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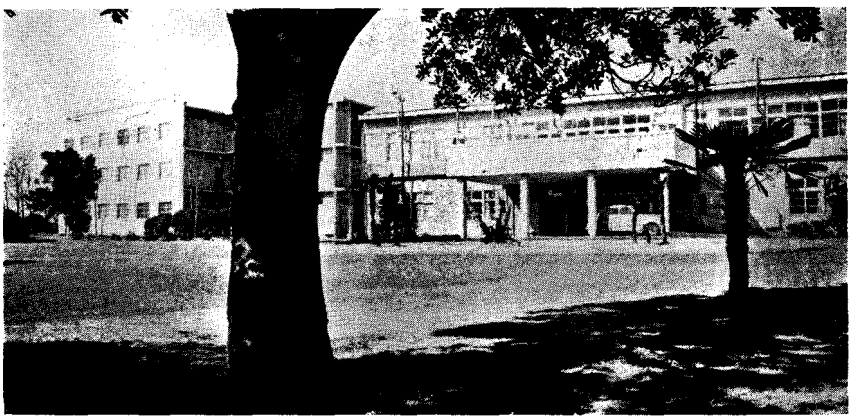

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GENERAL STATEMENT

From October 11 to November 7 of this year, we have had the Southeast Asian Regional Training Course on Genetics sponsored by the Ministry of Education and the Japanese Commission for UNESCO. There were 19 participants from 7 nations. Our staff members gave lectures on subjects from their own fields, covering biochemical, radiation, population as well as human genetics.

We believed that this course was successful for the participants not only in becoming acquainted with the principles of genetics but also in developing mutual understanding among themselves and the members of our Institute.

It was suggested that an advanced course in special fields of genetics would be organized in the future.

The main building of the Institute is not yet completed. It will take at least one more year.

Dr. Oshima, head of the Department of Physiological Genetics and Dr. Matsunaga, head of the Department of Human Genetics were awarded the prizes of Genetics Society of Japan and of Japan Society of Human Genetics, respectively.

To the Tenth Pacific Science Congress which was held in Honolulu from August 21 to September 2 we have sent several members.

ABSTRACT OF DIARY FOR 1961

Feb.	24.	90th meeting of Misima Geneticists' Club.
March	18.	91st meeting of Misima Geneticists' Club.
April	19.	92nd meeting of Misima Geneticists' Club.
May	12.	40th Biological Symposia
	19.	93rd meeting of Misima Geneticists' Club.
June	6.	41st Biological Symposia
	23.	94th meeting of Misima Geneticists' Club.
July	14.	95th meeting of Misima Geneticists' Club.
Sep.	15.	96th meeting of Misima Geneticists' Club.
Oct.	3.	97th meeting of Misima Geneticists' Club.
Nov.	24.	98th meeting of Misima Geneticists' Club.
Dec.	18.	42nd Biological Symposia
	19.	99th 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
   Bungo SAKAGUCHI, D. Ag.,
   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. and Yoshiaki YONEDA
3. Department of Physiological Genetics:
   Chozo OSHIMA, D. Sc., Head of Department
   Hitoshi HIHARA, D. Sc., M.J.A., Toshifumi TAIRA, D. Sc., Koichiro TSUNEWAKI, D. Ag., Ph. D. and Sadao SAKAMOTO (in U.S.A.)
4. Department of Biochemical Genetics:
   Mitsuo TSUJITA, D. Ag., Head of Department
   Yoshito OGAWA, M.D., Saburô NAWA, D. Sc. (in U.S.A.)
   Tetsuo IINO, Ph. D., Tôru ENDÔ, 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. (in U.S.A.), Akira MIYAZAWA, Takatada KAWAHARA
   Shinya IYAMA, D. Ag. (in U.S.A.) and Hiroko OKINO
6. Department of Induced Mutation:
   Seizi MATSUMURA, D. Ag., Head of Department
   Sôhei KONDO, D. Sc., Kiyoshi TUTIKAWA, Tarô FUJII, Terumi MUKAI Ph. D., and Hiromi ISHIWA
7. Department of Human Genetics:
   Ei MATSUNAGA, M. D., D. Sc., Head of Department
   Motoo KIMURA, Ph. D., D. Sc. (in U.S.A.)
   Yuichirô HIRAIZUMI, D. Sc., Akira TONOMURA, D. Sc. and Yasuko TONOMURA, D. Sc.

Part-time Staff and Research Associates

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

Research members under the Grant from the Rockefeller Foundation

Rice Research Group:
Hiroko OKINO, Keizô KATSUYA, Tomoo MABUCHI, Tadao KATAYAMA, Kôkichi HINATA and Akio SUZUKI.

Research members under the Grant from the National Institute of Health

Salmonella Research Group:
Itirô SASAKI and Masatoshi ENOMOTO.
Drosophila Research Group:
Sadao CHIGUSA and Keiko FUWA.

Department of Administration

Tanryû Otomo, Head of Department
Katsutarô Andô, Chief of General Affairs Section
Seiichi Koizumi, Chief of Finance Section
Hiroko Nakano, Ryôji Oyama, Kazuo Enomoto and Keiichi Kawashima
Clerks, Librarian, Typists, Chauffeur, Field Laborers and Janitors.....9

Misima Branch of Hatano Tobacco Experiment Station

Masao Tanaka, Head
Assistants..... 3

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Kenpo Tsukamoto, Director of National Institute of Radiological Sciences
Minoru Tachi, Director of Institute of Population Problems

PROJECTS OF RESEARCH FOR 1961

Department of Morphological Genetics

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

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 LILIENFELD)
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* (OSHIMA)
Physiological studies on eye-pigment formation in *Drosophila* (TAIRA and OSHIMA)
Population genetics of deleterious genes in natural populations of *Drosophila* (OSHIMA)
Studies on the origin of wheat (KIHARA and TSUNEWAKI)
Studies on nucleus substitution in wheat and related species (KIHARA)
Male sterility in wheat (KIHARA)
Production of polyploids and aneuploids by N_2O treatment (KIHARA and TSUNEWAKI)
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)
Genetics of virus (TSUJITA)
Immunogenetics of salmonella (IINO)
Genetics of multi-drug resistance in bacteria (IINO)

Department of Applied Genetics

Studies on breeding and genetics in poultry (YAMADA and KAWAHARA)
Theoretical studies on plant breeding techniques (SAKAI)
Studies on competition and migration in plants and animals (SAKAI, IYAMA and NARISE)
Genetic studies of alkaloid content in tobacco plants (SAKAI and IYAMA)
Biometrical study of cytoplasmic inheritance (SAKAI, IYAMA and NARISE)
Genetic studies on developmental stability in plants (SAKAI, NARISE and SUZUKI)
Polyploidy and sterility in fruiting plants (FURUSATO and MIYAZAWA)
Evolutionary-genetic studies in *Oryza* (OKA and OKINO (MORISHIMA))

Department of Induced Mutation

Radiation genetics of mice (TUTIKAWA)
Radiation population-genetics of *Drosophila* (MUKAI and CHIGUSA)
Studies on polygenic mutation in *Drosophila* (MUKAI and CHIGUSA)
Triploidy breeding of sugar beets (MATSUMURA)
Relation between the quality of radiations and mutations (MATSUMURA and KONDÔ)
Radiation genetics of cereals (MATSUMURA, FUJII, KATSUYA and MABUCHI)
Radiation genetics and its practical application (MATSUMURA, FUJII and MABUCHI)
Biophysical consideration of radiation genetics (KONDÔ)
Radiation dosimetry (KONDÔ and ISHIWA)

Department of Human Genetics

Prezygotic selection in ABO blood groups (MATSUNAGA and HIRAIZUMI)
Genetic studies on sporadic retinoblastoma in Japan (MATSUNAGA)
Cytogenetics in man (TONOMURA)
Sexual dimorphism in resting nuclei (TONOMURA (TOYOFUKU) and TONOMURA)
Theoretical studies of population genetics (KIMURA)
Effects of radiation-induced mutations on fitness (HIRAIZUMI)
Populational implications of meiotic drive with special reference to the *SD* locus in *D. melanogaster* (HIRAIZUMI)

RESEARCHES SUPPORTED BY FOREIGN GRANTS

1. JOINT RESEARCHES ON THE ORIGIN OF RICE SUPPORTED BY A GRANT FROM THE ROCKEFELLER FOUNDATION

Section 1. Collection and preservation of *Oryza* species (KIHARA)

a. Strains of 27 species so far collected amount to more than 4,100
b. Collection-tour was made this year by KATAYAMA to Philippines and New Guinea

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

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

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

a. Estimation of genetic variability among and within populations of wild rice (SAKAI, IYAMA and NARISE)
b. Comparative studies of seedling and flowering characters of wild and cultivated rice (NARISE and SAKAI)
c. Variation and inheritance of within-plant variability in seed size in wild and cultivated rice (SAKAI, NARISE and SUZUKI)
d. Variation studies of blast disease resistance in wild and cultivated rice (GOTOH and SAKAI)

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

a. Statistical-systematic studies of wild and cultivated rice strains (MORISHIMA and OKA)
b. Survey of variations between *O. perennis* and *O. sativa* f. *spontanea* (MORISHIMA, CHANG and OKA)
c. Crossing-experiments and sterility of hybrids between wild and cultivated rice strains (HINATA and OKA)
d. Studies of intermediate wild-cultivated forms in rice (OKA and CHANG)
e. Responses to growing conditions of wild and cultivated rice forms (OKA and CHANG)

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

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

II. STUDIES OF DELETERIOUS GENES IN DROSOPHILA POPULATIONS SUPPORTED BY A GRANT FROM NATIONAL INSTITUTE OF HEALTH, U.S.A.

