wild and cultivated *Oryza* species. A number of strains belonging to *O. sativa* (21 strains), *O. perennis* (42 strains, including *O. barthii* and *O. sativa* f. *spontanea*), *O. glaberrima* (29 strains) and *O. breviligulata* (29 strains) were tested by the *Mimosa* method. By this method, the drought condition in a pot is judged by the suspension of stimulation movement of a *Mimosa pudica* plant grown together with the plants to be tested. Thus the result shows the ability of plants to survive a strong drought. In *O. sativa*, as was previously known, strains of the Indica type were found to be generally more resistant than those of the Japonica type. Strains belonging to *O. perennis* showed a wide variation ranging from highly to very weakly

resistant, while those of the *spontanea* type were generally resistant. Strains of *O. glaberrima* and *O. breviligulata* were generally weak and most of them did not survive the test.

53. *Comparison of growth and fertilizer response between Oryza sativa and O. glaberrima*

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

Five *sativa* and five *glaberrima* varieties, early enough to be harvested before October frost in MISIMA, were tested in a field experiment with three fertilizer levels. A strain of *O. sativa* f. *spontanea* from India was added to this experiment, but it was too late to observe its yielding capacity. The experiment was designed as latin-square split plots with three replications, in which sub-plot represented a variety. Fertilizer treatment consisted of zero, standard (12 gm/m^2) and double nitrogen levels with the same amount of phosphoric acid and potash (both being 8 gm/m^2). Seedlings raised in a nursery-bed were transplanted at normal (25 cm × 25 cm) and high (12.5 cm × 12.5 cm) density.

The growth of plants in the normal-density plots was traced by the "growth analysis" method. It was found that the varieties of *O. glaberrima* generally had smaller "net-assimilation rate" than those of *O. sativa*. However, they had wider and thinner leaves than *O. sativa*, or a higher regression of leaf area on leaf weight. The large leaf area may compensate for the low assimilation rate resulting in the "relative growth rate" of this species approximately the same as that of *O. sativa*. This relation seemed to hold good at least in the earlier stages of growth when the leaves are not crowded. When the "leaf-area index" becomes high in later stage, especially in densely planted plots or in highly fertilized plots, *O. glaberrima* might have a lower assimilating power than *O. sativa*. It may then have shown smaller plant weight and smaller panicle number at maturity.

Regarding fertilizer responses, the Japonica varieties of *O. sativa* had a higher rate than the Indicas as is well known. In *O. glaberrima*, four of the five varieties tested showed a low rate as that of Indicas, but the remaining one had a high rate as that of Japonicas.

54. *Inheritance of mono and polyembryony in Citrus*

(By Kazuo FURUSATO)

Ten years ago, in the Experimental Citrus Farm in Kuzura, crosses have been carried out between mono- and polyembryonic *Citrus* varieties.

They were as follows:

Monoembryonic	Polyembryonic
C. Tamurana	*C. natsudaidai*
C. sulcata	*C. sinensis*
	C. Kinokuni
	C. reticulata
	C. leiocarpa
	C. kôkitsu

The F_1 could be examined now, after ten years, and the results are given in the following table.

Table 1.

Cross	Variety name	Number of F_1 plants	Polyembryonic	Monoembryonic
mono. × mono.	*C. Tamurana* × *C. sulcata*	2	0	2
mono. × poly.	*C. Tamurana* × *C. natsudaidai*	16	13	3
	C. Tamurana × *C. sinensis*	2	2	0
	C. Tamurana × *C. Kinokuni*	1	1	0
	C. sulcata × *C. leiocarpa*	3	2	1
poly. × poly.	*C. natsudaidai* × *C. reticulata*	1	1	0
	C. kôkitsu × *C. natsudaidai*	4	1	3

The number of F_1 plants is of course small but it may be concluded that the inheritance of this trait is controlled by an allele pair, mono- versus polyembryonic. The former allele is recessive, the latter is dominant. The two monoembryonic varieties must be recessive homozygotes. Of the polyembryonic strains *C. natsudaidai* could be a dominant homozygote. As to the other polyembryonic varieties the small numbers do not allow to draw a decisive conclusion as to their homo- or heterozygotic nature.

55. *Breeding of amphidiploids from a hybrid between Nicotiana rustica and N. tabacum*

(By Kazuo FURUSATO)

A cross between *Nicotiana rustica* and *N. tabacum* is usually very difficult, and the seeds obtained from either direction of the cross do not germinate.

In the spring of 1959 the cross with *N. rustica* as the female parent was repeated in a greenhouse kept at low temperature. From 1500 flowers pollinated 1300 capsules were obtained which yielded 134 viable seeds. But from the reciprocal cross, *N. tabacum*×*N. rustica*, carried out under the same conditions no viable seeds were obtained.

The F_1 seedlings were treated with colchicine and three tetraploid plants were obtained.

Pollen fertility in F_1 was about 1%; it amounted to about 89% in the tetraploids. But neither tetra- nor diploids produced capsules by self-pollination. Only when the tetraploids were backcrossed to *N. tabacum*,

Table 1.

Species	Plant height	Stem thickness	Leaf number	Largest leaf			Average weight of raw leaves from one plant
				Length	width	position	
	(cm)	(cm)		(cm)	(cm)		(g)
N. tabacum (Bright Yellow)	108.8	2.2	21.2	44.8	24.0	7.4	233
4x (*N. rustica* ×*N. tabacum*) × *N. tabacum*	131.4	3.0	20.3	50.8	32.8	6.2	480

Table 2.

Species	Chlorogenic acid (%)	Rustin (%)	Nicotine (%)
N. tabacum (Bright Yellow)	2.33	0.92	0.94
N. rustica (Brazilia)	0.69	0.72	1.44
4x (*N. rustica*×*N. tabacum*) × Bright Yellow	1.44	0.96	3.53

In Table 1 a few characters are given for Bright Yellow and for the 4x hybrid backcrossed to Bright Yellow.

In Table 2 the results of the chemical analysis are given for Bright Yellow, *Nicotiana rustica* and 4x hybrid×Bright Yellow.

capsules were produced, and the seeds were viable. The capsules contained on the average 80 seeds.

56. *Selection for low nicotine content*

(By Kazuo FURUSATO and Akira MIYAZAWA)

Synthetic tobacco obtained from a hybrid between *Nicotiana sylvestris* and *N. tomentosiformis* was crossed with the commercial variety Bright Yellow and selfing as well as backcrossing were practiced for five years. Yearly selection for nornicotine finally yielded 29 plants with high nornicotine and very low nicotine content. In addition, 3 plants among them lacked the cherry red leaf character.

The results of the chemical analysis of several plants were as follows:

Table 1.

Strain	Nicotine (%)	Nornicotine (%)
F_4- 33	0.28	1.32
F_4- 35	0.28	0.50
F_4- 2	1.72	0.37
F_4- 99	0.93	0.26
F_4-123	0.70	0.49
H- 8	2.38	0.22

57. *Heterotic vitality in natural populations of Trillium kamtschaticum Pall*

(By Yuichiro HIRAIZUMI, Takashi NARISE and Ichiro FUKUDA)

Vitality, one of the major components of fitness, was studied in natural populations of *T. kamtschaticum*. The main points studied were as follows.

1. The f value computed for chromosome E was chosen as an estimate of inbreeding coefficient for each population. This was on the assumption that this chromosome was relatively neutral regarding vitality.

2. With this f value, a method was proposed to measure the vitality of genotypes when only data from natural populations are available.

3. The method was applied to the actual data obtained from natural populations of *T. kamtschaticum* and it was concluded that the heterotic vitality is a general phenomenon in natural populations of this plant; if two (possibly more) types showing especially higher vitality in their hybrid are introduced or produced, they will be accumulated in populations to form the "specific local color."

58. *Cytogenetic studies in the genus* NICOTIANA *XIII*

(By Yô TAKENAKA)

The reduction division in PMC's was studied in 3 interspecific hybrids: *N. suaveolens* × *N. longiflora*, *N. suaveolens* × *N. plumbaginifolia* and 4× *N. tabacum* × *N. alata*.

1) F_1 of *N. suaveolens* (n=16)×*N. longiflora* (n=10)

At MI of PMC's of this hybrid, the number of bivalents ranged from 0 to 4, with the mode at 1. The frequency of PMC's with 0 bivalents followed that of PMC's with 1 univalent. PMC's with 2 and 3 bivalents were frequently found but those with 4 bivalents were very rare.

In the same hybrid, Kostoff (1943) found that the number of bivalents ranged most frequently from 0 to 3, and GOODSPEED (1954) also found the range from 0 to 4, although PMC's with 4 bivalents were very rare, but CHRISTOFF (1929) observed 26 univalents and no bivalent in the same hybrid. As KOSTOFF (1943) reported, quadrivalents are occasionally found.

Accordingly, it is difficult to speculate on the relationship between *N. suaveolens* which is an Australasian species and *N. longiflora* which is an American species, although these two species are much alike in external characters.

2) F_1 of *N. suaveolens* (n=16)×*N. plumbaginifolia* (n=10)

At MI of PMC's of this hybrid, the number of bivalents ranged from 0 to 2, with the mode at 0. The frequency of PMC's with one bivalent followed that of PMC's without bivalents. PMC's with 2 bivalents were rare.

In the same hybrid, KOSTOFF (1943) found that the number of bivalents ranged most frequently from 0 to 4, but CHROSTOFF (1929) observed 26 univalents and no bivalent in the same hybrid.

According to the above results, it is difficult to speculate on the relationship between *N. suaveolens* which is an Australasian species and *N. plumbaginifolia* which is an American species, although the resemblance in their external appearance is remarkable.

3) F_1 of 4× *N. tabacum* (n=48) × *N. alata* (n=9)

This hybrid was produced for the purpose of introducing a gene or genes for immunity against mildew and black shank, and for resistance against wild fire and anthracnose from *N. alata* into cultivated tobacco.

At MI in PMC's of this hybrid, the total number of bivalents and trivalents per cell was 24. The number of trivalents most frequently ranged from 1 to 4 but 0, 5 and 6 were rare.

At meiosis of the F_1 of *N. tabacum*×*N. alata*, the author (1956) observed 1–6 bivalents and the most frequent configulations found were $3_{II}+17_I$ and $4_{II}+15_I$. According to KOSTOFF's description (1943), the same

hybrid *N. tacacum*×*N. alata* contained 5-9 bivalents and also some polyvalents. Further, the number of trivalents in the hybrid, 4x *N. tabacum* ×*N. alata*, generally agreed with that of bivalents in the hybrid, *N. tabacum* × *N. alata*, observed by myself, but did not agree with KOSTOFF's findings.

At AI of PMC's, chromosome bridges and strayed chromosomes outside the spindle were frequently seen. At 2nd division, they form small nuclei and are the cause of polyspory at tetrad stage.

59. *Developmental studies in the genus Oryza* II *Further studies on embryo sac formation*

(By Yukio DOIDA)

Table 1. Materials and their origin

Species	Strain number	Origin
O. sativa var. *spontanea*	W0106	Phylankara, Cuttack, India
O. perennis	W0120	Cuttack, Orissa, India
O. officinalis	W0002 W0012	Bangkok Central Rice Res. Inst., Cuttack, India (C. R. R. I.)
O. latifolia	W0019	C. R. R. I.
O. minuta	W0045	Crop. Res. Div., USDA, Beltsville, Maryland, U.S.A.
O. eichingeri	W0015	C. R. R. I.
O. australiensis	W0008	C. R. R. I.
O. malabarensis	W0021	C. R. R. I.
O. granulata	W0004	Coimbatore, Madras, India
O. subulata	W0510	Chaco

In the previous report, the process of embryo sac formation was described for five *Oryza* species; *O. sativa*, *O. glaberrima*, *O. alta*, *O. breviligulata* and *O. ridleyi*.

Similar observations were carried out in ten other species listed in Table 1. No conspicuous differencies in the process were found. Eight nuclei were observed in the embryo sacs. Three formed the egg apparatus, two became polar nuclei and the remaining three were the antipodal nuclei. After fertilization an abortive development was observed of the antipodal cells which became multinucleate or polyploid and later degenerated.

60. *Cytological studies in the genus Polygonum* II

(By Yukio DOIDA)

Cytological studies have been continued with the view of discussing the problem of phylogeny of the Polygonaceae family.

The results are summarized in Table 1.

P. debile has sixteen chromosomes in root-tip cells. The chromosomes are markedly larger than those of other species. The basic chromosome number of this diploid species is 8. This number considered to be basic

Table 1. Chromosome numbers of the *Polygonum* species examined

Species name	Chromosome number (2n)	Previous reporters	Localities
Polygonum debile Meisn.	16		Nikko
P. weyrichii Fr. Schm.	20	Su: 20	Nikko*
P. senticosum (Meisn.) Franch et Savat.	24		Nagoya
P. nepalense Meisn.	48		Nikko
P. bistorta Linn.	(24)	J: 44 So: 46	Mt. Ibuki
P. bistorta Linn.	48		Nikko*

J: JARETZKY (1928), So: SOKOLOVSKAJA et al. (1938), Su: SUGIURA (1925)
*: Supplied by Nikko Botanic Garden of Tokyo University,

for the genus was found among other Polygonaceae only in the genera *Fagopyrum* and *Rumex*. The size of chromosomes in both, however, is considerably smaller than those of *P. debile*. The number n=8 was reported for the first time for a *Polygonum* species.

Pollen grains of *P. debile* belong to the "pore type" but those of *Rumex* and *Fagopyrum* species are of "furrow type". The relationship between *P. debile* and the two genera, *Fagoyprum* and *Rumex*, is considered to be rather distant.

P. bistorta was obtained from the Nikko Botanic Garden of Tokyo University. Fourty eight chromosomes were counted. Samples of the same species collected from Mt. Ibuki at Shiga-ken had twenty four chromosomes (cf. Ann. Rep. Nat. Inst. Genet., No. 10).

61. *Developmental studies in the genus Polygonum* IV *Further studies on the effect of gibberellin on microsporogenesis in three Polygonum species*

(By Yukio DOIDA)

The effects of gibberellin (GB) on the microsporogenesis of *Polygonum fagopyrum* was reported in the previous paper (Ann. Rep. No. 9), in which the range of gibberellin concentration used was 0, 0.1, 1, 10, 25, 50 and 100 p.p.m. The number of pollen grains was not strikingly altered by the lower concentrations, but was strongly reduced by the treatment with solutions of higher concentration than 10 p.p.m.

The effects of GB were further studied in 1960 with three *Polygonum* species: *P. fagopyrum* (buckwheat), *P. nodosum* and *P. blumei.*

The solutions used were 0, 0.01, 0.1, 1, 10, 25 and 100 p.p.m. which were sprayed on whole plants. Buckwheat was treated from seedling to flowering stage at about 3 day intervals. The other two species were collected in the state of nature and transplanted to pots May 17th. The treatment was applied at 5 day intervals from May 18th to July 25th.