1) Direct analysis of natural populations of *D. melanogaster* (OSHIMA and FUWA)
2) Experimental analysis
   a. The pre-adult viability of heterozygous flies for lethal or semi-lethal chromosome (OSHIMA and FUWA)
   b. The persistence of some natural lethal genes in experimental populations (OSHIMA and FUWA)
   c. The persistence of two recessive genes in experimental populations (MUKAI and CHIGUSA)
3) Studies on mutation rates
   a. Radiation-induced mutation rate (MUKAI and CHIGUSA)
   b. Spontaneous mutation rate (MUKAI and CHIGUSA)

III. STUDIES IN HUMAN GENETICS SUPPORTED BY A GRANT FROM THE ROCKEFELLER FOUNDATION

1. Selective factors maintaining human blood group polymorphism (HIRAIZUMI and MATSUNAGA)
2. Studies on dimorphism in human normal cerumen (MATSUNAGA)
3. Chromosome studies in patients with different congenital anomalies (TONOMURA)

FOREIGN VISITORS IN 1961

March	16.	Dr. Charlotte Auerbach (Edinburgh Univ., England)
	27.	Dr. H. B. BIRNBAUM (National Science Foundation, U.S.A.)
	28.	Dr. RICHARD K. ANDERSON and Cleo H. ANDERSON (Rockefeller Foundation, U.S.A.)
April	8.	Dr. VICENTE C. RODRIGUES (Bureau of Plant Industry, Philippines)
		Miss Payow Yimcharoen (Dept. Biology, Chulalongkon Univ., Bangkok, Thailand)
	27.	S. H. OU (FAO Regional Office, Bangkok, Thailand)
June	3.	Dr. G. L. McNEW (Boys Thompson Institute, New York, U.S.A.)
		Dr. A. W. NORDSKOG (Iowa State Univ., U.S.A.)
	6.	Dr. A. HOLLAENDER (Oak Ridge National Laboratory, U.S.A.)
July	18.	Dr. H. BOROUGHS (Inter American Institute of Agricultural Sciences, Costa Rica)
	23.	Dr. V. KOVDA (UNESCO, Paris, France)
		Dr. L. MATTSSON (UNESCO, Djakarta, Indonesia)
Aug.	3.	Dr. L. R. HOUSE (Rockefeller Foundation, New Dehli, India)
		Dr. T. S. DHILLON, (Dept. Botany, Univ. of Hong Kong)
	15.	Dr. S. UDAYACHALEM (Physics Dept., Chulalongkon Univ., Bangkok, Thailand)
Sep.	12.	Dr. K. SUVATABANDHU (BOTANY Dept., Faculty of Science, Chulalongkon Univ., Bangkok, Thailand)
		Dr. H. D. JORDAN (West African Rice Research Station, Sierra Leone)
		Dr. H. PARTHASARATHY (FAO Foundation, Bangkok, Thailand)
		Dr. N. MANGAMMAL (c/o FAO Foundation, Bangkok, Thailand)
Oct.	5.	K. R. STINO (Minister of Supply & Professor, College of Agriculture, Giza, Egypt, U.A.R.)

12. L. MATTSSON, (UNESCO, Djakarta, Indonesia)

Nov. 17. J. A. PATIL (Dept. of Agriculture, Mahapashtra State, Bombay, India)
B. B. CHAVDHARI (Dept. of Agriculture, Mahapashtra State, Bombay, India)

Dec. 7. V. G. PANSE (Institute of Agricultural Research Station, New Delhi, India)

18. Dr. T. MAKINODAN (Biology Division, Oak Ridge National Laboratory, U.S.A.)

RESEARCHES CARRIED OUT IN 1961

A. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF ANIMALS

1. *The persistence of some natural lethal chromosome of Drosophila melanogaster in experimental populations.*[1)]

(By Chozo OSHIMA)

The heterozygous flies for each of several lethal chromosomes isolated in 1959 from the large Katsunuma population or small Suyama population were introduced into experimental populations. The method described by Buzzati-Traverso (1947) was used. The decreasing frequencies of lethal heterozygotes have been followed up in the course of forty generations. To determine the frequency at an arbitrarily chosen generation, one hundred chromosomes sampled from the population were examined, to find out whether or not they have contained the lethal gene, by the test cross to the original lethal strain. The results are shown in Figure 1.

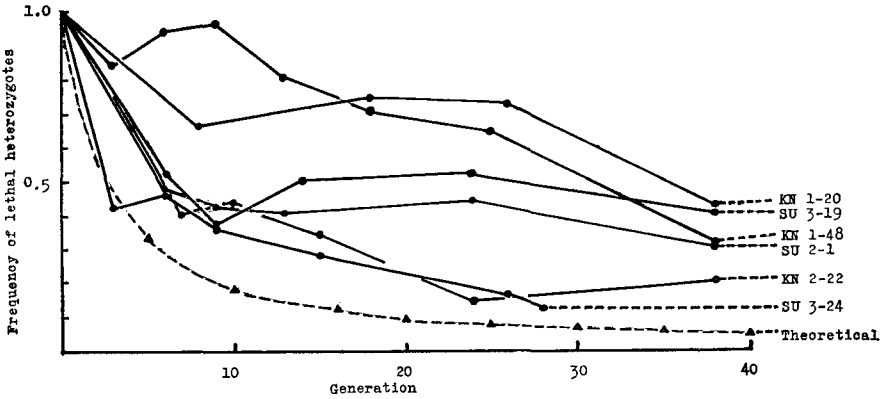


Fig. 1. Decreasing curves of natural lethal genes in experimental populations.

When the declining curves were compared with the theoretical curve, which was given by the no-selection coefficient ($s=0$), these six lethal genes could be assumed to show heterosis in the heterozygous state in experimental populations. The two lethal genes among them, SU 3-19

1) This work was supported by Grant RG-7836 from the National Institute of Health, U.S.A.

which was allelic to SU 2-1, and SU 3-24 were demonstrated in our previous paper (Oshima and Kitagawa, 1961) to have been maintained for at least one year in the Suyama population. As described in another report, two alleles of these two genes, respectively, were found repeatedly among the new lethal genes extracted from the same natural population in 1961. From this fact, these two lethal genes clearly showed the superiority of heterozygotes in fitness in both experimental and natural populations.

2. *Relative frequency of the second chromosomes bearing deleterious genes in natural populations of Drosophila melanogaster.*[1)]

(By Chozo OSHIMA, Keiko FUWA and Yoshiko IMAI)

Many flies were collected from natural populations in Suyama and Juriki Town in Shizuoka Prefecture on September 30th 1961. The second chromosomes of these flies were isolated individually by using the method of completely marked inversion. On the basis of the proportion of wild type flies to total flies emerged in the F_4 generation, the chromosomes bearing deleterious and normal genes were classified into four grades, namely no wild type flies: lethal, 0.0-16.7%: semi-lethal, 16.8-25.0%: subvital and more than 25.1%: normal chromosome. Such experiments have been repeated every year since 1959. The relative frequencies of chromosomes belonging to each grade are given in Table 1. No change was detected

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- (n)	
Suyama and Juriki (1960)	23	3	15	118	25	184
Frequency(%)	12.50 ± 2.44	1.63 ± 0.93	8.15 ± 2.02	64.13 ± 3.54	13.59 ± 2.53	
Suyama and Juriki (1961)	61	17	38	212	49	377
Frequency(%)	16.18 ± 1.90	4.51 ± 1.07	10.08 ± 1.55	56.23 ± 2.56	13.00 ± 1.73	

(1): lethal, (sl): semi-lethal, (sv): subvital, (n): normal.

1) This work was supported by Grant RG-7836 from the National Institute of Health, U.S.A.

in the relative frequencies of deleterious chromosomes isolated from natural populations in the same locality in 1959 and 1960 and they were maintained also in 1961 without significant fluctuation.

The lethal chromosomes have been maintained in the Cy balanced system in the successive generations. Diallel crosses between new 61 lethal strains and old 23 lethal strains are in progress for detecting any lethal genes which have been retained in the natural population during over one year. Four crosses among about four hundred crosses were found to be allelic. From the results, two lethal genes could be assumed to be retained for one year in the same natural population and other two lethal genes, which were demonstrated to persist from 1959 to 1960 by our experimental results, were observed to be allelic to two new lethal genes respectively. From this fact, the two lethal genes could be assumed to be retained for two years in the Suyama population.

3. *The pre-adult viability of heterozygous flies for lethal chromosome*[1)]

(By Chozo OSHIMA, Keiko FUWA and Terumi MUKAI)

Twenty-three lethal chromosomes and twenty-two normal chromosomes were isolated from a small natural population in 1960. The pre-adult viabilities of their heterozygotes were estimated with Cy-Pm technique deviced by B. Wallace (1956). This technique (mating scheme) was convenient to obtain heterozygous flies for two normal chromosomes in random combination, for one lethal and one normal chromosomes and for two different lethal chromosomes, and their strain-specific viability could be estimated by comparing it with that of Cy/Pm flies. The experimental results are shown in Table 1. The mean relative viability of natural

Table 1. Relative viability of lethal heterozygotes

Genotype	Pooled basis		Line basis	
	No. of counted flies	Relative viability	No. of linse	Relative viability
+/+	127,944	1.3530 ±0.0097	231	1.3677 ±0.0166
+/*l*	369,129	1.2643**±0.0052	506	1.3217*±0.0125
l/*l*	160,118	1.2768**±0.0080	242	1.3416 ±0.0181

The viability of Cy/Pm: 1.0000

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

1) This work was supported by Grant RG-7836 from the National Institute of Health, U.S.A.

lethal heterozygotes was significantly less than that of normal heterozygotes on either pooled or line basis, and that of double lethal heterozygotes within the same populations was significantly less only on the pooled basis. The mean coefficient of selection ($\bar{s}$) for lethal heterozygotes was 0.0336 on the line basis and the individual coefficients were distributed between +0.1906 and −0.1089. The selection coefficients of 8 lines among 23 lines showed negative values. It means that one third of lethal heterozygotes were not at a disadvantage in pre-adult viability. The relative viability of double lethal heterozygotes was rather better than that of single lethal heterozygotes. In contrast with this finding, the magnitude of decrease in the mean viabilities of single and double induced-lethal heterozygotes, having a relatively homozygous genetic background, was approximately proportional to the number of lethal genes per strain. Thus, it may be assumed that natural selection produces a genic system, *i.e.*, a co-adaptation system, in which the interaction of genetic backgrounds ordinarily reduces their detrimentality of deleterious genes.

4. *Seasonal changes in the frequency of chromosomes bearing a lethal gene in natural populations of Drosophila melanogaster*

(By Sumio MINAMORI,* Makoto SASAKI and Chozo OSHIMA)

A seasonal change in the frequencies of chromosomes bearing a lethal gene in some Russian populations of *D. melanogaster* was observed by Dubinin (1946), but such phenomena have not been detected by Ives (1954) and Goldschmidt *et al.* (1955) in natural populations of America and Israel. The effective size of natural populations in several Japanese localities was estimated to be smaller than that of American populations by Oshima and Kitagawa (1961) and Minamori and Azuma (1962). A number of second chromosomes were extracted by using the method of marked inversion from natural populations in Hiroshima locality in early summer and autumn of 1961. These chromosomes were classified into four grades; lethal, semi-lethal, subvital and normal chromosomes by using ratios of non-Curly and Curly flies in the F_3 generation. In these small populations, it was examined whether the frequencies of lethal genes would be increased or not at the last seasonal expansion of the population. The results of testing the frequencies of lethal and semi-lethal second chromosomes in flies collected in late July and early October are presented in the following table. From the results shown in Table 1, the frequencies

* Assistant Professor of Hiroshima University and temporary researcher upon recommendation of the Ministry of Education for one year from October, 1961.

Table 1. Frequencies of lethal and semi-lethal chromosomes extracted from three natural populations in Hiroshima locality in July and October

Population	Month of collection	No. of tested chromosomes	Frequency of lethal and semi-lethal chromosomes	Difference between two collections
Midori St.	July	222	14.0	$0.05<P<0.10$
	October	155	20.6	
Tera St.	July	216	12.5	$0.20<P<0.30$
	October	169	17.2	
Shiwa Town	July	236	5.5	$0.50<P<0.70$
	October	200	7.0	
Total	July	674	10.5	$0.025<P<0.05$
	October	524	14.4	

of deleterious chromosomes in flies collected in October were higher than those in July in all three populations and the difference between the pooled frequencies was significant. The accumulation of deleterious genes in these populations was found at the last expansion.

5. *Dieldrin resistance in Drosophila pseudoobscura*[1)]

(By Chozo OSHIMA)

In natural populations of *D. pseudoobscura* in America, a geographical cline from east to west has been observed in the relative frequencies of their chromosomal arrangements. PP(Pikes Peak) chromosome had been rarely found in California before 1947, but its occurrence has rapidly increased to about 10 per cent in 1957, and on the other hand CH (Chiricahua) chromosome has decreased from 20 per cent to 4 per cent during the ten years. Among several environmental factors that could cause such a change in the genetic structures of the California populations, the effect of insecticides was proposed by Professor Dobzhansky.

Ten homozygous strains for each chromosomal arrangement, ST (Standard), AR(Arrowhead), CH(Chiricahua), PP(Pikes Peak) and TL(Tree Line), which were extracted from the natural population in Mt. San Jacinto, California by Dobzhansky in 1959, were used in this experiment. Ten adult flies were exposed in small vials for 1 hour to 0.8, 0.4, 0.2, 0.1 and 0.05 per cent Dieldrin test papers, prepared by WHO and their

1) This work was supported by the Scientific Research Grant from the Ministry of Education.

mortality was established after 24 hours. Such test was replicated ten times for each dose with male and female flies separately. The experimental materials were homozygous flies, which were the offspring from crosses between the same chromosomal strains, and heterozygous flies, which were hybrids between two different chromosomal strains. The mortalities were transformed into arc-sine units and analyzed statistically. From the results of analysis of variance, the following conclusion was obtained; 1. there is no significant difference between the levels of resistance of homozygous and heterozygous flies for any chromosomal arrangement, 2. significant differences were observed between different homozygous or heterozygous chromosomal strains, 3. females were significantly more resistant than males, 4. the differences between mortalities at different doses were significant, 5. the interactions between dose and strain, and sex and dose were significant for both homozygous and heterozygous strains.

From these results, it follows that the increase of relative frequency of PP chromosome in California could not be caused by the effect of insecticides such as Dieldrin and might be attributed to the appearance of co-adapted gene complexes including the PP chromosome in the gene pool.

6. *Spontaneous polygenic mutation rate and the genetic structure of natural populations in D. melanogaster*

(Terumi MUKAI and Sadao CHIGUSA)

According to KIMURA's (1959) considerations based upon the classical hypothesis which assumes that most of the genetic load is mutational, the following conclusion may be drawn. Suppose that the number of loci controlling the fitness in *D. melanogaster* is 5000, and the mutation rate in them is 5×10^{-4}/locus/generation (ca. 50 times as large as the spontaneous mutation rate of major genes), then the survival rate becomes e^{-5}. This indicates that only 7 eggs out of 1000 become adults. This result is inconsistent with the actual situation in natural populations. Consequently, a proof that the spontaneous mutation rate of polygenes controlling the fitness is extremely high would be unfavorable to the classical hypothesis. Therefore, an experiment was started for the estimation of the mutation rate of polygenes controlling the viability, which is one of the major components of fitness. The experiment is still in progress but the result at hand will be reported here.

A single second chromosome was sampled from an isogenic line (BURDICK's *W*160) and multiplied to 104 by using *Cy*/*Pm* stock, whose genetic background had been completely substituted by *W*160, and 104 lines were established. In each line, the second chromosome has been maintained through a single male in the cross of $Cy/Pm(♀)\times Cy/+_i(♂)$ (i=1, 2, ..., 104) for the purpose of accumulating spontaneous mutations affecting the viability. In Generation 10, the homozygous viabilities of these lines were tested by the use of *Cy* technique with 4 or 5 replications for each line on the basis of counting about 150,000 flies. Among the 104 lines, 2 lethal and 3 subvital lines in homozygous condition were detected. These were clearly distinguishable from the other quasi-normal lines, on the basis of the distribution pattern of their viabilities.

The degree of differentiation due to polygenic mutations among 98 quasi-normal lines (1 line is missing) was tested in homozygous condition by the aid of the analysis of variance. The result is presented in Table 1. Although it was impossible to find significant differences in the viabilities of the 98 lines by this technique, the probability value was

Table 1. Analysis of variance with respect to the viabilities of 98 quasi-normal second chromosomes in homozygous condition

Source	S.S.	d.f.	M.S.	F
Between lines	0.1355	97	0.001397	1.27
Error	0.3612	328	0.001101	P≒0.06
Total	0.4967	425		

very close to 0.05. Therefore a further analysis was tried.

χ^2 test was conducted to compare in respect to viability the distribution pattern of homozygously quasi-normal lines in Generation 0 with that in Generation 10. In this test, it was assumed that the distribution in Generation 0 was normal with the mean of the mid-point between the highest and the lowest lines in Generation 10 [=(0.3633+0.2814)/2], and the variance (σ^2) which was estimated from the error variance ($\hat{\sigma}_e^2$) of Table 1 by the following appropriate procedure;

$$\hat{\sigma}^2=\hat{\sigma}_e^2\times\frac{\text{harmonic mean of fly number per one replication in one line}}{\text{harmonic mean of fly number per one line as a whole}}$$

The value of χ^2 thus obtained, 26.72 for d.f.=4, is highly significant, or the difference between the two generation is statistically significant. This indicates that there was a heterogeneity in the homozygous viabilities among 98 quasi-normal lines, and this heterogeneity was caused by many mutations having small effects on viability.

7. *Two cases of heterosis associated with the recessive genes sepia (se) and expanded wing (ew) in Drosophila melanogaster*

(Sadao CHIGUSA and Terumi MUKAI)

Two cases of single gene heterosis due to the recessive genes *sepia* and *expanded wing* have been tested. The latter was discovered in MUKAI and BURDICK's (1959) Population 1 and located at about 3-49±.

Four artificial population (Pop. A-1, Pop. A-2 for *se* and Pop. B-1, Pop. B-2 for *ew*) were established, and the gene frequencies of Pop. (A, B)-1 and Pop. (A, B)-2 were 0.5 and 0.1, respectively. The homozygosities of the genetic backgrounds in Pop. A's are extremely low, while those of Pop. B's are high. The populations have been maintained for 13-23 generations by the so-called Pearl's method. The frequencies of recessive homozygotes were estimated almost in every generation. Four populations are reaching equilibria where the frequencies of recessive homozygotes are about 0.07 in Pop. A's and about 0.05 in Pop. B's. These phenomena are supposed to have been caused by the superiority of the heterozygote over either homozygote.

The components of the fitnesses of heterozygotes were estimated and the superiority of heterozygotes seems to be due to better female fecundities of *se*/+ and *ew*/+ than those of the wild-type homozygotes, respectively. The relative female fecundities of *se*/+ and *ew*/+ to those of the wild-types were about 1.38 and 1.31, respectively. The experiments are still in progress.

8. *Transfer of the "sex-ratio" condition from D. willistoni to other species of Drosophila*

(By Bungo SAKAGUCHI and Donald F. POULSON*)

The maternally transmitted abnormal sex-ratio condition in *D. willistoni* was demonstrated by Malogolowkin, Poulson and Wright (1959) to be transferable to previously normal strains of this species. Furthermore Malogolowkin *et al.* (1960, 1961) demonstrated that the "sex-ratio" (SR) condition of *D. willistoni* can be transferred to other species, such as *D. equinoxialis* and *D. nebulosa*.

This has been accomplished for the female recipients of two other species, *D. melanogaster* and *D. pseudoobscura*. In the case of *D. melanogaster* two wild type strains, Oregon-R inbred and Sevelen inbred, a

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

triploid strain carrying attached-X chromosomes and a diploid strain with the same attached-X chromosomes ($\underline{y^2 scw^a ec}/Y$ ♀ $\times y^{31d} sc^8 dmB$ ♂) were used as recipients females. Hemolymph (0.05–0.08μl) from SR females of *D. willistoni* was injected by micropipette into the abdomens of 1-to 2-day old virgin females of the recipient strains. The injected females were mated individually to males from their own strains and transferred to new culture medium every two days.

Unisexual progenies began to appear in many instances even in the early broods of each experimental series in the case of *D. melanogaster*. From these, lines of SR have been established by mating with males from the corresponding normal strains. The maintenance of SR condition in some of these lines proved to be much more stable than in others. One of the best is a line derived from the attached-X strain demonstrating that the Y chromosome is not of primary importance in the lethal disturbances associated with the SR agent. That the persistance of the SR agent in *D. melanogaster* is dependent on the genotype of the flies is clearly demonstrated; for example, transfers to an inbred strain of Oregon-R took well and have shown a high level of stability in subsequent generations, while transfers into an inbred strain from Sevelen are much less stable suggesting a high degree of host resistance. The same results were obtained when the chromosomes of the attached-X strain and Oregon-R or Sevelen strain were exchanged.