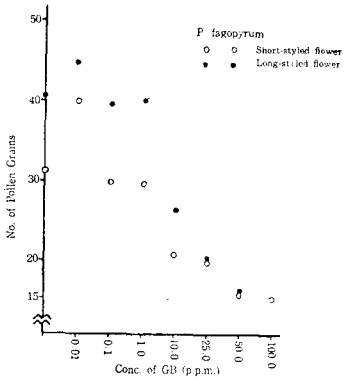


Fig. 1

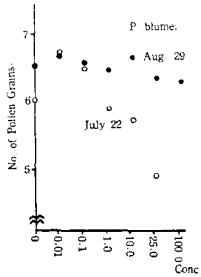


Fig. 2

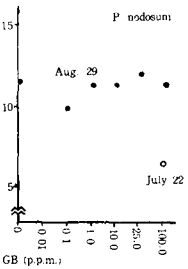


Fig. 3

Inflorescences were collected July 22nd and August 29th. *P. blumei* flowered July 22nd except for the plants which were treated with 100 p.p.m. solution, while on the same day of *P. nodosum* only plants treated with 100 p.p.m. flowered.

The results obtained as to the number of pollen grains are given in Figs. 1-3. The figures show a similar behavior for 2 species, *P. fagopyrum* and *P. blumei.* It was ascertained that the number of pollen grains increased by the treatment with 0.01 p.p.m. Other concentrations showed no effect or an inhibiting one. No effect of GB was observed for all con-

centrations Aug. 29th. All previously affected plants have recovered the ability to form the normal number of pollen grains.

The effects on the vegetative organs such as plant height, number of internodes and leaf size were also investigated. Plant height was increased at higher concentrations.

It may be concluded that GB stimulates cell divisions within a certain concentration range, effectiveness being different in vegetative and reproductive organs of the examined species.

62. *Studies on flower color variation due to anthocyanins*

(By Toru ENDO)

Cellular factors of flower color variation due to anthocyanins have been investigated. The present results were obtained by determination of the main component of anthocyanin, estimation of I_{30}/I_1 of aqueous extracts from fresh (and/or dry) flower petals with distilled water and comparison of absorption maxima of crude extracts and of pure anthocyanins in both 1% aqueous and 0.01% methanolic hydrochloric acid solutions. The latter procedure was employed to detect metal complex and co-pigmented anthocyanins. Some of the results are shown in Table 1, in which the degree of stability of the aqueous extracts is estimated by the agreement between the flower color and the color of the aqueous extract 30 min. after extraction, besides value of I_{30}/I_1. I_1 means optical density of the aqueous extract immediately after extraction from flower petals and I_{30} optical density 30 min. after extraction. For the estimation of I_{30}/I_1, 530, 560 and 580 mμ were used for reddish, purplish and bluish aqueous extracts, respectively.

As shown in Table 1. flower colorations can be apparently classified into two groups, stable and labile, according to the degree of stability of the aqueous extract. It is evident that the classification is not related either to the kind of the main anthocyanin component or the original flower color. Thus, it may be concluded that one of the main factors in flower color variation due to anthocyanins is based on the cellular colloidal system, which is basically composed of cell-sap soluble high molecular substances such as polysaccharides, proteins, polypeptides and some others, most of them being qualitatively and quantitatively different according to species or strain and strongly or weakly combined with the anthocyanins.

Table 1. Stability of colors in aqueous extracts from flowers of several plants

Plant name	Flower color	Main anthocyanidin	Color of aqueous extract	I_{30}/I_1	Stability	Note
Pansy	Reddish purple	Cyanidin	Red	0 (browing)	Labile	
Pansy	Deep purple	Delphinidin	Purple	0.61	Comparatively stable	
Pansy	Purplish blue	Delphinidin	Blue	0.74	Comparatively stable	Co-pigment
Torenia	Deep purple	Malvidin	Red	——	Labile	
Dahlia	Red	Cyanidin	Red	0 (browing)	Labile	
Morning glory	Bluish purple	Peonidin	Red	——	Labile	
Anchusa	Blue	Petunidin	Gray	——	Labile	
Commelina	Blue	Delphinidin	Blue	0.97	Stable	Metal complex
Rose	Red	Cyanidin	Red	0.96	Stable	
Cornflower	Blue	Cyanidin	Blue	0.90	Stable	Metal complex

63. *Anti-cancer activity of Gentiana extract*

(By Yō TAKENAKA, Yoshito OGAWA, Tōru ODASHIRO and Noriko KARUBE)

We reported that the extract from *Gentiana scabra* Bunge var. *buergeri* MAXIMOWITZ showed remarkable radiomimetic effects upon the living roots of *Allium scorodoprasum* var. *viviparum* and *Vicia faba*. This finding suggested the possibility of applying it with good results in the treatment of tumors, because *Gentiana scabra* var. *buergeri* is not poisonous to human body. The effect of the extract on Yoshida sarcoma, transplanted into a strain of rat, Wister (two months old), is reported in this paper.

Gentiana Radix Pulverata was treated with methanol. After evaporation of the solvent in vacuum at 50°C., a dark brown oil residue remained. Immediately after the transplantation of the ascites tumor, the obtained extract (0.5 mg. per 1 g body weight of rat) was injected at the axilla subcutaneously, and its influence on the sarcoma was observed in respect to the following three points: 1) Mitotic activity in sarcoma cells. 2) Systemic symptoms of the rat bearing the sarcoma (appetite, ascites, icterus and metastasis). 3) Prolongation of the host's life.

In the mitotic activity of sarcoma cells, a temporary decrease (significant et 5% level) was observed 48 hours after injection of the extract. But no significant difference was recognized between the treated group and the control in the frequency of dividing cells three days after transplantation. As to the systemic symptoms of the host rat, no difference was found between the treated and the control groups in the first five days, but seven days after injection, no ascites was found in the abdomen of the treated rat 15 days after injection, the treated group showed a remarkable recovery in systemic symptoms and 60% of the treated animals remained alive for more than 70 days after transplantation of the sarcoma (Fig. 1.), though the control group died out between the 10th and the 15th day. No toxicity of the extract applied in the above mentioned doses was oberved, except for some inflammation at the injected site observed only in the first three days after treatment.

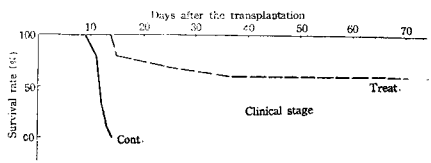


Fig. 1. The effect of Gentiana extract on life-prolongation of the host.

These experimental results seem to indicate that the effect of the *Gentiana* extract on sarcoma cells may be not direct but due to a secondary metabolite. Chemical the pharmacological research of this effective *Gentiana* component is now on the way.

C. MATHEMATICAL GENETICS

64. *An example of genetic system in which the gene frequency equilibrium is stable under negative overdominance*

(By Motoo KIMURA)

Let x_1 and $x_2(=1-x_1)$ be respectively the frequencies of a pair of alleles A_1 and A_2 in a random mating population and assume that the relative fitnesses of the three genotypes A_1A_1, A_1A_2 and A_2A_2 are expressed by linear functions of gene frequencies as follows:

Genotype	A_1A_1	A_1A_2	A_2A_2
Relative fitness (Malthusian parameters)	$-s_1(x_1-c_1)$	0	$-s_2(x_2-c_2)$

Here, we assume that s_1, s_2, c_1 and c_2 are all positive and constant. Such a situation may be realized if the environment consists of two different niches, e_1 and e_2, in which A_1A_1 is adapted to the former (e_1) and A_2A_2 to the latter (e_2), while the heterozygote A_1A_2 is adapted uniformly to both.

The average fitness of the population is

$$\bar{a}=-s_1x_1^2(x_1-c_1)-s_2x_2^2(x_2-c_2)$$

and the gene frequency equilibrium is attained when

$$s_1x_1(x_1-c_1)=s_2x_2(x_2-c_2)\ .$$

In order to simplify the situation, let us assume that $s_1=s_2\equiv s(>0)$. Then the equilibrium frequency of A_1 is

$$\hat{x}_1=\frac{1-c_2}{2-c_1-c_2}\ .$$

The necessary and sufficient condition for the equilibrium to be stable and non trivial is that both c_1 and c_2 lie between 0 and 1, i.e.

$$0<c_1<1\ ,\qquad 0<c_2<1\ .$$

Relative fitnesses of A_1A_1, A_1A_2 and A_2A_2 at equilibrium are

$$\hat{a}_{11}=\frac{-s(1-c_1-c_2)(1-c_1)}{2-c_1-c_2}$$

$$\hat{a}_{12}=0$$

$$\hat{a}_{22}=\frac{-s(1-c_1-c_2)(1-c_2)}{2-c_1-c_2}\ .$$

Here we may distinguish two cases:

Case 1. $0<c_1+c_2\leqq 1$. In this case, $\hat{a}_{11}<0$ and $\hat{a}_{22}<0$, so that the heterozygote A_1A_2 is advantageous over both homozygotes.

Case 2. $1<c_1+c_2<2$. In this case, $\hat{a}_{11}>0$ and $\hat{a}_{22}>0$, so that A_1A_2 is less advantageous than either homozygote (negative overdominance). It is interesting to note that in this case, the gene frequency equilibrium is stable under "negative heterosis".

In both cases, the equilibrium frequency of A_1 is generally different from the frequency in which the average fitness of the population is maximized, i.e.

$$(x_1)_{\max}=\frac{3-2c_2}{6-2c_1-2c_2}\ .$$

65. *Mutational load when the phenotypic effect of mutational damage is partially suppressed by developmental homeostasis*

(By Motoo KIMURA)

We will consider a random mating population of a diploid organism. No restriction will be made to the number of segregating loci except that the average number of mutant genes per individual is assumed to be small. Namely, it will be assumed that the frequency of mutant genes per locus is sufficiently low so that only mutant heterozygotes are required to be considered for the calculation of mutational load.

Let D be the total mutational damage per zygote measured in lethal equivalent (cf. Morton, Crow and Muller 1956). If the frequency of mutant gene in the k^{th} locus is $s^{(k)}$ and its selection coefficient is $-s^{(k)}$, then the average mutational damage may be expressed by

$$\bar{D}=\sum_k 2x^{(k)}s^{(k)}\ ,$$

where summation is over all relevant loci. The equilibrium gene frequencies are given by

$$\hat{x}^{(k)}=\frac{2\mu^{(k)}}{\left(\widehat{\frac{d\bar{a}}{dx^{(k)}}}\right)}\ ,$$

where $\mu^{(k)}$ is the mutation rate in the k^{th} locus, $\bar{a}$ is the average fitness of the population in which the fitness, measured in Malthusian parameters, of the normal genotype is taken as standard (0) and the "hat" ($\wedge$) on a letter designates that it is the value at equilibrium.

If there is no epistasis, fitness of individual genotype is proportional to genetic damage, i.e.

$$a=cD\ ,$$

where c is a positive constant (here corresponding to the degree of dominance of mutant genes in fitness). In this case it can easily be shown that the mutational load is equal to the total mutation rate per zygote:

$$\hat{L}_\mu=|\hat{\bar{a}}|=\sum_k 2\mu^{(k)}\ .$$

A simple but an interesting situation will be produced by assuming that fitness of an individual genotype is proportional to the square of genetic damage, i.e.

$$a=-cD^2\ .$$

Then the mutational load is given by

$$\hat{L}_\mu=M-c\hat{\bar{D}}^2\ ,$$

where M is the total mutation rate per zygote, i.e.

$$M=\sum_k 2\mu^{(k)}$$

and $\hat{\bar{D}}$ is determined by the relation

$$\hat{\bar{D}}=\sum_k \frac{2\mu^{(k)}}{c(s^{(k)}+2\hat{\bar{D}})}\ .$$

As an example, let $s=1$ for all loci (that is, all mutations are lethal), $M=0.4$ and $c=0.05$, then we have

$$L_\mu\approx 0.25\ .$$

Similar calculations may also be extended to the case of stronger epistasis such as

$$a=-cD^3\ .$$

66. *Population dynamics of selfing*

(By Terumi MUKAI)

Since the contributions of males to the fitness of a genotype are usually different from those of the females, it stands to reason that the fitness may not be a simple function of its component factors in random mating

populations. This should also hold true for selfing populations. Therefore, a study was carried out with respect to a single locus in order to clarify the effect of each fitness component on the relative frequencies of the genotypes.

In order to set up a mathematical model, the following parameters were defined: A and a=genes in the locus in question. m=relative pollen competitive ability of a-carrying pollen grains, that of A-carrying ones being 1. $s_1(s_2)$=relative seed productive ability of Aa individuals (aa individuals), that of AA being 1. $v_1(v_2)$=relative zygotic viability of Aa individuals (aa individuals), that of AA being 1 (germination percentage and selection before maturation). n=generation number.

The relative frequencies for mature plants (AA, Aa and aa) in F_n generation can be expressed as follows:

$$\left.\begin{aligned} f_{F_n}(AA)&=\frac{1}{L+Mv_1+Nv_2}\\ f_{F_n}(Aa)&=\frac{Mv_1}{L+Mv_1+Nv_2}\\ f_{F_n}(aa)&=\frac{Mv_2}{L+Mv_1+Nv_2}\end{aligned}\right\} \quad (1)$$

where $L=(s_1v_1-2s_2v_2)[2^{n-1}-(s_1v_1)^{n-1}]$, $M=(1+m)(2-s_1v_1)\times(s_1v_1-2s_2v_2)(s_1v_1)^{n-2}$ and $N=m(2-s_1v_1)[(s_1v_1)^{n-1}-(2s_2v_2)^{n-1}]$. ($s_1v_1\neq 2$ and $s_1v_1\neq 2s_2v_2$).

The relative frequencies for seeds for F_n generation can be easily obtained by equating $v_1=v_2=1$ in Formula (1) except for v_1 and v_2 in L, M and N.

Under the conditions of $s_1v_1>2$ and $s_1v_1>2s_2v_2$, the population reaches a stable equilibrium. The equilibrium frequency can be obtained by taking the limiting values of the formula given above when n is approaching infinity. Details of this work have been published in Seiken Jiho 11.

D. GENETICS AND BIOCHEMISTRY OF MICROORGANISMS

67. *Transductions between curly flagellar mutants in Salmonella*[1]

(By Tetsuo IINO)

In order to obtain recombinations between curly flagellar determinants in Salmonella (Annual Report, No. 9, 1959) transduction was carried out

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

from curly i-phase of SW577 to curly a-phase of SL23, and from curly enx-phase of SJ30 to curly 1.2-phase of either SJ167 or SJ168. SW577 is a mutant obtained from a diphasic strain of *S. typhimurium* R-14. SJ30, SJ167 and SJ168 were obtained from a phase-2 stable strain of *S. abortus-equi*, SL23, and its transductional derivative SJ25 (stable 1.2-type). In the first combination of the transduction, a phase-2 culture was used for the preparation of phage-PLT22 lysate and the transduction of $H_2^{1,2}$ was scored as control: when a phase-2 culture is used as a donor transduction of H_1 and H_2 are expected to be equal. In transductions from SJ30, SL23 was used as the donor in control experiments.