The persistance of the SR agent of *D. willistoni* in *D. pseudoobscura* has shown a high level of stability in subsequent generations.

It is indicated from this and other results (in this Annual Report) that the genotypes both of host and SR agent are of prime importance in the establishment, stabilization, and persistence of SR in the hosts.

9. *Comparative analysis of transmissive efficiency and pathological effects of "sex-ratio" agents of different origin in Drosophila*

(By Bungo SAKAGUCHI and Donald F. POULSON*)

A maternally transmitted condition known as "sex-ratio" (SR) has been demonstrated in a number of species from different geographical regions of *Drosophila*, in particular *D. bifasciata*, *D. equinoxialis*, *D. nebulosa*, *D. paulistorum*, *D. prosaltans*, and *D. willistoni*.

That the SR condition may be affected and even cured by elevated temperatures was first shown by Magni (1954) for the SR of *D. bifasciata*

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

and by Malogolowkin (1959) for the SR of *D. equinoxialis*. However, the SR of *D. willistoni* and *D. paulistorum* showed no such temperature sensitivity and none was apparent in the SR of *D. nebulosa*. Thus differences among SR agents of different origin are suggested, although the host species may also be involved in the temperature response.

In the present report the differences between the SR agents of different origin have been examined from the view points of transmissive efficiency into subsequent generations of the host flies and pathological effects of the agents. The SR strains of the four species, *D. bifasciata*, *D. equinoxialis*, *D. nebulosa* and *D. willistoni*, were used as the donors of SR agent and the inbred strain, Oregon-R, of *D. melanogaster* was used as a recipient for testing.

The SR agent of *D. nebulosa* is readily transferred and incubation from the time of injection to the manifestation of the SR agent is very short requiring only a few days. The killing effect of the agent against male zygotes is remarkably strong and has also proved to be very stable and persistent in subsequent generations in the females of the new host. The SR agent of *D. willistoni* is readily transferred having the same incubation time as the agent of *D. nebulosa* but is somewhat less stable and persistent in subsequent generations. But the agent of *D. equinoxialis* is much more difficult to transfer and shows very considerable instability and is very hard to maintain in *D. melanogaster* hosts. Furthermore other effects produced by the SR agents of the three species have demonstrated that there are differences in the patterns of developmental disturbances produced by each of them when transferred to new hosts.

In the case of the SR agent of *D. bifasciata* there has so far been no success in transferring the condition within the same species or to *D. melanogaster*. Poulson and Sakaguchi (1960, 1961) were able to show that the SR agents are small spirochetes, presumably *treponemata*, in each of the three species which they studied, *D. equinoxialis*, *D. nebulosa* and *D. willistoni*. In each case the transfer of the SR condition into a different strain of the same or of a different species was correlated with the presence of transferred *treponemata* in the hemolymph of SR females of the recipient strain and its descendants. On the other hand in the case of the SR agent of *D. bifasciata* it has not so far been possible to demonstrate the presence of typical spirochetes in the hemolymph of SR females. This suggests a wholly intra-cellular SR agent, highly integrated with the developmental system of the host.

These facts indicate differences among the SR agents of different species of *Drosophila* and from different geographical regions. It may then be possible to make clear the origin of SR agent and the significance of its biological evolution from a more detailed analysis of the differences among

the SR agents derived from various species of *Drosophila* collected from different geographical regions.

10. *Lethality and low viability induced by the segregation distorter locus (symbol SD) in Drosophila melanogaster*

(By Yuichiro HIRAIZUMI)

In a heterozygous *SD* male, *SD* breaks its partner chromosome in some stage of meiosis and thus more than 50% (usually 95% or more) *SD*-bearing chromosomes are transmitted to the next generation. Here the question arises whether the SD^+-bearing chromosomes found in the F_1-generation are 1) those which were not affected by *SD* action or 2) those which recovered from the break. If 2) is the case, then we may expect some changes, perhaps viability reduction, in the SD^+-bearing chromosomes from the heterozygous *SD* males. Accordingly, *SD/cn bw* (and SD^+/*cn bw* as a control) males were crossed to *cn bw/In*(2L) *Cy cn bw* females. The *cn bw* chromosomes in the heterozygous *SD* and SD^+ males in P-generation were derived from a single lethal-free chromosome and the remaining genetic background had been made uniform before experiments. From the F_1 of these matings *cn bw/In*(2L) *Cy cn bw* males were chosen to cross individually to *cn bw/In*(2LR) *Cy* females, and the F_2 *cn bw/In*(2LR) *Cy* sibs from each F_1 mating were mated to test the homozygote's viabilities of the *cn bw* chromosomes in comparison with their *Cy* heterozygotes. For the significance test the observed percentage of the *cn bw* homozygotes ($=r$) found in the F_3 in each culture vial was transformed according to the relation $r=\sin^2 R$. The results are summarized in Table 1. Figures

Table 1.

Exp. set No.	Original SD $\bar{R}$ (%)	No. of cultures	Recombinant SD $\bar{R}$ (%)	No. of cultures	SD^+ $\bar{R}$ (%)	No. of cultures
1	31.16 (26.8)	8 (+1 lethal)	31.49 (27.3)	20 (+1 lethal)	34.34 (31.8)	18
2	31.69 (27.6)	12	32.50 (28.9)	38	33.52 (30.5)	37
3	31.80 (27.8)	16	32.42 (28.8)	52 (+1 lethal)	33.38 (30.3)	31
Total	31.62±0.34 (27.5)		32.30±0.28 (28.6)		33.64±0.27 (30.7)	

in brackets indicate the percentages of *cn bw* homozygotes corresponding to each $\bar{R}$ (=average of R) value. The lethal-bearing *cn bw* chromosomes (indicated as +1 lethal etc.) were excluded from computing $\bar{R}$. The

number of chromosomes examined was very small but it is worth noting that 3 lethals were found among 148 chromosomes in the experimental sets while none appeared among 86 chromosomes in the control sets. Each experimental set was made at a different time, but in each set the *cn bw* chromosomes from the *SD*/*cn bw* male showed, on the average, reduced viabilities ($p<0.01$). It is interesting to note that the original *SD* lines (=*SD*-72 and *SD*-5; strong *SD*) caused more viability reduction than the recombinant *SD* (weak *SD*) lines did, although the difference was not statistically significant. Results of mapping tests suggested that all of the *SD*-induced lethals were located near the SD^+ locus (within 5% crossover units around SD^+).

The detailed mechanism for lethality and low viability induction is not fully understood, but a small deletion accompanied by the breakage-reunion event could be responsible.

11. *Cytological observation of the SD region in the salivary gland chromosome of Drosophila melanogaster*

(By Yasuko TONOMURA (TOYOFUKU))

It has been reported by Sandler and Hiraizumi that the segregation distorter of *D. melanogaster* is a complex locus (symbol *SD*) which locates close to the centromeric heterochromatin of chromosome II (probably in the right arm). The subject of the present report is a study of structure of the *SD* region by means of the salivary gland chromosome analysis.

(1) Inversions outside the *SD* region.

Sandler *et al.* (1959) reported that there were two types of the original *SD*-bearing chromosome, i.e., 1) singly inverted (=*SD*-72) and 2) doubly inverted (=*SD*-5), but they didn't indicate the positions of these inversions. The present investigation shows that the inversion in *SD*-72 includes sections from 51E to 55F in the right arm of chromosome II. In addition, *SD*-5 line carries one more inversion covering sections from 46A to 48F in the same chromosome.

(2) Structural abnormality at the *SD* region.

In addition to the inversion mentioned under 1), the *SD*-72 line carries a small aberration involving sections from 40A(?) (left arm) to 42A(right arm) in chromosome II. On the other hand no such aberration was observed in the *SD*-5 line. Since both *SD*-72 and *SD*-5 lines exhibit strong *SD* action, it is evident that this aberration is independent of *SD* action. The nature of the aberration has not yet been fully understood, but it is suggested that this is either 1) a small pericentric inversion or 2) a dupli-

cation. Since the aberrant segment is separated into two complementary recombinants by crossing over (Sandler and Hiraizumi, 1960), the second possibility, duplication, seems to be more likely. If this aberration is an inversion, then the "recombination" phenomenon within this region is not due to the usual crossing over, but has to involve some other complex mechanisms. Further cytological observations are in progress.

12. *Migratory activity of Drosophila as a genetic character*

(By Takashi NARISE and Kan-Ichi SAKAI)

A brief account of conclusions drawn from our genetic study on the migratory activity of *Drosophila melanogaster* is given as follows:

1) Genetic effect of chromosome substitution.

The second and third chromosomes of a weak migratory Samarkand strain were substituted by the corresponding chromosomes of IG-28 or IG-25. IG-28 is characteristic of high mass-migratory activity while IG-25 is high in random-migratory activity.

The genetic effect of the second and third chromosomes of IG strains on mass-migratory activity of Samarkand strain was quite apparent. The third chromosome was genetically more effective than the second. Random migratory activity of Samarkand was also increased either by the second or the third chromosome of IG strain, the third chromosome being again more effective than the second.

2) Observation of migratory activity in F_1 hybrids.

A number of strains differing in respect of mass- or random-migratory activity were reciprocally hybridized.

i) Mass-migratory activity of F_1 hybrids was in most cases intermediate between the two parents, though in some specific hybrids high heterotic effect was observed.

ii) Random-migratory activity was also found to show a partial dominance toward the side of the active parent. A remarkable heterotic effect was observed in some specific combinations of strains. Reciprocal difference was detected in all cross-combinations.

Thus, it has been concluded that (1) tendency to migration, either random or mass, is controlled by genes of rather polygenic nature, (2) the third chromosome contained more effective genes than the second and (3) a reciprocal difference was observed in the case of random-migration.

13. *Stimulating effect of a foreign genotype on migration in Drosophila*

(By Takashi NARISE and Kan-ichi SAKAI)

Flies of the mutant *vg*-strain of *Drosophila melanogaster* are very slow in migratory activities when they are in crowds by themselves. The present report deals with the discovery of a stimulating effect of wild flies, introduced into a population of *vg*-flies, on their migrating rate.

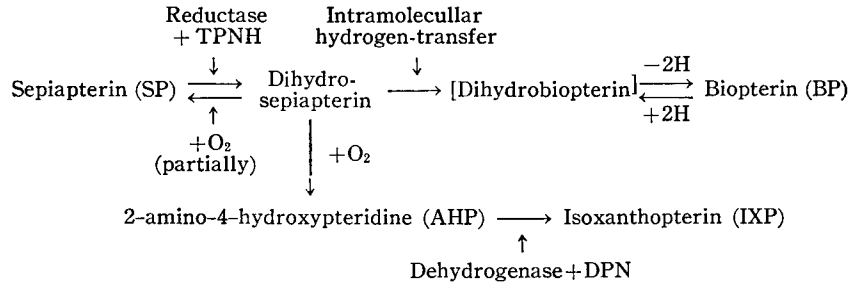
In this experiment, a mixture of *vg* and wild flies was used in proportion of 0 : 100, 10 : 90, 20 : 80, 30 : 70, 40 : 60, 50 : 50, 60 : 40, 70 : 30, 80 : 20, 90 : 10 and 100 : 0. The total number of flies in each mixture was made constant at 100. Migratory activity was measured in terms of the number of migrated flies from the original tube to the surrounding tubes. For the details about the method of experiment, the reader may refer to previous issues of this Annual Report. From this experiment, it was found that an admixture even of 10% of wild flies caused a sudden increase in migratory activity of otherwise inactive *vg* flies.

The migration of wild flies which is naturally higher than that of *vg* flies slightly increased in every mixture. The reason for the unexpected change in the migration rate of flies is not yet known.

14. *Pterine metabolism* in Drosophila melanogaster*

(By Toshifumi TAIRA)

On the basis of our recent data, the biochemical relationships among pteridines found in *D. melanogaster* are summarized in the following scheme :



At the present time, it is obvious, from our previously published reports, that the activities of reductase and dehydrogenase of these path-

* "Tetrahydrosepiapterin" expressed in our previous papers was corrected into "Dihydrosepiapterin".

ways are genetically controlled. The reductase step is restricted by Hn^{r-3} gene and the dehydrogenase step is suppressed by *ry* and *ml—l* genes. Isoxanthopterin in the above scheme seems to be the final product of pteridine metabolism, but biopterin is assumed to be an important intermediate rather than a final metabolite. The latter opinion is supported by two data, namely 1) the evidence that biopterin (possibly a derivative) is firstly revealed in the early drosophila embryo and 2) the evidence obtained by Ziegler and Nathan (1961) who found that a biopterin derivative (possibly riboside) found in *Drosophila* is converted into a sepiapterin-like substance through a tetrahydrobiopterin derivative by the supernatant extracted from *Critidia*, which requires biopterin as a growth factor. Therefore, they have suggested that the tetrahydrobiopterin derivative might be a precursor of sepiapterin. However, we have found that the supernatant prepared from *Drosophila* can not catalyze the reduction of free biopterin. Such contradictory results might be caused by the difference of the substrates and/or the experimental organisms in these two experiments, and this contradiction may be explained by clarifying whether or not the biopterin derivative is actually produced from a mixture of free biopterin and a monosaccharide.

The biosynthesis of drosopterin (DP) is our main project. As mentioned in our previous reports, the biosynthetic pathway of drosopterin is completely inhibited by *se* gene and also partially inhibited by *cl*, Hn^{r-1} and Hn^{r-3} genes, as summarized in Table 1.

Table 1. Relative contents of pteridines in the eyes of *D. melanogaster*

Strains	SP	BP	AHP	IXP	DP
Ore-R	±	±	±	±	+++
v and *cn*	±	±	±	±	+++
se	+++	+++	++	±	±
cl	++	+++	+++	±	++
Hn^{r-1}	+	++	++	±	++
Hn^{r-3}	++	+++	±	−	+

From this table and the given above scheme, it is assumed that a tetrahydro-form of pteridine might be the precursor of drosopterin. However, the chemical structure of drosopterin is not yet clarified. Therefore, the determination of the chemical structure of drosopterin is the immediate object of our study with the close cooperation of Dr. Matsuura (Dept. Chem., Nagoya Univ.).

15. *The fourth case of translocation between the W chromosome and an autosome in the silkworm*

(By Kimiharu ONIMARU and Yataro TAZIMA)

Three translocations involving the W chromosome have been known in the silkworm, *i.e.*, W·II (Tazima, 1941), W·III (Hasimoto, 1948) and W·X (Tazima *et al.*, 1951). In all these cases a translocated piece of the autosome behaves as if it were a part of the W chromosome in its transmission.

In the course of our study of spontaneous mutations, a new translocation was discovered between W and the fifth chromosome that bears the $+^{re}$ locus. The most characteristic feature of this translocation is that the translocated piece behaves as the fifth chromosome in contrast with the hitherto reported three cases.

Among 863,872 observed heterozygotes both for *pe* and *re*, + +/*pe re*, 87 *pe* individuals were found as presumable mutants for egg color. They were raised and 35 attained to maturity, among them 16 ♀♀ and 19 ♂♂. In order to discriminate the mutation-bearing chromosome from its homologue (*pe re*), these *pe* females were crossed to +*re* males. In F_1 segregation occurred as expected with respect to egg color at the ratio of 1 normal : 1 red. After discarding the red eggs, only normal eggs were left in each batch for further raising. Among 8 mutant lines thus raised 7 showed the normal sex-ratio of 1 : 1, whereas in the remaining one all individuals were female. This clearly suggested that translocation must have occurred in the ancestor of this line.

Regarding the occurrence of translocation two possibilities may be considered : (A) translocation of a small piece of the fifth chromosome to W and (B) translocation of a small piece of W that contains the female determining gene to the fifth chromosome. The breeding experiments with this translocated chromosome led us to assume that the latter possibility is more plausible than the former. If this proves true, the important conculsion might be drawn that the female determining gene or genes are located in a very limited segment of the W chromosome.

16. *Studies on the manifestation mechanism of dilute lemon gene in the silkworm*

(By Mitsuo TSUJITA)

A silkworm strain showing dilute yellow larval color was derived from the F_2 generation of the cross between YD_4 yellow lethal (lem^l/+) and

lemon (*lem/lem*) individuals. The results of mating this mutant (*d-lem*) with another dilute lemon mutant which was reported by Chikushi (1961) have proved that these two genes are allelic and that this character is due to the presence of gene *d-lem* on chromosome II in addition to lemon gene on chromosome III. Thus, the genetic constitution of the lemon strain was confirmed as *lem/lem*; $+^{d\text{-}lem}/+^{d\text{-}lem}$ and that of dilute lemon strain as *lem/lem*; *d-lem/d-lem*. For the purpose of studying the manifestation mechanism of dilute lemon gene the present experiments were carried out.

1. *Measuring of the amount of pteridines*

For the separation of yellow pigment and isoxanthopterin two-dimensional paper chromatography was used. Full grown larvae in the fifth instar of normal, lemon and dilute lemon were used as materials. The hypodermis taken from 5 larvae of each strain was dried and ground into powder. 100 mg of the powder and 0.7 ml of 0.5 N perchloric acid were mixed, and heated for two minutes on a water bath at 80°C. After centrifugation, 0.3 ml of each supernatant was placed on filter paper (Toyoroshi #51 40×40 cm) and chromatographed by the solvent of a mixture of *n*-propanol and 1% aqueous ammonia (2 : 1) for the first and 5% aqueous acetic acid for the second dimension. Each separated pteridine was eluted with 5% sodium bicarbonate solution. The relative amounts of pteridines of each strain were estimated by the intensity of fluorescence. The standard fluorescence was determined by a solution of 0.5 g. of synthesized isoxanthopterin in 1 ml of 0.5% sodium bicarbonate solution. As the yellow pigment is very unstable in alkali solution, the amount of this substance showing yellow fluorescence was represented by the quantity of its photo-decomposed pteridine, 2-amino-4-hydroxypteridine-6-carboxylic acid, showing blue fluorescence. Measuring of each sample was repeated 3 times.

i) *Amount of yellow pigment*

The amount of yellow pigment extracted from lemon and dilute lemon larvae in full grown stage of 5th instar is given in the following table.

Genotype \ Amount of yellow pigment	μg/100 mg dry weight
lem/lem; $+^{d\text{-}lem}/+^{d\text{-}lem}$	13.5 ± 1.0
lem/lem; *d-lem/d-lem*	4.5 ± 0.5

The amount of yellow pigment obtained from lemon larvae is about three times larger than that from dilute lemon larvae.

ii) *Amount of isoxanthopterin*

The relative amount of isoxanthopterin extracted from normal, lemon and dilute lemon larvae in full grown stage of 5th instar is shown in the following table.

No. of samples / Genotype	1	2	3	4	5	Total	Mean	μg/100 mg dry weight
+/+	50	52	51	49	51	253	50.6	16±2.5
lem/lem; +/+	15	16	17	14	15	77	15.4	5±0.5
lem/lem; *d-lem/d-lem*	13	12	10	12	10	57	11.4	4±0.5

The amount of isoxanthopterin obtained from the lemon larvae as well as from the dilute lemon larvae is far smaller than that obtained from the normals.

2. *Injection of AHP into the body cavity of 5th instar larvae*

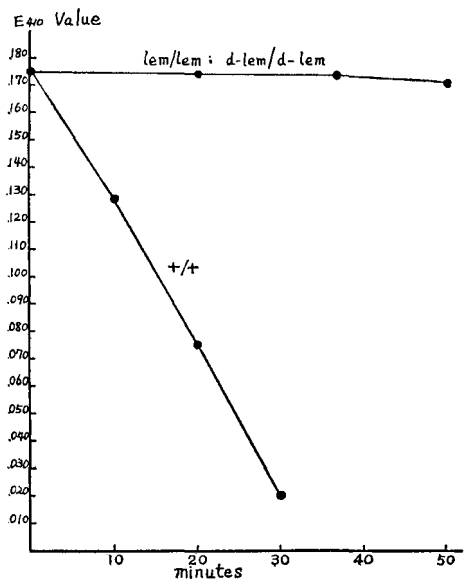


Fig. 1. Enzyme activity of adipose tissue of normal and dilute lemon larvae.