So far, the normal flagella type which carry antigen type of the donor has not been obtained from any combinations, while in each control combination over 500 transductional recombinants were obtained.

It suggests that the mutant sites of these curly types are very closely linked or identical. As demonstrated by Kerridge (1959), curly flagella are produced when phenyl-alanine is replaced by p-fluoro phenyl alanine in flagellar regeneration. Therefore it might be quite possible that the curly flagellar mutants were produced by replacement of a certain amino acid in flagellin by the other; such mutants might occur by mutation of a specific site in H_1 or H_2.

In a combination of the control experiments, in which SL23 was used as a donor and SJ168 as a recipient, 126 normal flagellar type clones with enx-antigen were detected. Among them, two clones were found to be agglutinated by anti-1.2 serum as well as enti-enx serum. They were inhibited spreading on nutrient gelatine-semisolid plates by either anti-enx serum or anti-1.2 serum. Their segregation to the pure enx- or 1.2-type cells in successive subcultures has not been observed. In these two clones recombination might have occurred in an antigen type determinant H_2 coupled with the flagellar shape determinant. Agglutination tests with single factor antisera on these recombinants are in progress.

68. *A non-flagellated mutant which produces flagellar protein in Salmonella*[1]

(By Tetsuo IINO and Ichiro HARUNA[2])

S. abortus-equi SJ28 is a non-motile mutant obtained from a phase-2 (enx-type) stable strain SL23. The mutant does not produce flagella and is not agglutinated by anti-H (enx) serum. The mutant character of

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

2) Virus Research Institute, Kyoto University.

SJ28 is very stable; so far, the reversion to a motile tipe has not been observed.

Antiserum was prepared against a 0.5% formaline-saline suspension of SJ28 cells, which contained 5×10^6 cells per ml: 0.5 to 2.0 ml of the suspension was injected intravenously to a rabbit seven times at 3 or 4 days' interval. The anti-SJ28 serum thus obtained showed both H- and O-agglutinations when it was mixed with the cells of SL23. After complete absorption of the anti-SJ28 serum by SL25, which is an 1.2-type transductional derivative of SL23, and by heated SL23, an enx-specific antiserum fraction (tube agglutination titer: 16,000) remained. The specificity of the anti-enx serum fraction was further confirmed by a precipitation test with isolated flagellar proteins of different antigen types.

The chromatographic fraction (fraction-f) which corresponds to flagellar protein was obtained from SJ28 by 'sonication-DEAE method'. Though the amount of protein in the fraction-e from SJ28 is less than one twentieth of that from SL23, the presence of enx-specific antigens in it was demonstrated by precipitation tests. Furthermore, on a rabbit immunized by the fraction-e of SJ28, the production of anti-enx serum of titer 8,000 was observed.

From these results, it is inferred that SJ28 is a mutant which can synthesize protein with the same antigenicity as a flagellar protein of the parent strain SL23, but the mutant cannot organize such protein molecules into a flagellum. A part of the enx-type protein produced by SJ28 may leak out from the cells.

In contrast with SJ28, proteins with H-antigenicity have not been detected from a deletion type of non-flagellated (*Fla*$^-$) mutant SJ157.

69. *Host-range mutants of chi-phage*[1]

(By Itiro SASAKI)

It has been known that chi-phage attacks Salmonella having flagellar antigen with the exception of the following two distinct kinds of flagellated types: one which has flagella but is not motile (so-called paralysed type), and the other which has g-antigenic flagella. In the latter type, the phage resistance is generally antigenic phase specific.

As a new type of chi-resistant bacteria, motile bacteria resistant in both flagellar antigenic phases were obtained from *S. panama* (lv:1,5), *S. lomita* (eh:1,5), *S. reading* (eh:1,5).

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

Three phage types which can attack chi-resistant SJ 26 (gp:(1,2)), SJ 181 (lv:1,5), and SJ 8 (mt:) were obtained from chi-phage developed in serial mixed cultures of phage-sensitive strain, SL 23 (a:enx) and each of the resistant bacteria strains. It was proved that these phages are indistinguishable from original chi-phage in serological specificity, host specificity (:they attack flagellated bacteria but not non-flagellated bacteria), growth characteristics on the common host SL 23, and electron-microscopic morphology of their particles. These phage types originated from SJ 26, SJ 191, and SJ 8 were designated h1, h2, h3, respectively.

Host-range and efficiency of plating are approximately the same between h1 and h3 on most of the bacterial strains having g-type flagella. They can not attack SJ 181. In contrast with h1 and h3, h2 attack SJ 181 but for cannot attack g-group bacteria. Phage-h1 propagated through SL 23 can grow in every cell of SL 23 but only in about one of 10^6 cells of SJ 26 (gp:(1,2)). The same phage-h1 propagated through SJ 26 (gp:(1,2)) can grow in all cells of SL 23 and in about one of 10 cells of SJ 26 (gp:(1,2)). However, after its propagation through SL 23, its infectivity to SJ 26 is reduced again to one in 10^6 cells. Thus, a remarkable host controlled variation by SL 23 and SJ 26 was observed on h1. On the contrary, h3 does not show the variation. When a suspension of h3 is plated with SJ 26 (gp:(1,2)), it produces a smaller number of plaques than with SL 23.

70. *The composition of ribonucleic acid in Yeast irradiated with ultraviolet light*

(By Saburo NAWA)

The nucleotide composition of ribonucleic acid synthesized in the irradiated cells of yeast was determined. After the incubation of *UV* irradiated cells in the minimal medium containing ^{32}P (orthophosphate) for 30 min., RNA was extracted using the Davidson and Smellie procedure and digested with potassium hydroxide. The nucleotides were chromatographically separated using a column of Dowex $1-8x$ formate and the activity of ^{32}P of each nucleotide was measured.

The nucleotide composition of the RNA fraction from the irradiated cells showed a higher value of $C/A(C+G/A+U)$ and a similar value of C/G or U/A as compared with RNA from non-irradiated cells. It was observed that the composition of RNA synthesized in irradiated cells was affected by the supplementation of certain nucleic acid precursors to the irradiated medium. The ratio C/A decreased when adenosine and guanosine were in combination supplied to irradiated cells, although addition of ribonucleotides had no effect when they were singly supplied. It is

noticeable that addition of deoxyribosides could modify the composition of RNA in irradiated cells. Supplementation of deoxyadenosine plus thymidine let to normalization of the ratio C/A. This suggests that the synthesis of the abnormal RNA in irradiated cells involves some turnover of DNA. No effect was observed of above supplementation on the nucleotide composition of RNA in non-irradiated cells.

71. *Nuclear structure of yeast*

(By Yoshiaki YONEDA)

The nuclear structure and the division process of yeast were investigated. The materials used were haploid, diploid and tetraploid strains of the Carbondale stocks of *Saccharomyces*.

As previously reported, the extravacuolar nucleus of haplo-, diplo- and tetraploid strains is composed of two parts; one is the Feulgen-positive chromatin region and the other is the Feulgen-negative karyoplasm. Aceto-orcein staining revealed that the reddish stained chromatin is situated on one side of the nucleus which is attached to the central vacuole. At the nuclear division stage, the chromatin divides at first into two bodies, whereupon the nucleus becomes constricted.

Later, the detailed structure of the chromatin region was observed by staining with Feulgen and Giemsa methods; it could be also clearly observed with Feulgen and aceto-orcein combined staining. In the center of the chromatin region, a small granule was found, which was Feulgen-positive and tentatively termed as central granule. A ring of homogeneous chromatin surrounded this granule at the resting stage.

At the first stage of the division, some parts of the chromatin ring changed to condensed structures, from which chromosomal bodies originated in later stages. The chromatin condensation was remarkable through the last stage of the non-budding phase and during bud sprouting. Before a bud grew in length to one third of the mother cell, metaphase chromosomal bodies appeared and anaphase was carried out. The number of the metaphase chromosomal bodies was not proportional to ploidy. About three such bodies were distinguished in all haplo-, diplo- and tetraploid strains and at anaphase six or more bodies were observed. Simultaneously, the division of the central granule took place. When the bud increased to half the size of the mother cell, the chromatin was completely separated into two daughter chromatin bodies. Thereafter, the elongation and constriction of the whole nucleus occurred in the mother cell or

1) This work was supported partly by Grant RF 57178 from The Rockefeller Foundation.

between it and the bud. The daughter chromatin body, moving to the bud, was compact and after settling in the bud, assumed a diffused appearance.

Recently, YUASA and LINDEGREN (1959) reported the existence of "centrioles" in yeast nuclei, the number of which corresponded to the ploidy of the cell. The central granule may be comparable to those "centrioles" in regard to its position, though only one granule was found in the strains studied.

72. *The effect of nucleoside supplementation on mutation*[1]

(By Sayaka NAKAI)

Previous studies of the author in yeast and of Doudney and Haas in *E. coli* have shown that there are several steps in mutation which are correlated with macromolecular synthesis after irradiation. The present experiments are concerned with nucleic acid base requirements in the mutative steps after UV irradiation by metabolite supplementation techniques for the examination of molecular properties of UV induced mutations.

The mutation studied is a reversion of the isoleucine-requiring to the non-requiring allele ($il^- \rightarrow il^+$) in the haploid strain A9 of *Saccharomyces cerevisiae*. In order to conduct this study, various nucleosides (50 μg/ml), singly or combined, were supplemented to the medium at 30 minute intervals from 0 to 3 hours after irradiation. The results obtained are summarized as follows:

1) Supplementation of pyrimidine, especially, thymine deoxynucleoside immediately after UV irradiation increases the surviving fraction (about 20%), but deoxypurine nucleoside supplement has no such effect.

On the other hand supplementation of purine ribonucleoside increases the surviving fraction (about 20%), but not pyrimidine nucleoside.

2) Purine deoxynucleoside supplement, especially, in combination of deoxyadenosine and deoxyguanosine remarkably affects the mutation frequency, changing it to a bimodal pattern as a function of time intervals between irradiation and supplementation of nucleoside. The first peak appears at 30 minutes and the second peak at 120 minutes after addition of the above compounds. On the contrary, pyrimidine deoxynucleoside supplement does not show such relationship.

Effect of ribonucleoside supplementation on mutation frequency is somewhat different from that of deoxynucleoside supplementation. In general, pyrimidine ribonucleoside has a similar effect to that of purine deoxynucleoside.

1) This work was supported partly by Grant RF 57178 from The Rockefeller Foundation.

The results lead to the following hypothesis: Genetic damage caused by UV irradiation primarily occurs more frequently at the site of pyrimidines in DNA molecule. Supplemented deoxypurines may partly substitute for the damaged pyrimidines in the process of mutation induction.

73. *Variations in the number of micronuclei found in the wild populations of Paramecium polycaryum*

(By Susumu MURAMATSU)

Paramecium polycaryum is commonly found in foul water containing large amounts of organic substances, and is characterized by the number of micronuclei. It has been known that the number of micronuclei varies from one to eight, although four is the typical number.

The present report deals with the variation of micronuclei counts of ten wild populations of the species collected in Hakodate, Hokkaidô. The micronuclei counts in these samples varied in a wide range from one to thirteen. From Table I, it is found that the majority of animals prossess three to five micronuclei. As compared with other populations, however, HM, HG and HA populations which were collected in foul water without any contamination by sea-water or hot-spa, exhibited a rather small amount of variation in the micronuclei counts and had also a low incidence of aberrant and polymicronucleate animals. On the other hand, the other populations, HK, HN, HT, HS, HD, HY and HU which were collected in the habitat considerably contaminated by sea-water or warm-water drainage from hot-spa, exhibited comparatively large range of variation in the micronuclei counts, and had a high in cidevant of aberrant and polymicronucleate animals.

From the above investigations, it seems that a certain relationship exists between the numerical variation of the micronuclei and the environmental factors. At present, it seems that differences in water temperature and salinity are most important factors in the variability of the counts.

The analysis is now in progress of the seasonal change of variability within populations.

Table 1.

Populations	Nos. of Individuals Observed	Aberrant Animals*	Number of Micronuclei													Mean value of Micronuclei	Coef. of Variation
			1	2	3	4	5	6	7	8	9	10	11	12	13		
			%	%	%	%	%	%	%	%	%	%	%	%	%		
HM	2049	0.2	1.4	5.2	7.3	76.7	4.2	2.1	2.0	0.5	0.2	0.01	—	—	—	3.959±0.939	0.236
HG	1908	0.1	1.5	10.8	12.3	67.2	3.1	2.8	2.1	0.1	—	—	—	—	—	3.773±0.993	0.263
HA	1250	0.2	2.0	9.1	11.7	69.3	6.3	1.2	0.2	—	—	—	—	—	—	3.733±0.835	0.223
HK	1761	1.6	9.3	16.6	14.1	19.8	14.7	9.6	7.5	4.3	1.8	0.5	0.3	0.1	—	4.231±1.897	0.448
HN	1372	1.7	7.6	14.5	16.3	18.6	13.7	12.8	10.9	3.1	0.5	0.2	0.1	—	—	4.285±1.785	0.416
HT	2381	2.0	7.7	11.3	18.1	15.0	15.6	12.2	7.9	5.1	2.7	1.8	0.3	0.1	0.2	4.515±2.256	0.499
HS	1150	0.4	6.8	16.3	15.6	19.3	16.5	9.8	8.3	4.9	1.2	0.4	0.2	0.2	0.1	4.243±2.045	0.481
HD	1895	3.4	8.6	14.8	14.5	14.3	13.8	10.5	7.9	5.7	3.6	2.0	0.4	0.3	0.2	4.502±2.419	0.537
HY	1892	1.3	8.4	23.2	14.7	30.1	12.6	9.5	0.2	0.1	0.03	—	—	—	—	3.467±1.453	0.419
HU	2109	1.6	2.8	8.1	24.3	11.0	14.1	26.6	9.8	0.6	0.8	0.1	0.1	0.04	0.1	4.570±1.764	0.385

* Aberrant animals involved are "ghost", "amicronucleate" and "amacronucleate" animals.

E. RADIATION GENETICS IN ANIMALS

74. *Two types of dose-rate dependence of radiation-induced mutation rate in spermatogonia and oögonia of the silkworm*[1]

(By Yataro TAZIMA, Sohei KONDO and Toshihiko SADO)

Studies on the dose-rate dependence of radiation induced mutation rate have been carried out with silkworm spermatogonia and oögonia by irradiating them with different dose-rates of X- and γ-rays.

Both sexes of a wild type strain, C108, were exposed to X-rays or Cs-137 γ-rays of high dose-rate or to Co-60 γ-rays of low dose-rate at a definite stage or during a definite period in early larval stages. The dose-rate ratios between acute and chronic irradiation varied from 2500:1 to 7600:1, with the same total doses of 950r or 1000r. For the estimation of mutation rates we applied the specific loci method. The results obtained are as follows.

a) A type of dose-rate dependence consistent with the findings of Russell, *i.e.*, lower mutagenic effectiveness of chronic than of acute irradiation, was found in the silkworm only in very early stages of spermatogonia and oögonia, when the primordial germ cells are prevailing in the testis (Type I).

b) A new type of dose rate dependence, *i.e.*, higher mutagenic effectiveness of chronic than acute irradiation was found in later stages of spermatogonial development (Type II).

For an explanation of these apparently contradictory findings, the following assumptions were made.

1) Primary mutation rates differ according to the stage of germ cells. They may be highest at the stage of primordial germ cells, lowest at the primary spermatogonial stage and intermediate at the secondary spermatogonial stage.

2) Repair of primary mutations as postulated by Russell for chronic irradiation may take place in the germ cells with a long mitotic cycle but is less frequent in those with a short mitotic cycle. Primary and secondary spermatogonia are supposed to have a much shorter cell cycle than the primordial germ cells. Hence, recovery is supposed to play an important role only in the primordial germ cells.

3) Among the three types of cells, the secondary spermatogonia, which supposedly correspond to type B spermatogonia of mouse, are extraordinary sensitive to radiation. They are selectively killed by intense dose rates,

1) This work was supported by Grant RF57178 from The Rockefeller Foundation.

while primordial germ cells and primary spermatogonia can survive under acute dose rate conditions such as were used in our experiments. Under chronic irradiation all three types of cells can survive and are transmitted to the next generation.

With these assumptions, both types of dose rate dependence could be explained without contradiction.

75. *Frequency of accumulated recessive embryonic lethals in a popular commercial race, C115, of the silkworm and their spontaneous mutation rate*[1)]

(By Yataro TAZIMA and Takao KOBAYASHI)

An estimate of the frequency of accumulated recessive embryonic lethals was made in a highly popular commercial race, C115, of the silkworm. The materials used were the original P_1 eggs of this race, which were produced for distribution purpose by the National Sericultural Experiment Station in 1959. 200 P_1 layings, selected at random, were raised separately in the spring of 1960. By sib-mating within the same P_1 separate batch,

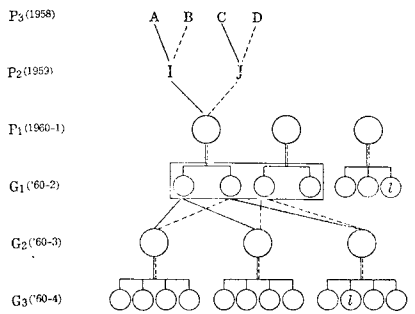


Fig. 1. Breeding system utilized for extraction of lethal free strains and estimation of spontaneous lethal mutation rate.

about 100 layings of G_1 generation were obtained for each batch. They were subjected to an embryonic lethal test, after treatment with hot hydrochloric acid to induce hatching. Within each P_1 line, the number of G_1 layings with about 25%, 43.8% or more dead embryos was scored. From the ratio of lethal segregating layings to total layings, the number of recessive lethals carried by the grandparents (P_2) was estimated. The

1) A part of the work carried out under the Contract No. 28 between I.A.E.A. and Dept. of Morphological Genetics.

results are shown in Table 1.

From the data the frequency of lethals that had been transmitted from P_3 to P_2 were calculated. Since the number of gametes produced by P_3 and transmitted to both parents (P_2) of each P_1 batch are four, the frequency of accumulated lethals (1_a) per gamete produced by P_3 is

$$1_a = 0.6011/4 = 0.15026$$

Table 1. Detection of accumlated recessive embryonic lethals in C115

Total tested	Number of P_1 lines				Total No. of lethals detected	Freq. of lethals per P_1 line
	Detected lethals per line					
	0	1	2	3		
188	97	75	16	2	113	0.6011

This figure may also be taken as representing the frequency of spontaneous lethals accumulated and maintained, in every generation, in a large population of this race.

Among 95 lethal free lines, a few lethal free strains were extracted. With one of those, an experiment was carried out to estimate the spontaneous lethal mutation rate.

By random mating of G_1 moths of this strain, G_2 layings were raised separately. By sib-mating within each G_2 batch, G_3 layings were obtained and they were tested for their embryonic lethality by the same procedure as described above.

Among 309 G_2 lines observed 10 were lethal yielding. Therefore the frequency of lethals per gametes transmitted to both parents of each G_2 batch is calculated as

$$\frac{10}{309 \times 4} = 0.008091$$

As all lethals accumutated down to P_3 generation had already been eliminated by sib-mating of P_1, and lethals newly arisen in G_1 and G_2 could not have been detected in the present test, the observed lethals must have occurred during P_2 and P_1.

The calculated mutation rate per gamete per generation is, therefore,

$$\mu = \frac{0.008091}{2} = 0.004046$$

This may be regarded as an estimate of the spontaneous mutation rate of recessive embryonic lethals in this race.

76. *Sensitivity and time of degeneration of spermatocytes irradiated at different stages of meiosis of the silkworm*[1]

(By Toshihiko SADO)

An extremely sensitive stage to the sterilizing effect of X-rays has been revealed in the silkworm early in the fifth instar of the male larva. Hypersensitivity of secondary spermatogonia (SADO 1959) cannot satisfactorily account for this, because at this stage most germ cells are already differentiated into spermatocytes.

Spermatocytes, especially during first meiotic prophase, represent a very long-lived stage in the silkworm. The majority of germ cells in the testis of the early fourth instar larva are spermatocytes at synaptic and/or pachytene stage, while in the early fifth instar most are in late meiotic prophase. To investigate the effect of X-rays on the spermatocytes at different developmental stages both early fourth and early fifth instar larvae were exposed to X-rays of 1000r and 2000r.

Most of the spermatocytes irradiated with 1000r at an early meiotic stage showed no degenerative figures at meiosis. Fertility tests showed that they developed into functional sperm. However, those exposed to 2000r showed structural abnormalities and degenerated during the spermiogenic stage.

The majority of spermatocytes irradiated at late meiotic prophase with 1000r were normal in appearance at metaphase and anaphase, but seemed to be damaged, for spermatids with necrotic nuclei were observed frequently 5 days after exposure. Still later in the pupal period eupyrene spermatozoa with structurally abnormal heads (nuclei) appeared in the testes. Thus spermatocytes in late meiotic prophase are more sensitive to irradiation than those in early meiotic prophase.

It is highly probable that the majority of the observed structural abnormalities occurred in cells that carried gross chromosomal disturbances. Actually chromosome aberrations have been observed in some of the irradiated testes. However, the detection of such aberrations is not easy in the silkworm, because the chromosomes are too small and numerous ($n=28$) for cytological analysis.

77. *Reduction in number of sperm after irradiation of male silkworms*[2]

(By Toshihiko SADO)

The foregoing experiment suggests that the number of sperm produced in the testes irradiated during the early fifth instar would be reduced.

1) This work was supported by Grant RF57178 from the Rockefeller Foundation.
2) This work was supported by the Grant-in-Aid from the Ministry of Education.

A quantitative histological study was carried out to confirm this prediction from counts of the numbers of eupyrene and apyrene sperm bundles in the irradiated and control testes. Throughout the pupal period, the number of bundles of apyrene spermatozoa was found to be greatly decreased in the irradiated testes. This, however, cannot account for the observed sterility of the treated males, because even in nonirradiated males apyrene spermatozoa are not considered to play a role in fertilization.

In irradiated testes, the number of eupyrene sperm bundles on the third day after pupation or later was about 50 per cent of that in the nonirradiated ones. Furthermore, about 30 per cent of the eupyrene sperm bundles in the treated individuals had structurally abnormal nuclei.

Though a considerable amount of sperm was contained in the testes of the irradiated male moths and most of them seemed to have been ejaculated into the bursa copulatrix of the female, only very few of them could reach the receptaculums eminis. This show that most of them are functionless.

In conclusion, the excessive sterility of males irradiated during the early fifth instar can be explained by the fact that cells in late meiotic prophase are highly sensitive to irradiation and either degenerate during spermiogenesis or form spermatozoa many of which are non-functional.

78. *Differences between times of death of the F_1 after irradiation of oögonia or mature oöcytes*[1)]

(By Yataro TAZIMA and Kimiharu ONIMARU)

Marked changes in radiation-induced mutation rates between the stages of gametogenesis have been known in the silkworm both for visible recessive mutations and dominant lethals. The induced mutation are recovered with relatively low frequency at spermatogonial and oögonial stages, while the maximum frequencies are observed at the spermatid stage in the male and in mature oöcytes in the female. In mature oöcytes the observed mutation rate is several times as high as that in the oögonia.

Moreover it was found by the specific locus method with egg color mutants that the induced rates rise linearly with increasing radiation doses in oögonia, while they increase exponentially with doses when mature oöcytes are irradiated. This suggests that most of the mutants induced in oögonia may be accompanied by gross chromosomal aberrations, and that the aberration bearing cells are eliminated afterwards during the long course of gametogenesis. On the contrary, most of the aberrations induced in mature oöcytes can not eliminated during the short time from irradiation to fertilization. It is, however, likely that most of the aberration

1) This work was supported by the Grant-in-Aid from the Ministry of Education.

bearing individuals die some time during early development. Earlier experiments had, in fact, shown that the F_1 from the irradiated spermatids and irradiated mature oöcytes shows lower viability than the F_1 from irradiated spermatogonia and oögonia. In order to confirm this, mortality was measured at various developmental stages of the F_1 of irradiated oögonia and irradiated mature oöcytes as in controls. Doses given were 1000r of Cs-137 γ-rays. The experiment was carried out in three replications.

Table 1. Mortality in F_1 of irradiated oögonia, irradiated mature oöcytes and non-irradiated control

Treatment	No. of zygotes observed	Per cent died during					
		Embryo	I-II Stadium	III-V Stadium	Spinning-Pupation	Pupation-Emergence	Total
1. Non-irrad. ♀ ×non-irrad. ♂	2064	1.1%	2.4%	2.3%	3.2%	2.4%	11.4%
2.* Irrad. oögonia ♀ ×non-irrad. ♂	1851	2.3	4.1	2.2	5.2	2.0	15.8
3.* Irrad. mature oöcytes ♀ ×non-irrad. ♂	2213	21.4	9.0	6.1	5.8	2.5	44.8

* Irradiation dose: 1000 r of Cs-137 γ-rays

The results were completely in accordance with our expectation: a considerable part of the F_1 individuals of irradiated mature oöcytes were found to be eliminated during embryonic and larval stages, especially in earlier stages.

79. *Recessive lethal mutations induced in mice by chronic irradiation during the whole development in three successive generations*

(By Tsutomu SUGAHARA, Kiyosi TUTIKAWA, Susumu MURAMATSU, and Yoshiko TAKEDA)

We studied by Haldane's method recessive lethal mutations in mice induced by chronic irradiation of 80 day duration, starting immediately after fertilization and continued through three successive generations. The result obtained seems to indicate that the genetic effect of radiation is cumulative, even at a very low dose rate.

Multiple recessive mice were irradiated chronically with gamma-rays of total dose of about 103r for three successive generations. In each generation, the mice were raised until maturity in the radiation field, then

mated by placing a pair in a cage at the end of the irradiation. To search for recessive lethal mutations, the offspring from irradiated ancestries were mated with mice of a wild-type strain, and the segregation for the recessive marker genes in the F_2 progenies was examined for each F_1 pair. The criteria used for scanning linked recessive lethals were the same as those used by CARTER (1959) which were computed for the probability of false clue $P \leqslant 0.01$.

The "wavy" stock and the CBA strain were used as the multiple recessive and the wild-type strain, respectively. The "wavy" stock which is homozygous for six loci, *a*, *b*, c^{ch}, *d*, *p* and *se*, was built up in 1957 by K. TUTIKAWA. However, only four out of six loci were used as markers in the present experiment, because *d*—*se* and c^{ch}—*p* are closely linked. The mice were chronically exposed to ^{60}Co-gamma-rays for 80 days per each generation with the dose rate of 0.43r/day. The results obtained are summarized in Table 1.

Table 1.

	Irradiated	Non-irradiated
Number of P_1 mice analysed	74	46
Number of suspected recessive lethals	2	0
Total length of map scanned	5112.688 cM	4553.172 cM
Mutation rate		
Observed	6.8×10^{-3}/r/Total autosomes	
Expected	$3.3\text{-}6.6\times10^{-3}$/r/Total autosomes	
Pilot experiment (1959)	4.3×10^{-3}/r/Total autosomes	

The total length of linkage map for a male mouse was estimated from the cytological data to be about 1824 centimorgans (SLIZYNSKI, 1955). Hence, the total map length scanned in the irradiation series, *i.e.* 5113 centimorgans, and that in the non-irradiated series, *i.e.* 4553 centimorgans, were equivalent to 2.8 and 2.5 autosome sets, respectively, provided that the length of the linkage map of the female mouse is the same as that of the male. Two suspected linked lethals were found in the P_1 mice of the irradiated series, and no lethals were found in those of the non-irradiated series.

The expected mutation rate given in Table 1 is based on the value estimated by HALDANE (1956), and the difference in mutation frequencies between males and females is neglected. Since, in the present experiment, the gametes irradiated at various stages of germ cells must have contributed to fertilization, the expected mutation rate has to make allowance

for doubling Haldane's estimated value, taking account of the higher mutation frequency in the postgonial cells.

The observed rate is in good agreement with the expected value.

80. *Shift of secondary sex ratio among the progeny of chronically irradiated male mice (F_1 generation)*

(By Kiyosi TUTIKAWA)

Reports that the secondary sex ratio in man is modified in the progeny of irradiated fathers led us to the examination of sex distribution in the progeny obtained from the above experiment. According to Schull and Neel, who summarized the available human evidences, irradiation of the father leads to a preponderance of males in the progeny.

CSMF_1 mice used in the present experiment were produced by mating C57BL/6 females with SM/Rr males. The males were chronically irradiated with ^{60}Co-gamma-rays of total doses of 100r (dose rate 2r/day), and 400r (does rate 8r/day) for 50 days. In order to explore the possibility of the influence of age, two sets of experiments were carried out in which two groups of 30- and 60-day-old CSMF_1 males were irradiated and two months later, after completion of irradiation, were mated with non-irradiated females of the same F_1.

In brief, for each dose and age groups, the sex ratio (fraction of males) at birth was as follows:

(1)	0 r		1103 mice, sex ratio, 0.5512
(2)	100 r	30-day-old	519 mice, sex ratio, 0.5241
		60-day-old	484 mice, sex ratio, 0.5351
(3)	400 r	30-day-old	418 mice, sex ratio, 0.4928
		60-day-old	501 mice, sex ratio, 0.4830

The two age groups given the same doses, did not differ significantly from each other in the sex ratio. However, the low sex ratio of the exposed groups did significantly differ from that of the controls. Statistical tests (χ^2) showed significant heterogenity among the three dose groups ($0.01<P<0.025$).

Recently, KOHN (1960), showed for the CAF$_1$ males used in his experiment, that X-rays have no effect on the sex ratio as tested by linear regression for absorbed doses in the range 0–720 rads.