A small amount (0.2 cc per individual) of 2-amino-4-hydroxypteridine (AHP) was injected into the body cavity of each of 30 normal and 30 dilute lemon larvae on the 3rd day of 5th instar and the amount of isoxanthopterin produced in their hypodermis was periodically measured

after 1, 2, 4, 8 and 24 hrs. However, no significant change in the amount of isoxanthopterin could be observed in the larvae of both strains. It seems, therefore, that the permeability of the hypodermal cells of dilute lemon larvae to AHP or isoxanthopterin is not different from that of the normals.

3. *The activity of pteridine reductase*

Various tissues taken from larvae, pupae and imagoes of normal, lemon, and dilute lemon were used as materials. 0.6–1.0 g of fresh tissue was

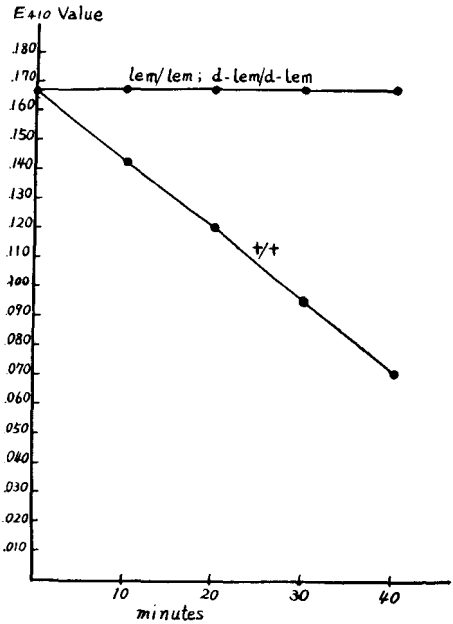


Fig. 2. Enzyme activity of integument ot normal and dilute lemon larvae.

mixed with a phosphate buffer solution three times the tissue weight, homogenized under ice cooling and centrifuged at 12,000–18,000 r.p.m. according to the nature of the tissue cells. The supernatant was used as a crude enzyme solution. The yellow pigment purified from the lemon larvae having genotype *lem/lem*^{l} and purified sepiapterin from the sepia strain of *Drosophila melanogaster* were used as substrate. The activity of pterine reductase of the enzyme solution mentioned above was measured according to the procedure described in my previous paper (Tsujita 1961). No enzyme activity could be detected in several tissues of the larvae, pupae and adults of dilute lemon. But, adipose tissue and integument exhibited a weak or very weak enzyme activity. Some of the experi-

mental results are shown in Figs. 1. and 2. Thus, no significant difference has been found in the enzyme activity between lemon and dilute lemon strains.

4. *Function of d-lem gene*

In the dilute lemon larvae the activity of pterine reductase acting on the step from yellow pigment (dihydropterin) to tetrahydropterin is very weak just like that of lemon larvae. In spite of this fact the amount of yellow pigment accumulated in the hypodermis of the former is very small, amounting to about one third of that of the latter. To explain this small accumulation of yellow pigment in dilute lemon larvae two hypotheses can be assumed. One of them is that owing to the defective function of *d-lem* gene the amount of the precursor of yellow pigment is small. The other hypothesis is that though the yellow pigment is normally produced, a large part of it is lost from the hypodermal cells owing to the defective system caused by the *d-lem* gene which keeps this compound within the cells. If the first hypothesis were correct, the production of isoxanthopterin in the hypodermal cells of the larvae with the genotype $+^{lem}/+^{lem}$; *d-lem/d-lem* should be far smaller than that in the larvae with the genotype $+^{lem}/+^{lem}$; $+^{d\text{-}lem}/+^{d\text{-}lem}$. To test this possibility a cross between normal and dilute lemon strain was carried out in order to find out whether or not segregation occurs as to the amount of isoxanthopterin among the F_2 individuals. However we could not get clear-cut results. It is inferred from the experimental results so far obtained that the second hypothesis is more reasonable than the first one. Therefore, we are now planning to study the exact mechanism according to the former hypothesis.

17. *Amount of isoxanthopterin in the larvae of the silkworm*

(By Mitsuo TSUJITA)

Glassman and Mitchell (1959) who studied a mutant of *Drosophila* deficient in xanthine dehydrogenase suggested that the *ma-1*$^+$/*ma-1*$^+$ females are passing a substance which is not produced in *ma-1* flies but can be transmitted by this mutant to the progeny with the egg plasma. They thought that this substance may be a precursor of xanthine dehydrogenase. We succeeded in showing a clear-cut maternal effect of $+^{lem}$ on pterine reductase in the silkworm. In order to obtain information on the maternal effect of the substances pertaining to pteridine metabolism, studies of the amount of isoxanthopterin, an end product of pteridine metabolism, produced by single larvae, were carried out.

Materials and methods: Normal larvae (Daizo, P_{22}) and larvae of F_1 and F_2 of a hybrid between A strain producing a large amount of isoxanthopterin and B strain producing a small amount of this compound were used as materials. To measure the amount of isoxanthopterin contained in the hypodermis of single individuals, each of the larvae was cut open from the dorsal side and almost all of the inner organs were removed such as digestive tube, Malpighian tubules, adipose tissue, silk glands and others.

The remaining hypodermal tissue was spread on a filter paper and dried. After drying the weight of each individual hypodermis was determined. Each larval skin was cut into two or three pieces, which were placed in a small test tube with 2 cc distilled water and heated for 20 minutes on a water bath at the temperature of 95°–100°C in order to extract isoxanthopterin. The extraction was repeated twice and the total amount of isoxanthopterin in the extracted solution was measured by Beckman's spectrophotometer. This procedure is relatively simple so that a large number of larvae can be examined for the amount of isoxanthopterin.

Experimental results: 1. *Difference of the amount of isoxanthopterin among races or strains*

It was found that the amount of isoxanthopterin varied from race to race or from strain to strain. An example is shown in the following table.

Table 1. Amount of isoxanthopterin in the hypodermis of a full grown larva

Items examined / Strains	Weight of hypodermis (average) mg	Relative amount of isoxanthopterin (average) 50≒6γ	R. amount of IXP / Weight of hypodermis (average)
Daizo	45.9±7.0	76.6±15.5	1.7±0.4
P_{22}	52.5±6.5	65.7±12.0	1.3±0.2
S-strain	48.6±4.3	101.6± 7.1	2.1±0.3

2. *Change in the amount of isoxanthopterin in development stages*

Using P_{22} and a hybrid the amount of isoxanthopterin contained in each larva was determined in the course of development from the third day of the third instar to the end of the fifth instar. There was variation in the size of larvae at the same stage, so that the weight of hypodermis of each individual was ascertained and the ratio isoxanthopterin amount/hypodermis weight was calculated. The experimental results are shown in Fig. 1.

Fig. 1 shows that the absolute amount of isoxanthopterin increases day by day with the development from the beginning to the end of each of the 4th and 5th instars, but decreases to some extent during the 4th

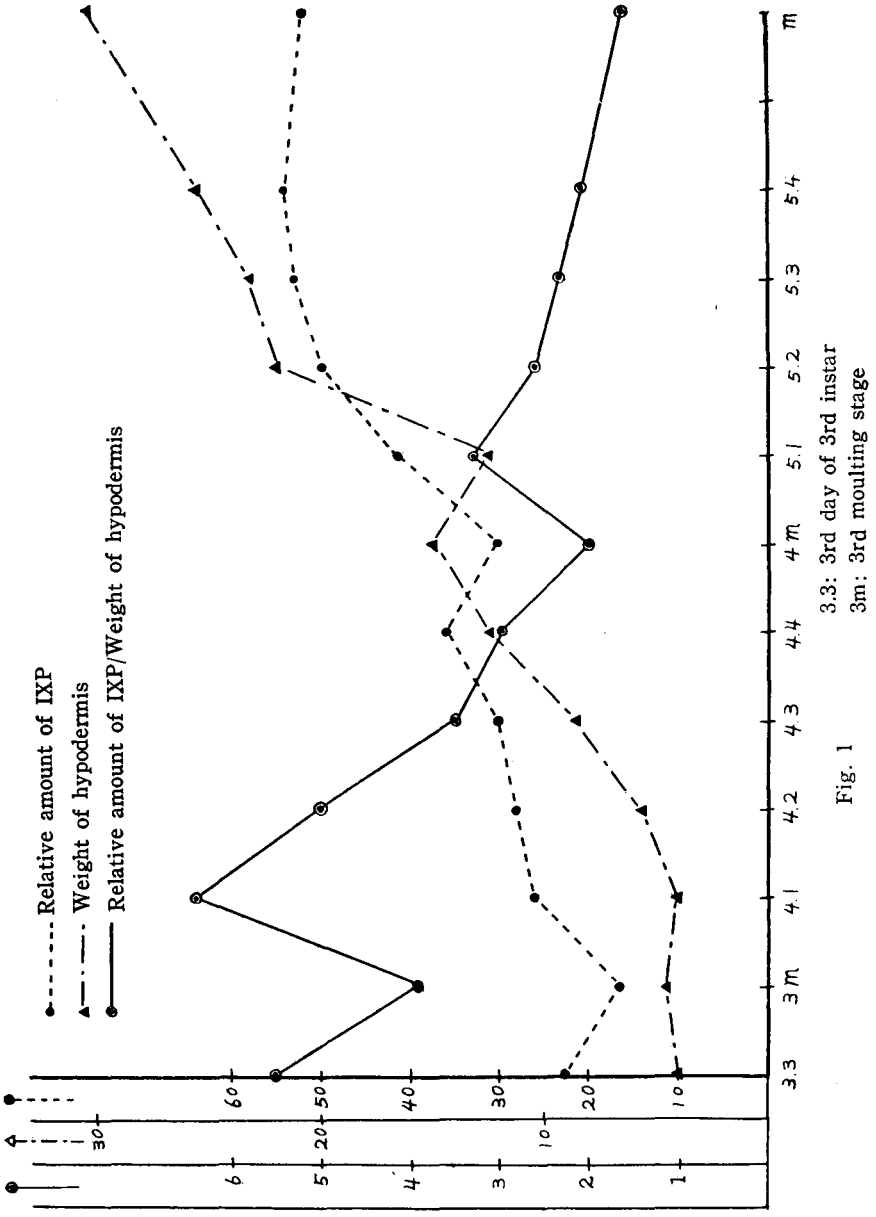


Fig. 1 3.3: 3rd day of 3rd instar
3m: 3rd moulting stage

moulting stage and increases again markedly immediately after moulting. Although the weight of the hypodermis is smallest at the beginning of

the fourth and fifth instars it gradually increases with the development and reaches the maximum at the end of these instars, and then it slightly decreases immediately after the fourth moulting. The ratio isoxanthopterin amount/hypodermis weight in single individuals is highest at the beginning of the fourth and fifth instars and gradually decreases with the development and attains the minimum at the end of these instars.

It is concluded from the experimental results so far obtained that the appropriate stage for the comparison of races or strains, or for surveying segregation of F_2 larvae in the cross between A and B strains with respect to isoxanthopterin amount and its relation to hypodermis weight, is the beginning and middle stages of the fourth or fifth instar.

2. *Results of the reciprocal crosses between A and B*

Reciprocal crosses were made between A strain producing large amounts of isoxanthopterin and B strain producing small amounts of this compound. Three batches of each of the reciprocal crosses were reared and using 30 larvae of the fifth instar in each batch the amount of isoxanthopterin was determined. It was observed that the mean value of isoxanthopterin amount and its ratio to hypodermis weight in F_1 larvae, when A was the female parent, tended to be larger than in the reciprocal cross. Furthermore, the same tendency was confirmed for the larvae of the F_2 generation of the same crosses. However, whether or not cytoplasmic effects are involved is not clear. The study will be continued.

18. *Pteridines in the compound eyes of the silkworm moth, Bombyx mori*

(By Mitsuo TSUJITA)

Five strains of the silkworm with the following genetic constitutions were used as materials for the detection of pteridines in the compound eyes of the moths.

Materials	Genotype	Color of compound eyes	egg color
1	$+^{lem}/+^{lem}; +^{d\text{-}lem}/+^{d\text{-}lem}; +^{w_1}/+^{w_1}$	black	black
2	$lem/lem; +^{d\text{-}lem}/+^{d\text{-}lem}; +^{w_1}/+^{w_1}$	black	black
3	$lem/lem; d\text{-}lem/d\text{-}lem; +^{w_1}/+^{w_1}$	black	black
4	$lem/lem; +^{d\text{-}lem}/+^{d\text{-}lem}; w_1/w_1$	yellow	white
5	$lem/lem; +^{d\text{-}lem}/+^{d\text{-}lem}; w_2/w_2$	light reddish brown	light reddish white

Two-dimensional paper-chromatography was used for the separation of several pteridines found in the compound eyes of *Bombyx mori*. The compound eyes of 70 moths of each strain were removed from the heads and homogenized with 0.5 ml of 30% acidic ethanol (pH 2.0) and heated for two minutes on a water bath at 80°C. After centrifugation, 0.05 ml of each supernatant was put on filter paper (Toyo-roshi #51, 40×40 cm) and chromatographed by the solvent of a mixture of n-propanol and 1% aqueous ammonia (2 : 1) for the first and 5% aqueous acetic acid for the second dimension. The experimental results are as follows:

(1) *Normal compound eyes* ($+^{lem}/+^{lem}$; $+^{d\text{-}lem}/+^{d\text{-}lem}$; $+^{w_1}/+^{w_1}$). Several spots were separated and some of them identified as isoxanthopterin, biopterin and as xanthopterin-like substance. Besides, a large greenish-blue spot and a small bluish green spot were observed.

(2) *Compound eyes of the moths with the genotype lem/lem*; $+^{d\text{-}lem}/+^{d\ lem}$; $+^{w_1}/+^{w_1}$. Spots of isoxanthopterin, biopterin and xanthopterin-like substance were observed. A clear-cut purplish-blue spot was observed near the spot of xanthopterin-like substance. This may be a characteristic of the *lem/lem* strain lacking the gene for white egg color.

(3) *The compound eyes of the moths with the genotype lem/lem; d-lem/d-lem.* Separation of isoxanthopterin, biopterin and xanthopterin-like substance was made. A purplish-blue spot, smaller than that found in material (2), was observed.

(4) *Yellow colored compound eyes of the moths with the genotype lem/lem*; w_1/w_1. In addition to the spots of isoxanthopterin, biopterin and xanthopterin-like substance a large spot appeared showing yellow fluorescence. This spot showed yellow color in visible light but in day light it changed into its photo-decomposed pteridine, 2-amino-4-hydroxypteridine-6-carboxylic acid. A large spot of kynurenine and a small spot of 3-hydroxy-kynurenine appeared. The purplish-blue spot noticed in the above-mentioned two materials (2) and (3) could not be observed.

(5) *Compound eyes of the moths with the genotype* (*lem/lem*; w_2/w_2). Although almost the same pattern of chromatogram as that of material (4) was observed, the spot of yellow pigment was smaller. The reason why the eggs with this genotype show light reddish brown color is not clear. It may be due to the presence of some other similar pigment in addition to dihydropterin. Further studies on the eye pigments of this type are necessary.

Briefly, it may be said that each of the five strains has its own feature in respect to the pteridines of the compound eyes. The yellow pigment which is accumulated in the larval hypodermis can be scarcely detected in the compound eyes of the genotype *lem/lem*; $+^{w_1}/+^{w_1}$. However, in the presence of the gene for white egg color the compound eyes show

distinctly yellow or light reddish brown color owing to the accumulation of large amounts of yellow and some other similar pigments.

19. *Influence of temperature on the syntheses of contractile proteins in Triturus embryo**

(By Yoshito OGAWA)

The influence of temperature on the syntheses of contractile proteins, actin and myosin, in early embryos of *Triturus pyrrhogaster*, BOIE was examined.

Approximately 1,000 embryos were divided into five equal groups which were raised separately at five different temperatures (10, 15, 18, 20 and 25°C). The determination of the first detectable trace of actin and myosin formation in embryos was carried out by serological technique as before in the period of 12 hrs. after fertilization.[1),2)] Anti-sera against G-Actin and myosin were prepared by injecting intravenously into rabbits a solution of G-Actin and myosin, isolated from the skeletal muscle tissue of *Triturus pyrrhogaster* by Szent-György's method, and were raised to their serological specificity by resorption tests with saline extracts of liver, spleen and skin tissue of *Triturus*. Titres of both sera were adjusted to 1 : 512 before the precipitin reaction with a saline extract of embryos. Constant volumes of sera and progressively decreasing amounts of antigen were used in order to avoid an excess of the latter.

It was found that actin first became detectable 84, 120, 132, 144 and 156 hrs. after fertilization in embryos raised at 25, 20, 15 and 10°C., respectively. As to myosin formation the first detectable trace was found 108 hrs. after fertilization in embryos raised at 25°C., 168 hrs. when raised at 20°C., 180 hrs. at 18°C., then again 168 hrs. at 15°C. and 132 hrs. at 10°C., as shown in Fig. 1. Thus, actin formation in early embryos was markedly suppressed with dropping temperature under the above experimental conditions. On the contrary, myosin formation was most strikingly suppressed at 18°C. and from there on was clearly promoted independently of falling or rising temperature.

This finding is unexpected but the result is noteworthy, because the temperature of the natural breeding season of *Triturus* is 18°C. It may be imagined that many metabolic pass-ways are in competition for the materials they need to keep a balance necessary for the growth of the embryo. For instance, there may be a metabolite present which at the

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

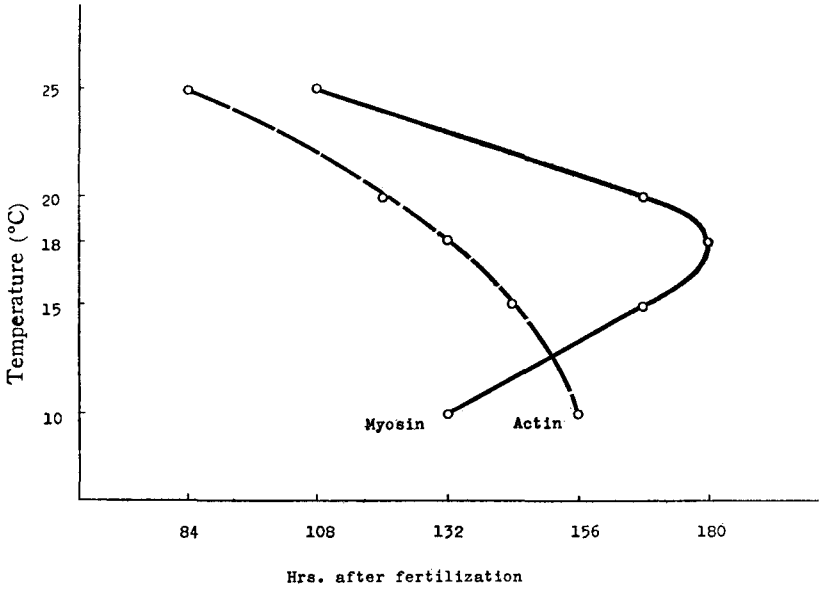


Fig. 1. The influence of temperature on the syntheses of muscle proteins in the early embryo of *Triturus pyrrhogaster*.

optimum temperature for growth appropriates the materials necessary for myosin formation.

The order of actin and myosin formation in embryos raised at 10°C is opposite to that found in natural development. This result furnishes another evidence of the independence from each other of actin and myosin formation as already pointed out in another report by this writer.[2),3)]

1) OGAWA, Y. Nature **182** (4645): 1312. 1958
2) OGAWA, Y. Nature **186** (4718): 77. 1960
3) OGAWA, Y. Med. and Biol. **58**: 185. 1961

20. *Protective action of sodium-glucuronate on the formation of the contractile muscle protein, actin, inhibited by X-ray irradiation in Triturus embryo**

(By Yoshito OGAWA)

When the effect of sodium-glucuronate on the growth of animal embryos was studied, it was found that those raised in the solution of sodium-

* This work was supported by a Grant-in-Aid from Tokyo Biochemical Research Foundation.

glucuronate were generally not influenced by the environment. The effect of sodium-glucuronate protecting the skeletal muscle tissue of X-irradiated *Triturus* embryos from abnormal differentiation was therefore examined. The qualitative and quantitative analyses of the developing skeletal muscle protein, actin, during early embryonal stages under various experimental conditions were carried out by the same serological technique as before.[1),2)]

G-Actin was isolated from the skeletal muscle tissue of adult *Triturus* by Szent-György's method. This protein (100 mg) was injected intravenously into rabbits and the obtained rabbit anti-serum was heated with saline extracts of liver, spleen and skin tissue of *Triturus* to eliminate non-specific antibodies. The titre of anti-serum was adjusted to 1 : 512 before carrying out the precipitin reaction with saline embryo extract. Immediately after fertilization, the embryos were raised at 20°C. in 0.01% solution of sodium-glucuronate (most effective concentration for promoting the growth of *Triturus* embryos[3)]), and X-irradiation of the embryos with 50 r, 200 r and 500 r was carried out 108 hrs. after fertilization. After irradiation, the treatment with sodium-glucuronate was continued whereupon the analyses of actin were immediately carried out. Non-treated and irradiated, treated and non-irradiated, and normal (non-treated and non-irradiated) groups were prepared for control.