In our experiment, although gamma-rays had no effect on the mean litter size, birth weight and skeletal development of new born animals, the sex ratio decreased linearly with the doses. An attempt to explain

the result was made under the assumptions that (1) difference from 1:1 sex ratio of the control is probably due to the difference in competitive ability between X- and Y-carrying sperm, (2) the frequency of non-disjunction in gametogenesis could have been increased by irradiation. In this case, the frequencies were calculated to be 4% for 100r, and 12% for 400r respectively. However, some doubt remains as to whether such high rates can be induced in gametogenesis by irradiation.

81. *A radiation genetical consideration concerning the genetic structure of natural populations in Drosophila melanogaster*

(By Terumi MUKAI)

There are two main mutually contradictory hypotheses concerning the genetic structure of natural populations, (DOBZHANSKY, 1955), *i.e.*, the classical hypothesis based upon the superior viability of homozygotes over heterozygetes and the balance hypothesis founded on the superiority of heterozygotes over either homozygote.

If one accepts the classical hypothesis, he would not expect to find any real difference between autosomes and sex chromosomes in polygenes governing viability, that is, most of the loci would be occupied by dominant adaptive genes in homozygous condition, and an inbreeding depression is not expected.

If one accepts the balance hypothesis he can expect the accumulation of variability in heterozygous condition in autosomes while not in sex chromosomes because of the hemizygosity in males. Therefore, for the autosomes inbreeding depression is indicated but none for the sex chromosomes.

A study on the heterozygous effects of irradiation-induced mutations occurring in artificially extracted isogenic lines would bring a considerable amount of information as to whether the classical or the balance hypothesis is well-grounded. It is well known that most mutations are deleterious in homozygous condition (BRIDGES and BREHME, 1944). If this is the rule, it is impossible on the basis of the classical hypothesis to increase the viability of heterozygotes by the induction of mutations either in autosomes or in sex chromosomes. However, if one adheres to the balance hypothesis, the results should be slightly different. In the sex chromosomes, the situation would be essentially the same as indicated by the classical hypothesis. (Indeed, DOBZHANSKY, KRIMBAS and KRIMBAS (1960) have reported a tendency for the supervital chromosomes in *D. pseudoobscura* not to show heterosis when they are combined with chromosomes having loboratory-origin dominant genes.) But, contrary to expectation under

the classical hypothesis, the heterozygotes due to induced mutations in autosomes under the balance hypothesis would show heterosis in viability in the genetic backgrounds where the subvital genes are accumulated.

The statistical analysis of unpublished experimental data (BURDICK, MUKAI and KRAWINKEL) has brought the following conclusion: There are two kinds of loci in *D. melanogaster*, and the balance hypothesis is applicable to one kind, the proportion of which is about two thirds. The classical hypothesis holds true for the other one third of the loci. Thus, it is impossible to make an alternative judgement but the frequency of loci where the balance hypothesis is valid is higher than that where the classical hyphothesis holds true.

82. *Radiation-induced polygene mutation rate*

(By Terumi MUKAI)

When the characteristics of the distributions of treated and control individuals are employed, the proportion of heterotic and non-heterotic radiation-induced polygene mutations in heterozygous condition can be estimated under the following assumptions:

(1) There is no epistasis. This is too bold, but not dangerous if the number of mutations exceeds one in an individual in only few cases.
(2) Each mutation has the same effect either in the positive or the negative direction.
(3) The positive and negative mutations distribute independently as Poisson distributions.

In order to set up a mathematical model, the following paramaters are defined: a=heterozygous effect of each mutation. $p(q)$=mean number of negative (positive) mutations in one set of chromosomes.

From the differences of means, variances and the third moment about the mean between the irradiated and the control group, the following simultaneous equations can be obtained:

$$a(q-p)=A$$
$$a^2(p+q)=B$$
$$a^3(q-p)=C$$

where A, B and C stand for the differences of statistics between the irradiated and the control group, respectively.

The heterozygous effect of radiation-induced mutations (acute 100r) on the viability of the second and third chromosomes in *D. melanogaster* was estimated, based upon the sample sizes, n=296 for the treated group, n=279 for the control (BURDICK, MUKAI and KRAWINKEL, unpublished).

On the basis of the estimates A=0.025000, B=0.009973 and C=0.000660,

a, p and q were estimated to be $a=0.16$, $p=0.11$ and $q=0.27$. If we assume that the number of loci is 3000, we can estimate polygene mutation rate to be 1.26×10^{-6}/locus/r. This is extremely high compared with so called major-gene mutation rates.

83. *Frequency of chromatid breaks at various doses of X- and γ-rays*[1]

(By Tosihide H. YOSIDA, Teiichiro TAKAHASHI, Yoshikazu MATANO and Kazuhiko UTSUMI)

YOSIDA and TAKAHASHI (1959) demonstrated that 30 minutes after X-irradiation chromatid or isochromatid breaks considered to be one hit aberrations occurred at the highest frequency in all samples obtained immediately after, 30 minutes and 8 to 72 hours after 250r irradiation. In order to investigate the relation between the frequency of chromatid breaks and dose, 250, 500 and 750r of X-rays and 1,000r of γ-rays

Table 1. The number of chromatid breaks per cell at various doses of X- and γ-rays

Source	X-rays			γ-ray
Dose (r)	250	500	750	1000
No. of breaks per cell	2.6	5.3	6.14	8.5

(Ce^{137}) were applied to tumor bearing mice. γ-rays were used for short exposures with 1000r. As shown in the table the frequency of chromatid breaks by X- and γ-rays was increasing in proportion to the dose, irrespective of the radiation source.

84. *Effect of DNP (2,4-dinitrophenol) on the frequency of X-ray induced chromatid breakes in Ehrlich tumor cells*[2]

(By Yoshikazu MATANO)

In the present study, the effect of DNP on the frequency of X-ray induced chromosome aberrations was examined, using hyperdiploid Ehrlich carcinoma cells (chrom. no. 44) of mice.

Doses of 100r, 250r and 500r were given to tumor-bearing mice, which were held separately in plastic cylindrical vessels. 0.2 ml of 10^{-2} M DNP

1) This work was supported by Grant RF57178 from The Rockefeller Foundation.
2) This work was supported by Grant RF57178 from The Rockefeller Foundation.

was injected to intraperitoneal cavities 30 minutes before X-irradiation. Samples of ascites fluid were taken 30 minutes after irradiation. As control the same dose of DNP was injected to non-irradiated mice and samples were taken one hour after the injection. For the observation of metaphasic chromosomes squash method with water pretreatment (MAKINO, 1959) was adopted, and the number of chromatid and isochromatid breaks was scored. The result of the experiment is given in Table 1. With the exception of 100r-irradiation, the breaks were significantly increased by pretreatment with DNP. The result of this experiment indicates that DNP inhibits the restitution of the breaks.

Table 1. Frequency of X-ray induced chromatid breaks by pretreatment with DNP for 30 minutes. Each of samples was taken 30 minutes after the exposure

Dosage	Treatment with DNP	Total cells analysed	Isochrom. breaks	Chromatid breaks	Total breaks	Breaks per cell
Control	Non-treated	198	2	40	42	0.2±0.05
	Treated	55	1	36	37	0.7±0.15
100 r	Non-treated	26	1	68	69	2.7±0.66
	Treated	33	0	80	80	2.4±0.63
250 r	Non-treated	50	2	130	132	2.6±0.48
	Treated	56	1	568	569	10.2±1.43
500 r	Non-treated	23	0	121	121	5.3±1.28
	Treated	32	1	290	291	9.1±2.21

Further experiments were made to investigate the time relation between breaks and restitutions. In these experiments only 250r irradiation was employed. The results of the experiments are shown graphically in Figure 1A and 2B. In the irradiated experiment, two kinds of treatments were given, namely DNP pretreatment 30 minutes before irradiation and DNP post-treatment immediately after irradiation; no DNP (only X-irradiation) was applied in the control. Samples were taken immediately, 10 minutes and 30 minutes after irradiation. In the experiments with DNP pretreatment, a significant increase in the frequency of breaks was observed immediately after irradiation. Further increases of breaks were observed in the following 10 and 30 minutes. However, no visible change was found in the frequency of breaks between irradiated only and the post-treated mice with DNP after 10 and 30 minutes. The results of DNP

post-treatment indicate that breaks produced by X-rays are restituted in a short time. However, when DNP was applied as pretreatment, restitutions were inhibited and some of the breaks remained open for at least 30 minutes. The remarkable increase in breaks observed 30 minutes after irradiation indicates that a certain stage, 30 minutes before metaphase, is more sensitive to X-ray irradiation than other stages preceding at least by 10 minutes the metaphase. On the other hand, the same non-irradiated material was used for confirmation of the effect of DNP pre-treatment. The three kinds of treatments were applied in non-irradiated material. The frequency of breaks is shown Figure 1B. From the comparison of the results with irradiated and non-irradiated material it follows that the striking number of breaks found after 30 minutes in DNP pretreated material is almost entirely due to the inhibition of restitution of breaks produced by X-rays.

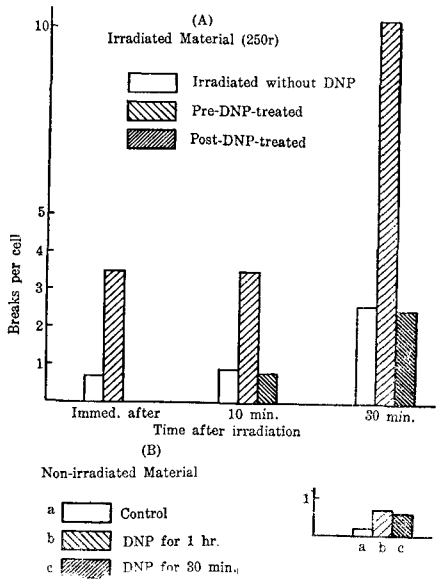


Fig. 1A. Relation of chromatid breaked to time after X-irradiation, with exposures made after and before DNP-injection (0.2 ml of 10^{-2} M). 1B. Non-irradiated material treated in similar way.

85. *Effect of sodium azide (NaN_3) on X-ray induced chromatid breaks*[1)]

(By Kazuhiko UTSUMI)

It is known that sodium azide (NaN_3) is a respiration inhibitor. As stated above, DNP, another respiration hinibitor, caused a remarkable increase in the number of chromatid breaks in Ehrlich tumor cells when it was injected to tumor bearing animals before X-irradiation.

In order to compare the effect of NaN_3 with that of DNP, 0.2 ml of 10^{-2} M and 2×10^{-2} M NaN_3 were injected into the intraperitoneal cavity of tumor bearing mice 30 minutes before 250r X-irradiation. The frequency of breaks per cell was in the control (250r irradiation only) 0.16% and

1) This work was supported by Grant RF57178 from The Rockefeller Foundation.

2.86% immediately and 30 minutes after irradiation, respectively, and in batches pretreated with NaN_3 it was similar or lower than in the control batch. It can be concluded that the effect of NaN_3 on X-ray induced chromatid breaks is markedly different from that of DNP.

A possible mechanism of ineffectiveness of NaN_3 on chromatid breaks is discussed in this annual report by Moriwaki, one of our collaborators, from the view point of ATP content.

86. *Changes of ATP content in Ehrlich tumor cells after treatment with DNP and NaN_3*[1]

(By KAZUO MORIWAKI)

Measurements of ATP content in cells after treatment with DNP and at the same time observations of chromosomal aberrations in the same material have as yet not been carried out. In order to estimate the ATP level, the 10′P content of tumor cells was measured following the treatments with DNP and X-rays.

Before X-irradiation no remarkable change was found in 10′P content in the tumor cells after treatment with 10^{-2}M DNP *in vivo*. But following irradiation an increase in X-ray induced chromosomal aberrations could be observed after the same treatment with DNP. In order to estimate accurately changes in ATP content and in ^{32}P incorporation after DNP treatment, the gradient chromatograph method was employed.

The results indicate an appreciable inhibition of cell metabolism by DNP, that is, a decrease in ATP content, an increase in ADP and inorganic phosphates (Pi) and an increase in ^{32}P in corporation into ADP and Pi. No significant changes were found in ^{32}P incorporation into total nucleic acid and protein.

The reducing effect of DNP on ATP content can be markedly increased by X-ray irradiation. To avoid individual variation in tumor bearing mice, one ml from a pool of ascites tumor cells of four mice was used in this experiment. Probably because of the incubation of tumor cells without addition of nutrients and oxygen, a considerable decrease in 10′P content was found in the control tube, and more markedly in a tube treated with DNP. Furthermore, there was beyond doubt an additive effect of X-rays on the 10′P decrease by DNP. A similar effect of X-rays also appeared in a tube treated with NaN_3, although the decline of 10′P level occurred to a smaller degree.

In view of the above results it is conceivable that in animal cells an

1) This work was supported by Grant RF 57178 from the Rockefeller Foundation.

oxidative metabolism with its concurrent formation of ATP is necessary for rejoining broken ends as previously stated by WOLFF and LUIPPOLD (1955).

87. *Cytological observations of ascites tumors treated with chronic γ-radiation*[1]

(By Tosihide H. YOSIDA and Teiichiro TAKAHASHI)

Acquired radioresistance of cancer cells has been discussed clinically and experimentally by several authors. Some of them reported a development of radio resistance, whereas others noticed no change in radiosensitivity following irradiations.

The present study was aimed at investigating whether ascites tumor cells acquire or not radioresistance at chronic irradiation. γ-rays were chronically given to the whole body of mice and rats bearing Ehrlich ascites carcinoma and YOSHIDA sarcoma. A hyperdiploid strain of the Ehrlich ascites carcinoma was transplanted into the peritoneal cavity of mice which were divided into four experimental groups. Each group consisted of 3 or 4 animals. One group as a control experiment was placed in the usual mouse room and the other groups were chronically irradiated with 50r, 100r and 150r γ-rays per day (22 hours) for 70 days (10 transplant generations). The total number of mitotic cells gradually decreased; the number of metaphasic cells was also reduced.

The frequency of anaphasic aberrations, however, increased in the course of γ-irradiations. In order to examine wheter the tumor cells which were pretreated with γ-rays have acquired radioresistance, acute irradiation with 100, 300 and 500r X-rays was applied to the total body of tumor bearing mice. The frequency of cells containing micronuclei, binuclear and polyploid cells, and the mitotic index in pretreted and non-treated tumor cells were observed from immediately after irradiation to 96 hours later. The mitotic index in the non-treated tumor line decreased markedly after 100 to 500r acute X-irradiation, whereas that in pretreated tumor lines decreased remarkably already after 100r and 300r X-irradiation, but after 500r X-irradiation it was not reduced as strikingly as in the control. The frequency of anaphasic aberrations in the non-treated tumor line was after acute X-irradiation significantly higher than in the pretreated tumor lines. It is interesting that the frequency of tetraploid or triploid tumor cells in the diploid Ehrlich strain decreased by continuous γ-radiation. It is assumed that stem cells have much stronger resistance to γ-radiation than other tumor cells.

1) This work was supported by Grant RF57178 from The Rockefeller Foundation.

YOSHIDA rat sarcoma was chronically irradiated with 2r and 50r per day (22 hours) at a γ-field from ^{60}Co source for 160 days. The chromosome constitution of tumor cells after this continuous radiation was observed. 52 per cent of tumor cells were characterized by the tumor stem karyotype. Although there were observed various abnormal cells, the stem line karyotype was not changed by chronic radiation.

Based on the above investigation, it can be concluded that the karyotype of tumor stem line does not change by chronic radiation, and that these cells may have stronger resistance to radiation than other non-stemline cells. Tumor lines which were chronically treated by γ-rays showed slightly stronger resistance to acute X-radiation in respect to the occurrence of abnormal mitosis. Such radiation resistance seems to be due to selection of tumor stem cells which have stronger cell-activity than other non-stemline cells.

88. *A theory for the frequency distribution of cluster mutants*[1]

(By Sohei KONDO)

Many mutants are often found in the offspring of an animal or plant when irradiation was applied at the early embryonic stage. It is noteworthy that the mutation rate estimated from the observed number of mutants to a high degree depends on how they are treated.

A general theory for the frequency distribution of the number of mutants per irradiated individual has been developed on the basis of the following assumptions: 1) Presence of two types of germ cells with different multiplication factors in each individual when exposed to radiation, 2) Poisson distribution of the initial mutative events, 3) Cell multiplication without fluctuation, and 4) Random sampling of gametes giving rise to the offspring. The frequency distribution function of mutants depends on four parameters which are to be adjusted to fit the experimental data.

The theory has been applied to TAZIMA's (see TAZIMA's paper in this report) experimental data on silkworm irradiated at their gonial stage. Comparison of the theoretical distributions with the experimental data shows a very good agreement.

From the theory we have also derived a method for estimating the number of the two types of the germ cells; they are of reasonable magnitudes. As to the estimation of mutation rate, we have reached the conclusion that each mutant of a mutant cluster should be counted as if it were a single mutant observed per insect.

(Published in Jap. Jour. Genet. 36: 6-17, 1961)

1) This work was supported by Grant RF57178 from The Rockefeller Foundation.

89. *A biophysical consideration on radiation mutagenesis*[1)]

(By Sohei KONDO)

The sequence of events which leads from the absorption of radiation to the eventual recovery of mutants, is presented in perspective. The radiation-initiated mutagenesis has been classified into four steps: (I) Induction of Premutation. (II) Modification of Premutation. (III) Fixation of Mutation and (IV) Detection of Mutation. A model of the mechanism of mutational events in microorganisms, modifiable by post-irradiation treatments, is proposed and applied to the interpretation of the differences in mutation rate in various stages of germ cells of the silkworm.

(Published in Progress Theor. Phys. Suppl. No. **17**: 129–142, 1961)

F. RADIATION GENETICS IN PLANTS

90. *Radiation effects of beta- and gamma-rays in Triticum monococcnm*[1)]

(By Seiji MATSUMURA)

Dormant seeds of *Triticum monococcum flavescens* were soaked in water or ^{32}P and ^{131}I solutions for 1 and 2 days just before sowing. Radioactive solutions contained 0.2–1.2 mc/gr of ^{32}P and 0.8–1.6 mc/gr of ^{131}I. To compare the beta-radiation effects of these solutions with those of gamma-rays, the seeds were subjected to chronic gamma-radiation from a ^{60}Co source at 2, 4 and 6 kr during water-soaking. The germination rate, seedling heigh 4 weeks after sowing, survival rate, mature plant height, seed fertility and chromosome aberrations in PMC's of the treated plants, and the chlorophyll mutations in the X_2 were compared. As to the first 5 characters, the effects of beta-radiation from 0.2 mc/gr ^{32}P solution for 2 days corresponded roughly to those of 4 kr gamma-radiation for 2 days, and were slightly higher than those of 1 day beta-radiation from 0.4 mc/gr ^{32}P solution and of 1 day gamma-radiation at 4 kr. Also the effects of 1 day beta-radiation from 0.8 mc/gr ^{32}P solution coincided roughly with those of 6 kr gamma-radiation for 1 day. Further one day beta-radiation from 1.6 mc/gr ^{131}I solution for 1 day was considerably less effective than 2 kr gamma-radiation for 1 day and showed similar effects as 1 day beta-radiation from 0.2 mc/gr ^{32}P solution.

In general, the effects of gamma-radiations on chromosome aberrations

1) This work was supported by Grant RF57178 from The Rockefeller Foundation.

and gene mutations were unexpectedly small, compared with those of corresponding beta-radiations from ^{32}P and ^{131}I solutions.

If we assume that the effects of beta-radiation are confined to the embryo, we find by calculation that the 0.4 mc/gr ^{32}P solution for 1 day or the 0.2 mc/gr ^{32}P for 2 days equals 5 or 4.8 krad, respectively. This, too, will account for the obtained data.

91. *Segregation ratio and viability of several chlorophyll mutants in einkorn wheat*

(By Tarô FUJII)

The segregation ratio often showed a wide range of variation in the X_2-generation. The mutant homozygotes appeared very often in too small numbers, for instance 14:2 in *albina*-5002, 12:2 in *albina*-5005 and 19:4 in *xantha*-5041. Occurrence of more than 25 per cent of recessive homozygotes, as 1:5 in *chlorina*-5040, was very rare. But the usual 3:1 ratio could be found in the successive generations of 35 of 45 chlorophyll mutant strains. Among the remaining 10 strains, such as *albina*-5008, -5012, *striata*-5057, *basi-viridis*-5059, the segregation did not fit the 3:1 ratio. Low germination ability of the mutant homozygotes in some strains (*striata*-5057, *basi-viridis*-5059) is partly responsible for their deficient numbers. In all investigated cases the mutation from dominant to recessive concerned single genes. But in some strains, such as *basi-viridis*-5077, *virido-albina*-5072, *virido-xantha*-5100 etc., *albina* and *striata* plants were segregated from the heterozygotes. A peculiar phenomenon was observed in *virido-xantha*-5100, namely the occurrence of 76 *virido-xantha*s, 9 *albina*s and 6 *striata*s besides 59 normal plants. These strains will be further investigated.

Viability was examined in 65 strains of several chlorophyll mutants, such as *albina*, *chlorina*, *basi-viridis*, etc. Ability recover the chlorophyll content was mostly observed in bi-colored mutants, to such as *basi-viridis* and *virido-albina* and did not occur in uniformly colored ones, such as *chlorina*, *xantha* and *albina*. Neither could the *striata* mutant recover the chlorophyll content. The recovery occurred only when they were grown in the greenhouse or in the phytotron and was restricted in the field to the warm season. In most of the mutants, except *chlorina* and *striata*, occurred a more pronounced dilution of green color in the field during the winter months than at seedling stage and they all died out. But the time rate of chlorophyll content recovery was different by the strain. Also

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

differences within the same mutant type were observed, for instance in the recovery speed and viability. May be different steps in the genetic make-up of chlorophyll production are blocked in the various strains.

92. *Preliminary experiments on the relation between dose rate and radiation effect in einkorn wheat*[1)]

(By Tarô FUJII)

10 and 20 kr irradiations with three different dose rates were applied to einkorn wheat seeds. The highest dose rate was 1,000 r/min of X-rays and the lowest was 0.75 r/min of 10 kr and 1.5 r/min of 20 kr gamma-radiation. 33 r/min irradiations at 10 and 20 kr were used with X- as well as gamma-rays for the check of RBE differences. Then seedling height, fertility and chlorophyll mutation rate in the X_2-generation were examined (Table 1).

Table 1. Effect of radiations with different dose rates

Dosage (kr)	Dose rate (r/min)	Seedling height (cm)	Fertility in X_1 (%)	Chlorophyll mutations in X_2 (%)
0	—	11.03	66.54	0
10	1,000	8.52	45.89	8.51
	33 (X-ray)	7.20	44.02	5.96
	33 (γ-ray)	8.64	43.94	5.42
	0.75	9.03	58.31	4.14
20	1,000	1.68	died	—
	33 (X-ray)	1.63	13.07	0
	33 (γ-ray)	3.72	30.76	8.19
	1.5	2.97	31.81	4.55

In all irradiated lots the seedlings were shorter and the fertility on the plants was reduced in comparison with the normal lot, this effect being milder in the batch of lowest dose rate than in the other three irradiated lots. 20 kr lot with the highest dose rate was the most severely damaged and all plants died after germination. The differences were not clear between intermediate and highest dose rates, especially at 10 kr.

The higher the dose rate at 10 kr, the higher was the chlorophyll mu-

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

tation rate in the X_2-generation. This tendency was also observed in 20 kr gamma-irradiations. That no mutation was found at the intermediate X-ray dose rate will be due to the small number of X_2 head progenies. Similar mutation rates were obtained at 10 kr from the intermediate dose rate in X- and gamma-rays. RBE of X- and gamma-rays was about the same as for mutation rates.

93. *Relation between radiation effects and dose rates in rice*

(By Tomoo MABUCHI, Tarô FUJII and Seiji MATSUMURA)

In order to investigate the relation of radiation effects to dose rates, dry seeds of upland rice were irradiated by gamma-rays from a ^{60}Co or ^{137}Cs source at the doses of 5, 10, 20 and 30 kr and three different dose rates of 10 r/hr, 300 r/hr and 10,000 r/hr. Only the 10 r/hr irradiation at 30 kr was not applied. These chronic, moderate and acute irradiations were terminated almost simultaneously before sowing. The germination rate, seedling height 20 days after sowing, seed fertility and contents of free amino acids and reduced sugar were investigated. At 20 and 30 kr the germination rate was slightly lower, especially at chronic 20 kr and acute 30 kr irradiations, than in the untreated lot and those with 5 and 10 kr irradiations. Unexpectedly, chronic irradiation, which was applied from the start, was most effective in inhibiting the growth of seedlings and in decreasing the fertility, especially at 20 kr. Thus an intensification of radiation damage due to storage was found.

Contents of free amino acids and reduced sugar were examined in 5 grams of fresh leaves 20 days after sowing. There was no marked difference between the content of the first and the second leaf. Neither the contents were noticeably influenced by the dosage. But at the same dosage chronic irradiations showed higher contents of amino acids than the acute ones, while the contents of reduced sugar were not clearly different by the dose rates. The relation between growth of seedlings and dose rates was reversed to that between amino acid contents and dose rates. From these findings it is suggested that the protein synthesis from amino acids is depressed at low dose rates, while high dose rates inhibit the amino acid metabolism from carbohydrates through organic acids, *i. e.* the preceding steps of protein synthesis.

94. *Effects of radiation on the susceptibility of wheat and rice seedlings to leaf rust*

(By Keizô KATSUYA)

In *Triticum Spelta*, *T. Timopheevi*, *T. vulgare* and *Oryza sativa* a batch of seedlings with 2 leaves was irradiated by X-rays and gamma-rays at 10, 30, 50 and 70 kr. The seedlings were inoculated 1 and 7 days after irradiation by spraying with an aqueous uredospore suspension (*Puccinia triticina* 21B) and the effects of radiation on the susceptibility to leaf rust were observed. In the remaining seedlings of the same age the first and second leaves were cut off and the amounts of amino-nitrogen and reduced sugar were measured.

Effects of irradiation on leaf characters, namely stomata size, internal structure and viability, and sporulation capacity on irradiated leaves were examined. Rust susceptibility of irradiated plants (*T. Spelta* and *T. vulgare*) was increased, as compared with the normals. In *T. Timopheevi* that was inoculated 7 days after irradiation, the third leaf was susceptible, while the first and second leaves of the same plants were mostly resistant. Rust susceptibility of irradiated rice plants remained unchanged.

In *T. Timopheevi* seedlings, the amount of amino-nitrogen increased in the seedlings 1 day after irradiation, as compared with that of the normals, while it decreased 7 days after irradiation. The amount of reduced sugar increased in seedlings 1 and 7 days after irradiation in comparison with the normals. Stomata size and internal structure of irradiated plants (*T. Spelta* and *T. vulgare*) did not change noticeably, as compared with the normals. Effects of irradiation on the viability of leaves were tested; when the growing point suffered radiation damage, the already developed leaves remained longer fresh than those of the normals. The uredosori of leaf rust on irradiated *T. vulgare* were larger than on normal plants. Relationships between rust susceptibility and radiation await further experiments.

95. *Calculation of absorbed dose delivered to wheat seeds soaked in* ^{32}P *and* ^{131}I *aqueous solutions*[1)]

(By S. KONDO and H. ISHIWA)

We have already confirmed that the coats of wheat seeds soaked in ^{32}P or ^{131}I aqueous solutions prevent strongly radioactive isotopes from penetrating into the interior of the seeds at least for one to two days[1)].

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

Therefore the dosimetry of seeds soaked for one or two days can be made with a good accuracy by taking account of the contribution of the isotopes assumed to be homogeneously distributed outside the seeds. Roughly speaking, the seeds of *Triticum monococcum flavescens* soaked in water for 24 hrs have an ellipsoidal shape with the three axes 2.8, 3.8, 7.9 mm long. In this report, however, we will calculate the average embryo dose and the average seed dose based on simplified models.

[1] *Average Embryo Dose*: Assume that the dose to the embryo of thickness h (g/cm^2) can be approximated by that to a surface layer of thickness h of an infinite plane slab made of a tissue-like material with infinite thickness whose plane surface is in contact with an infinite aqueous solution with homogeneously distributed β-emitting isotopes. Then, integrating Loevinger's empirical formula[2], the average dose $\bar{D}(\bar{h})$ is given by

$$\left.\begin{aligned}\bar{D}(\bar{h}) &= \frac{1}{2} D_\beta \frac{\alpha}{h}\left\{ c^2\left[3h - \frac{c}{\nu}(e - e^{1-(\nu h/c)}) - \frac{5}{4}\frac{\nu h^2}{c} - \frac{1}{2}\frac{\nu h^2}{c}\ln\frac{c}{\nu h}\right] \right.\\ &\quad \left. + \frac{1}{\nu}(e - e^{1-\nu h})\right\}; \qquad 0 < h \leq \frac{c}{\nu}\\ &= \frac{1}{2} D_\beta \frac{\alpha}{h}\left\{ \frac{c^3}{\nu}\left(\frac{11}{4} - e\right) + \frac{1}{\nu}(e - e^{1-\nu h})\right\}; \qquad \frac{c}{\nu} < h\end{aligned}\right\} \quad (1)$$

where ν (cm^2/g) is the apparent absorption coefficient of the β rays, α and c are the characteristic constants of the β-emitting isotopes and the D_β is the absorbed dose in the interior of the isotope solution. The D_β is given by

$$D_\beta = 51.1 \bar{E}_\beta a_0 \left(\frac{1 - e^{-\lambda t}}{\lambda}\right) \text{rad} \quad (2)$$

where $\bar{E}_\beta$ (Mev) is the average β-ray energy, λ (day^{-1}) the disintegration constant, a_0 (μc/g) the initial concentration of the β-active isotopes in the solution and t (days) the soaking time.

Since, for ^{32}P, $\alpha = 1/3$, $c = 1$, $\nu = 9.2$ cm^2/g, $\bar{E}_\beta = 0.694$ Mev and $\lambda = 0.693/14.3$ day^{-1}; and for ^{131}I, $\alpha = 0.26$, $\nu = 40$ cm^2/g, $\bar{E}_\beta = 0.187$ Mev and $\lambda = 0.693/8.0$ day^{-1}, from equations (1) and (2) we have for $h = 0.063$ g/cm^2, the average thickness of the embryo soaked for one day:

$$^{32}\text{P}: \quad \overline{D_{\text{embryo}}} = 0.35_5 D_\beta = 13.1 \text{ kr/day/mc/ml} = 25.6 \text{ kr/2 days/mc/ml} \quad (3)$$

$$^{131}\text{I}: \quad \overline{D_{\text{embryo}}} = 0.14_3 D_\beta = 1.3_9 \text{ kr/day/mc/ml} = 2.6_6 \text{ kr/2 days/mc/ml} \quad (4)$$

where we have used the value of 0.945 rad/r for Co-60 γ rays calculated for the elemental composition[4] of the seeds.

[2] *Average Seed Dose*: Assume the average dose to the whole seed mass can be approximated by that to a sphere with radius b. Then, using the published numerical calculations[2], we can easily calculate the average seed dose $\overline{D_{sph}(b)}$. If we assume $b=0.22$ (g/cm^2), we have

$$^{32}P: \quad \overline{D_{seed}}=0.54D_\beta=19.2 \text{ kr/day/mc/ml}=37.5 \text{ kr/2 days/mc/ml} \qquad (5)$$

$$^{131}I: \quad \overline{D_{seed}}=0.14D_\beta=1.36 \text{ kr/day/mc/ml}=2.60 \text{ kr/2 days/mc/ml} \qquad (6)$$

[3] *Comparison with Experimental Data*: Comparing with MATSUMURA's[3] data for soaking the seeds in ^{32}P and ^{131}I aqueous solutions for two days and two day exposure of soaked seeds to ^{60}Co γ rays, we obtain the following table.

Considering the partial shielding of β rays by the proximity of seeds

Table 1. Comparison of the data of γ-irradiated ^{32}P or ^{131}I β-rayed experiments with the theoretical estimations

Isotope	mc/ml	Experimental equivalent roentgen (kr)		Calculated equivalent roentgens (kr)	
		Seedling heights	Fertility	Embryo dose	Seed dose
^{32}P	0.15	2.0	3.7	3.9	5.6
^{32}P	0.30	4.8	4.4	7.8	11.3
^{131}I	0.6	1.6	1.9	1.6 (1.9)*	1.6 (1.8)

* The figures in the parentheses represent the total exposure doses corrected for γ rays of ^{131}I.

touching each other when they are soaked in the isotope solution in batches of 20 seeds per a gauze pouch, from Table 1 we may conclude that the present calculation gives a fairly good estimation of the absorbed in the soaked seeds, because the RBE values of these β rays relative to Co-60 γ rays will be close to unity.

1) S. Kondo et al. Ann. Report, National Inst. Genetics (Japan) No. 8, 98 (1958).
2) R. Loevinger et al. in Radiation Dosimetry (G. J. Hine and G. L. Brownell, ed.) Academic Press. 1956, p. 694.
3) S. Matsumura. Ann. Report, National Inst. Genetics (Japan) No. 10, 136 (1960).
4) S. Kondo. Ann. Report, Nationl Inst. Genetics (Japan) in Japanese, No. 9, 134 (1959).

96. *The effect of X-irradiated pollen grains on the formation of empty hybrid seed in nicotiana*

(By Masao TANAKA)

In the cross, *Nicotiana tabacum* (♀)×*N. alata* (♂) in which only empty seeds are expected, X-irradiated pollen grains increase the percentage of filled seeds markedly. The author had found in the ovaries of tobacco dusted with pollen grains of N. alata, that auxin levels were very low—almost at the same level as those of the ovaries kept without pollination—and that in the ovaries dusted with X-irradiated pollen grains, the levels of auxin rose a little higher.

In order to reveal the action of growth regulators on the fullness of seeds, the levels of auxins and IAA-inhibitors in the ovaries were investigated. When the partly purified extract from immature capsules is developed on chromatograms using isobutanol-metanol-water (80:5:15) as a developing solvent, two spots of auxins usually appear in the position of Rf 0.19 and 0.97 after spraying with Salkowski-Link's reagent. Two yellowish white spots of IAA-inhibitors appear in the position of Rf 0.20 and 0.28 on the red background of the paper strips when the original extract has been developed by the same solvent and treated with Salkowski-Link's reagent after spraying with a diluted solution of IAA. The IAA-inhibitors are supposed to be ferment-like substances from the fact that they are fluorescent in ultra-violet light and inactivated by KCN and furthermore, they give purple colour reaction by spraying with a solution of nynhydrin.

The areas of the spots were compared among the extracts of the capsules, *N. tabacum* selfed, *N. tabacum* dusted with non-irradiated as well as irradiated pollen grains of *N. alata*. The spots of auxins from the capsules dusted with irradiated pollen grains were obviously larger than those from non-irradiated pollen grains though they were much smaller than those from the capsules of selfed *N. tabacum*. No differences were observed in the areas of the spots of IAA-inhibitors among the capsules of selfed *N. tabacum* and those obtained from dusting with non-irradiated pollen grains of *N. alata*. But the spots from the capsules obtained by dusting with irradiated pollen grains were obviously smaller than those from the other two kinds of pollination.

Summarizing the results mentioned above, we may draw the conclusion that the empty hybrid seeds arise partly by a decrease of auxin level which might be caused by a physiological disharmony between paternal and maternal genomes in the endosperm. Irradiated pollen grains are supposed to overcome the decrease of auxin levels by suppressing the

action of an inhibitor producing systems and will work toward increasing the filled condition of seeds.

G. TECHNICAL NOTE

97. *A preliminary calculation of depth dose curve of* ^{60}Co γ*-ray irradiation*

(By S. KONDO and H. ISHIWA)

It is well known that the absorbed dose varies appreciably with depth in the surface region of materials exposed to high energy photons. A theoretical calculation of the depth dose curve for Co-60 γ irradiation of tissue-like materials has been carried out.

Assume a thin block of material with an infinite plane surface. Take the origin of x coordinates at the surface with the positive x axis parallel to the inward normal and expose the block to γ rays coming parallel to the x axis from the negative side. Then, the absorbed dose dD/dx at the depth x (g/cm^2) due to recoil electrons originating from the surface and within the solid angle element $d\Omega$ in the direction of the angle of electron recoil, θ, is given by

$$\frac{dD}{dx}=c\int E(\theta)P\left(\frac{x}{\cos\theta},\ E\right)\frac{f(\theta)}{R(E)\cos\theta}d\Omega \qquad (1)$$

where c is the proportionality constant, $E(\theta)$ the energy of recoil electrons, $R(E)$ the maximum range of electrons with energy E, $f(\theta)d\Omega$ the number of the above recoil electrons and $P(x/\cos\theta,\ E)$ the fraction of energy released by the above electrons per unit thickness at distance $x/\cos\theta$ from the surface along the θ direction. The relative depth dose $P(\xi, E)$ at the depth $\xi(=x/\cos\theta)$ for electrons with incident energy E and angle $\theta=0$, has been calculated by SPENCER[1]. However, to simplify further the calculation, we will use as a preliminary method NAKAI's approximation[2] which fits well SPENCER's theory and the experimental results for aluminum:

$$P(\xi, E)=\exp\left[-4.532(\xi-0.336R)^2/R^2\right] \qquad (2)$$

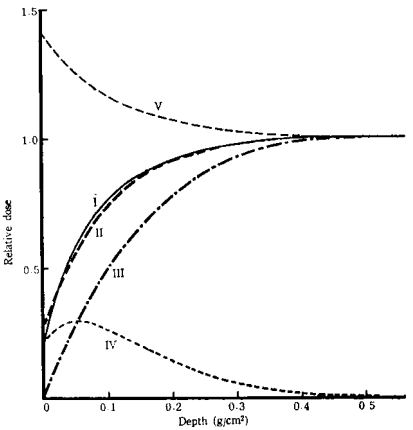
where, for simplicity, the extrapolated range R used by NAKAI[2] has been replaced by the maximum range R.

Now, the relative depth dose $I(x)$ at depth x is given by

$$I(x)=\int_0^x\frac{dD(x)}{dx}dx\Big/\int_0^\infty\frac{dD(x)}{dx}dx \qquad (2)$$

The depth dose curve calculated from equation (3) is given by Curve III in Fig. 1. Using the experimental spectrum of secondary electrons obtained for aluminum at $\theta=0°$ and 15° by AGLINTSEV *et al.*[4], we have obtained the relative depth dose curve IV (see Fig. 1) for the external secondary electrons originating from the materials outside the dosimeter.

The final curve I is the sum of curves III and IV where curve IV has been adjusted to fit the experimental curve II obtained by ONAI *et al.*[3] at $x=0.2$. The agreement between the theoretical and experimental curves is satisfactory. An improvement of the present calculation by using SPENSER's theory is now under way.



An example of a very high contamination by external secondary electrons is given in curve V for the case of the ^{60}Co γ irradiation facility with no filter in National Institute of Genetics. Irradiation of small animals or plant seeds should be carried out by encasing the materials in a container with electronic equilibrium wall thickness not only for the secondary electron equilibrium but also for elimination of the external secondary electrons.

1) L. V. SPENCER: N. B. S. (U. S. A.) Monograph **1** (1959).
2) Y. NAKAI: unpublished.
3) Y. ONAI et al.: Rinsho Hoshasen **3**, No. 9 (1958) 56.
4) K. K. AGLINTSEV et al.: Atomnaia Energia **2**, Feb. (1957) 66.

BOOKS AND PAPERS PUBLISHED IN 1960 BY STAFF MEMBERS

CHANDRARATNA, M. F. and K. SAKAI 1960 A biometrical analysis of matrocinous inheritance of grain weight in rice. Heredity **14** Parts 3 & 4, 365-373

DOIDA, Y. 1960 Developmental studies in the genus *Polygonum* I. Microsporogenesis of *Polygonum persicaria* L. Bot. Mag. **73**: 278-282

——— 1960 Cytological studies in *Polygonum* and related genera I. Bot. Mag. **73**: 337-340

ENDO, T. 1960 Biochemical genetics in higher plants (in Japanese). Protein, Nucleic acid and Enzyme **5**: 75-86

——— 1960 Paperchromatography and its application to the plant science in "Experimental Methods in Cytogenetics" (ed. by I. NISHIYAMA Yoken-dô) 381-434

FUJII, T. 1960 Radiosensitivity in plants IV. Experiments with several mutant strains of einkorn wheat. Jap. Jour. Genet. **35**: 110-119

——— 1960 Mutations in einkorn wheat induced by X-rays VI. Segregation ratio and viability of several chlorophyll mutants. Seiken Zihô **11**: 12-20

——— and Y. ONO 1960 Amino acid content of einkorn wheat chlorophyll mutant (Preliminary report). Proc. Jap. Acad. **36**: 612-617

FURUSATO, K. and A. MIYAZAWA, 1960 Breeding of amphidiploid by cross between *Nicotiana rustica* and *N. tabacum* (in Japanese). Hatabako Kenkyu **25**: 82-85

——— 1959 A new method to produce easily rooting cuttings (in Japanese). Seiken Zihô **10**: 95-97

——— 1960 Investigation of agriculture in Africa (in Japanese). Kankitsu **11**: 46-50, **12**: 108-111; 60-64; 56-61

——— 1960 Investigation of *Citrus* in Africa (in Japanese). Kajitsu Nihon **15**: 4-5; 53-55

——— 1960 An example of seedless fruits by gibberellin treatment (in Japanese). Iden **14**: 61

HIRAIZUMI, Y. and JAMES F. CROW 1960 Heterozygous effects on viability, fertility, rate of development, and longevity of *Drosophila* chromosomes that are lethal when homozygous. Genetics **45**: 1071-1083

———, L. SANDLER, and JAMES F. CROW 1960 Meiotic drive in natural populations of *Drosophila melanogaster* III. Populational implications of the Segregation-Distorter locus. Evolution **14**: 433-444

——— and ——— 1960 Meiotic drive in natural populations of *Drosophila melanogaster* IV. Instability at the Segregation-Distorter locus. Genetics **45**: 1269-1287

———, I. FUKUDA, T. NARISE and M. KURABAYASHI 1960 Evolution and Variation in *Trillium* VI. Migrations among natural populations of *T. kamtschaticum* across the Ishikari Depression. Evolution **14**: 224-231

KAO, K. N., C. H. HU, W. T. CHANG and H. I. OKA 1960 A biometrical-genetic study of irradiated populations in rice: Genetic variances due to different

doses of X-rays. Bot. Bull. Acad. Sinica **1**: 101-108

KATAYAMA, T. 1960 Studies on the intercellular spaces in rice I. Proc. Crop Sci. Soc. Jap. **29**: 229-233

KATSUYA, K. 1960 Susceptibility of the *albina* mutant of Einkorn wheat to leaf and stem rusts, *Puccinia triticina* and *P. graminis*. W. I. S. No. **11**: 16

KAWAHARA, T. and S. ICHIKAWA 1960 Studies on heterosis in the domestic fowl II. A comparative analysis of body weight in F_1 hybrids and their purebreds (in Japanese with Eng. résumé). Jap. Jour. Zootech. Sci. **31**: 109-114

——— 1960 Studies on heterosis in the domestic fowl III. Mortality and susceptibility to leucosis in F_1 hybrids (in Japanese with Eng. résumé). Ibid. **31**: 115-118

KIHARA, H. 1960 Principles of genetics I (in Japanese). Iden **14**: 54-56

——— 1960 Principles of genetics II (in Japanese). Iden **14**: 52-55

——— 1960 The rice plant in Sikkim. A report of the scientific mission. Seiken Zihô **11**: 46-54

———, Y. OHTA and N. YUTANI 1960 Comparison of hereditary variegation and breaking by viruses (in Japanese). Jap. Jour. Gen. **35**: 274

——— and K. TSUNEWAKI 1960 Production of polyploid wheat by nitrous oxide. Proc. Jap. Acad. **36**: 658-663

KIMURA, M. 1960 Genetic load of a population and its significance in evolution (in Japanese with English summary). Jap. Jour. Genet. **35**: 7-33

——— 1960 Optimum mutation rate and degree of dominance as determined by the principle of minimum genetic load. Jour. Genet. **57**: 21-34

——— 1960 Outline of population genetics (in Japanese). Baifû-kan

——— 1960 Relative applicability of the classical and the balance hypotheses to man. Especially with respect to quantitative characters. Jour. Radiation Res. **1-2**: 155-164.

KONDO, S. 1960 Radiation Dosimetry for Biology and Chemistry (in Japanese). Biophys. Biochem. Symp. **5**: 229-245 Nanko-do

——— 1960 Neutron response of silver-activated phosphate glass. Health Phys. **4**: 21-24

——— and M. L. RANDOLPH 1960 Effect of finite size of ionization chambers on measurements of small photon sources. Rad. Res. **13**: 37-60

——— 1960 Absolute dosimetry with silver-activated phosphate glass for liquid and solid containing β-ray sources (in Japanese). Proc. 3rd Jap. Isotope Congress 465-469

——— 1960 Dosimetry for radiation chemistry (in Japanese). Kô-bunsi **2**: 3-14

——— 1960 Principles or radiation dosimetry (in Japanese). Gensiryoku Kôgyô **6**: 74-80

MATSUMURA, S. 1960 Radiation genetics in wheat V. Influence of irrradiation time and temperature on the genetic effects of ionizing radiation in diploid wheat. Jap. Jour. Genet. **35**: 197-204.

——— 1960 Radiation effects of fast and thermal neutrons on wheats I. Genetic effects of neutrons on einkorn wheat. W. I. S. No. **11**: 5-7

——— 1960 Comparison of radiation effects of beta- and gamma-rays on einkorn

wheat. W. I. S. No. **11**: 12-13

——— and T. FUJII 1960 Induction of bud sports of radiation (in Japanese). Proc. 3rd Conf. Radioisotopes 1959: 1005-1008

———, ——— and S. KONDO 1960 Co^{60} gamma-irradiator and its use in plant genetics. Large Radiation Source in Industry, IAEA **2**: 179-193

MIKAMI J. and Y. OGAWA 1960 Effects of Glucuronic acid on the function of liver after partial hepatectomy (in Japanese). Advances in Glucuronic acid Res. 1955-1959, 123-126

MORISHIMA, H. and H. I. OKA 1960 The pattern of interspecific variation in the genus *Oryza*: Its quantitative representation by statistical methods. Evolution **14**: 153-165

MORIWAKI, K. 1960 Effects of the content of energy-rich phosphate esters on the swelling in mouse diaphragm. Ann. Zool. Jap. **33**: 211-216

MUKAI, T. 1960 Destiny of heterotic lethal genes in populations (in Japanese). Jap. Jour. Genet. **35**: 278-279

——— 1960 Population dynamics of selfing. Seiken Zihô **11**: 43-45

NAKAI, S. 1960 Experimental methods in research of radiation genetics. "Experimental Methods in Cytogenetics" (ed. by I. NISHIMURA) Yokendo 435-533

NAWA, S. 1960 The structure of the yellow pigment from *Drosophila*. Bull. Chem. Soc. Japan **33**: 1555-1560

——— 1960 Biochemical genetics of insects (in Japanese). Protein Nucleic Acid Enzyme **5**: 87-94

NEZU, M. 1960 Radiation effects of fast and thermal neutrons on wheat II. Relation of ploidy to chromosome aberrations. W. I. S. No. **11**: 7-8

———, T. KATAYAMA and H. KIHARA 1960 Genetic study of the genus *Oryza* I. Cross ability and chromosomal affinity among 17 species. Seiken Zihô **11**: 1-11

OGAWA, Y. 1960 Growth, differentiation and regeneration XVI. Synthesis of myosin in the regenerating limb of *Triturus pyrrhogaster* (in Japanese). Med. and Biol. **54**: 189-192

——— 1960 Growth, differentiation and regeneration XVII. Effect of X-irradiation on the synthesis of actin in early embryo of *Triturus pyrrhogaster*, Boie (in Japanese). Med. and Biol. **55**: 34-36

——— 1960 Synthesis of contractile proteins in the X-irradiated embryo. Nature **186**: 77-78

——— 1960 Individual differences of taste-sensitivity to citbittol A, a bitter substance extracted from the fruit of Colocynthe (in Japanese). Med. and Biol. **55**: 91-94

——— 1960 Effect of Glucuronic acid on the growth of embryo and the regenerating limb tissue of *Triturus* (in Japanese). Advances in Glucuronic acid Res. 1955-1959, 25-31

——— 1960 Growth, differentiation and regeneration XVIII. Growth promoting effect of Na-glucuronate on the early embryos of *Triturus pyrrhogaster*, Boie (in Japanese). Med. and Biol. **55**: 161-164

——— 1960 Growth, differentiation and regeneration XIX. Synthesis of skeletal muscle protein in the regenerating tissues of fore limb and tail of *Triturus*

pyrrhogaster, Boie (in Japanese). Med. and Biol. **56**: 37-39

—— 1960 Taste-ability for phenyl-thio-carbamide of Japanese (in Japanese). Med. and Biol. **56**: 156-159

—— 1960 Growth, differentiation and regeneration XX. Effect of Natrium glucuronate on tre impediment of growth of X-irradiated embryos of *Triturus pyrrhogaster*, Boie (in Japanese). Med. and Biol. **57**: 23-26

—— 1960 Growth, differentiation and regeneration XXI. Effect of X-irradiation on the synthesis of myosin in skeletal muscle of early embryo of *Triturus pyrrhogaster* (in Japanese). Med. and Biol. **57**: 133-136

—— 1960 Effects of gluculonic-acid and related compounds on the growth of the embryo of *Triturus pyrrhogaster* (in Japanese). Med. **17**: 894-899

OHTA, T. and S. MATSUMURA 1960 Cytological and histological observations on the male sterility in sugar beet (in Japanes with Eng. résumé). Seiken Zihô **11**: 77-81

ONO, S. and S. KONDO 1960 Molecular theory of surface tenion in liquids. Handbuch der Physik)Springer-Verlag/Berlin) 134-280

OKA, H. I. 1960 Variation in competitive ability among rice varieties (Phylogenetic Differentiation in Cultivated Ric. 19). Jap. Jour. Breed. **10**: 61-68

—— and W. T. CHANG 1960 Survey of variations in photoperiodic response in wild *Oryza* species. Bot. Bull. Acad. Sinica **1**: 1-14

OSHIMA, C. and O. KITAGAWA 1960 Frequencies of second chromosomes, having deleterious effects on viability when homozygous in natural population of *D. melanogaster*. D. I. S. **34**

—— and —— 1960 The pre-adult viability of heterozygotes of *D. melanogaster* second chromosomes that are lethal and semi-lethal when homozygous. D. I. S. **34**

SAKAI, K. I. 1959 Practical purity and the method of maintenance of established varieties of rice. News Letter, International Rice Commission **8**: 7-13

—— —— 1960 Studies on the method of selection in the coconut palm. Bull. Fac. Agr., Tamagawa Univ. 63-72

SHIMOTUMA, M. and Y. OGAWA 1960 Cytogenetical studies in the genus citrullus III. Bitter substances in fruits of C. Colocynthis Schrad. and C. vulgaris Schrad. Jap. J. Genet. **35**: 143-152

TAIRA, T. 1960 A biochemical study on allelism at *Henna* locus in *Drosophila melanogaster*. Jap. Jour. Genet. **35**: 344-350

—— —— 1960 Is the $Hn^{r3}ry$, double recessive mutant a synthesized lethal strain. D. I. S. **34**:

TAKENAKA, Y. 1960 The northern limit of distribution of tropical and subtropical species in Japan. Biol. Jour. of Nara Women's Univ. **10**: 36-39

TAZIMA, Y. 1960 Application of radiations for animal breeding (in Japanese). Kagaku **30**: 22-26

—— —— 1960 Comparison of radiation-induced mutation rates between acute and chronic irradiation in silkworm. United Nations Document A/Ac, 82/G/L, 473

TSUJITA, M. 1960 Alkaloids in tobacco plant and sericulture. Kinugasa Gakuhô **8**: 3-5

TSUNEWAKI, K. 1960 Monosomic and conventional gene analysis in common wheat

III. Lethality. Jap. Jour. Gen. **35**: 71-75

——— and E. G. HEYNE 1960 The transmission of the monosomic condition in wheat var. Chiness Spring. Jour. Hered. **51**: 63-68

——— and B. C. JENKINS 1960 A comparative study of various methods of root-tip preparation in screening wheat aneuploids. Cytologia

YAMADA, Y. 1960 Observations of genotype-environment interaction in production traits in chickens. Jap. Jour. of Breeding **10**: 23-28

——— 1960 Optimum stracture of poultry breeding flock which maximizing genetic improvement by selection. Jap. Joor. of Genet. **35**: 187-196

YOSIDA, T. H., T. ISHIHARA and T. HAMADA 1960 Chromosomal alteration and the development of tumors III. Change in ploidy of the stemline chromosomes with a transplantability shift. Gann **51**: 55-65

———, ——— and T. ODASHIRO 1960 Ibid IV. Comparative idiogram analysis in cells of normal rat liver and rat ascites hepatoma No. 7974. Jap. Jour. Genet. **35**: 35-40

——— 1960 Ibid V. Temporary proliferation of tetraploid cells in the Yoshida sarcoma and their origin. Gann **51**: 359-369

——— and S. TSUJI 1960 Ibid VI. Difference in invasiveness of the diplo- and tetraploid cells of the Yoshida sarcoma. Jap. Jour. Genet. **35**: 377-381

———, T. TABATA and H. HIRUMI 1960 Cytological studies on radiation effects I. Chromosome breaks in the three types of chromosomes of Yoshida sarcoma cells and their localization. Jour. Rad. Res. **1**: 1-9

——— and H. HIRUMI 1960 Cytological study on the effects of 8-azaguanine and related compounds on the Yoshida sarcoma cells. Gann **51**: 345-357

———, T. TABATA, T. TAKAHASHI and H. HIRUMI 1960 Effect of X-rays on cell muclei of ascites tumors, with special emphasis on the chromosome breaks and reunion (in Japanese with Eng. résumè) Symp. Cell. Chem. **10**: 203-220

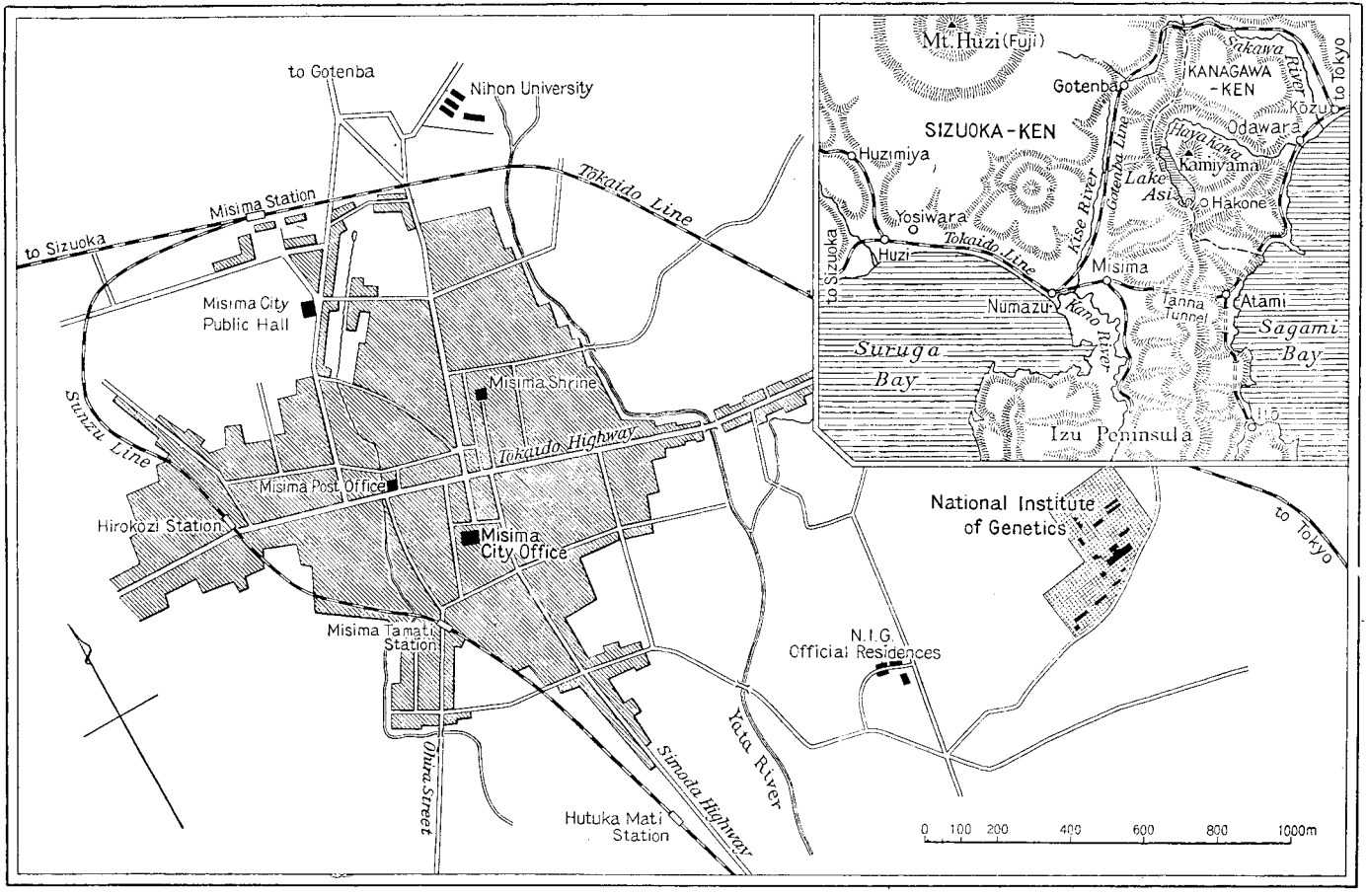
——— and T. ODASHIRO 1960 The walker rat carcinoma having a triploid stemline (in Japanese with Eng. résumè). La Kromosome 44-45

ACKNOWLEGMENTS

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

国立遺伝学研究所年報　第11号
昭和36年10月20日　印刷
昭和36年10月25日　発行

発行者　木　原　　均
国立遺伝学研究所内
編集者　小　川　恕　人
国立遺伝学研究所内
印刷者　笠　井　康　頼
東京都新宿区山吹町184
印刷所　株式会社　国際文献印刷社
東京都新宿区山吹町184
発行所　国立遺伝学研究所
静岡県三島市谷田1,111
電・(三島)(5) 0771, 0772


to Gotenba
Nihon University
Misima Station
to Sizuoka
Tōkaido Line
Misima City
Public Hall
Misima Shrine
Sunzu Line
Tokaido Highway
Misima Post Office
Hirokozi Station
Misima
City Office
Misima Tamati
Station
Ohira Street
Simoda Highway
Yata River
Hutuka Mati
Station
N.I.G.
Official Residences
National Institute
of Genetics
to Tokyo
0 100 200 400 600 800 1000m
Mt. Huzi (Fuji)
Gotenba
KANAGAWA
-KEN
Sakawa River
to Tokyo
Kōzu
SIZUOKA-KEN
Odawara
Haya Kawa
Huzimiya
Kamiyama
Gotenba Line
Aise River
Lake
Asi
Hakone
Yosiwara
to Sizuoka
Huzi
Tokaido Line
Misima
Numazu
Kano
River
Tanna
Tunnel
Atami
Sagami
Bay
Suruga
Bay
Izu Peninsula
to Tokyo