In normal embryos, actin first became detectable 132 hrs. after fertilization. In the case of irradiation, actin was detected 156 hrs. after fer-

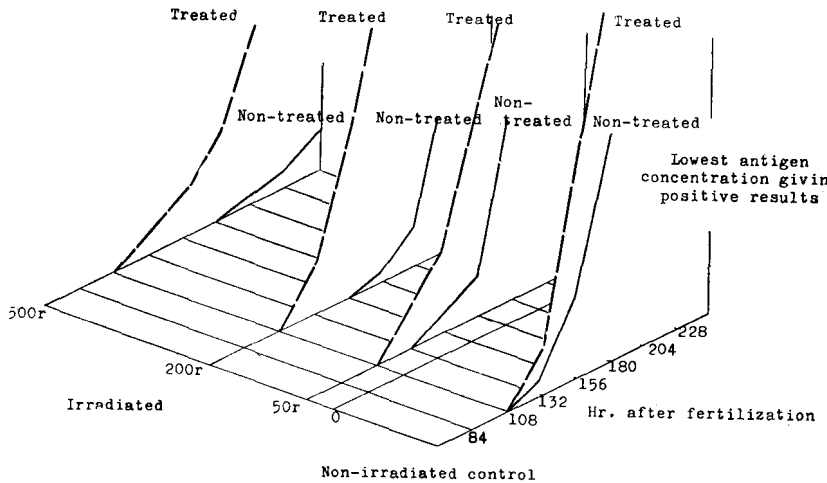


Fig. 1. Effect of sodium-glucuronate on the synthesis of actin in X-irradiated *Triturus* embryos.

tilization with 50 r, 180 hrs. with 200 r and 204 hrs. with 500 r.[2] Thus, the synthesis of actin is markedly suppressed not only as to the rate of formation but also as to its amount and the decline is proportional to the irradiated X-ray dosage as shown in Fig. 1.

In the treated embryos with sodium-glucuronate, the influence of X-irradiation could be scarcely observed either in the rate of actin formation or in its increasing amount. (Fig. 1.)

It was therefore proved that sodium-glucuronate markedly protects from a decline of actin synthesis inhibited by X-irradiation in early embryonal stages.

1) OGAWA, Y. Nature **182** (4645): 1312, 1958
2) OGAWA, Y. Nature **186** (4718): 77, 1960
3) OGAWA, Y. Ann. Rep. Natl. Inst. Genet. **10** : 150, 1959

21. *Effects of glucuronic acid and related compounds on the chemo-differentiations of muscle tissue in regenerating hind limb of Triturus**

(By Yoshito OGAWA)

The effects of sodium-glucuronate, glucosamine and glucuronolacton on the synthesis of the skeletal muscle proteins, actin and myosin, in the regenerating hind limb tissue after amputation at the knee were examined in *Triturus pyrrhogaster*, Boie.

About 800 of good-sized adult *Triturus* specimens were divided into four equal groups. One was used as the non-treated control. The remaining three were subcutaneously injected, respectively, with solutions of sodium-glucuronate, glucosamine and glucuronolacton at a dosage of 1.4 mg per 1 g body weight an 24 hours intervals immediately after the amputation until the removal of the granula. The animals were kept at 18°C. From 10 to 30 days after operation, the granulated tissue of the regenerating limb was removed at intervals of two-three days, and a saline extract was made. The identification of actin and myosin was carried out by means of serological technique.[1]

In the non-treated control, actin and myosin first became detectable in the regenerating limb tissue 20 and 27 days after the operation, respectively.[1] As to the treated group with sodium-glucuronate, actin and

* This work was supported by a Grant-in-Aid from Tokyo Biochemical Research Foundation.

myosin were first detectable 14 and 20 days, respectively, after operation. Thus, sodium-glucuronate remarkably promoted the development of muscle tissue. When a solution of glucosamine was injected, both proteins were investigated 16 days after operation. In another treated group, injected with a solution of glucuronolacton, the formation of actin was found 24 days and that of myosin 16 days after operation. Therefore, glucuronolacton supresses the synthesis of actin and promotes that of myosin.

The differentiation of muscle tissue is not accomplished until both actin and myosin formations coincide. The time of chemo-differentiation of muscle tissue in the regenerating limb tissue treated with sodium-glucuronate, glucosamine and glucuronolacton were 20, 16 and 24 days after operation, respectively. Above results show a similar tendency to those found in the experiments on chemo-differentiation of muscle tissue in early embryonal stages of *Triturus*.[2]

When treated with glucuronolacton, the order of development of actin and myosin is reversed to that found in the normal control. Such a reversal was already found also in regenerating limb tissue after X-ray irradiation with 2,500r, 18 days after amputation at the knee.[3] The order of synthesis of actin and myosin is therefore easily reversible. These results support the view that the processes of synthesis of actin and myosin in regenerating tissue are independent of each other as they were found to be also in early embryonal stage.[4]

1) OGAWA, Y. Nature **182** (4645): 1312, 1958
2) OGAWA, Y. Ann. Rep. Natl. Inst. Genet. **11** : 40, 1961
3) OGAWA, Y. Ibid. **11** : 38, 1961; Med. and Biol. **58** (1): 8, 1961
4) OGAWA, Y. Nature **186** (4718): 77, 1960

22. *Two factors influencing sensitivity to X-ray in the synthesis of myosin during early embryonal stages**

(By Yoshito OGAWA)

Last year, two factors (A and B) were reported influencing during early embryonal stages the sensitivity to X-ray in the synthesis of the muscle protein, actin.[1] This paper deals with myosin formation, another important contractile protein in muscle tissue, with respect to X-irradiation at various stages of development of *Triturus pyrrhogaster*, Boie.

* This work supproted by a Grant-in-Aid for Fundamental Scientific Research (No. 710267) from the Ministry of Education in Japan.

Approximately 15,000 embryos at different development stages were exposed to single doses of X-rays of 5r, 10r, 30r, 50r, 200r, 500r, 1,000r and 2,500r and examination for the first detectable trace of myosin after fertilization was carried out in periods of 24 hrs. after irradiation by using the same serological technique as before.[2),3)] Myosin, prepared from adult *Triturus*, was injected into rabbits and the obtained anti-serum was heated with saline extracts of liver, spleen and skin tissue of *Triturus* to eliminate non-specific antibodies. The titre of anti-serum was adjusted to 1 : 512 before carrying out the precipitin reaction with saline extracts of X-irradiated embryos.

In normal embryos, myosin first became detectable 176 hrs. after fertilizations as already reported by the present author.[2)] (In the present

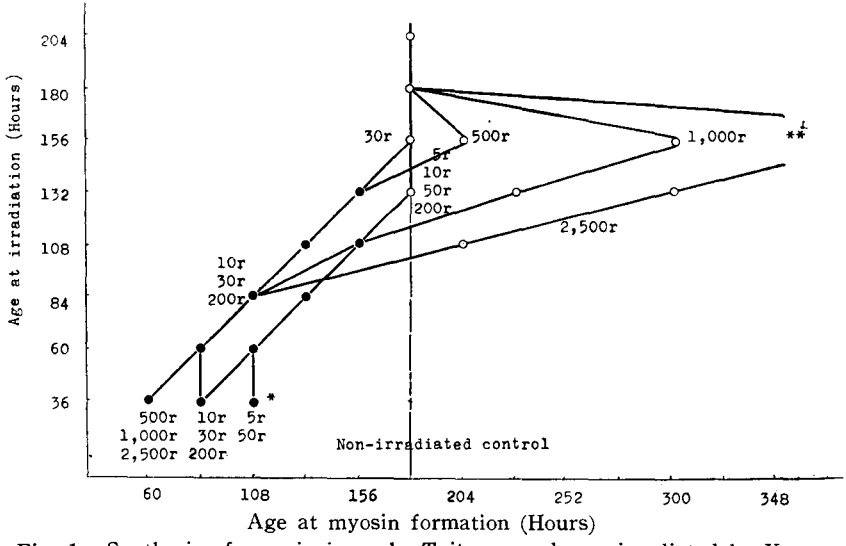


Fig. 1. Synthesis of myosin in early *Triturus* embryos irradiated by X-rays applying doses from 5r to 2,500r.

experiment, the myosin formation in the non-irradiated control was first recognized 180 hrs. after fertilization, because the examinations were carried out in periods of 24 hrs.) The time of myosin formation after fertilization in the treated embryos is shown in Fig. 1. A dose of 5r given 36 hrs. after fertilization changes the time of myosin formation to 108 hrs. after fertilization (Fig. 1*) but in the case of irradiation 156 hrs. after fertilization with a heavy dose of 2,500r, no myosin formation was recognized before the necrosis of the embryos (300 hrs. after fertilization) (Fig. 1**).

The curves shown in Fig. 1 suggest a biochemical similarity of the response to X-rays in actin and myosin syntheses, indicating the presence of two factors sensitive to X-rays in the synthesis of myosin as well as actin during early embryonal stages. One (A) suppresses the synthesis of muscle proteins and the other (B) markedly promotes it after X-irradiation. The points found on the right of the vertical control line indicate the effect of the X-ray inhibiting factor A (Fig. 1 ○), and those on the left refer to the X-ray promoting factor B (Fig 1 ●). But the two factors of actin synthesis (Aa, Ba) may be different from those of the myosin synthesis (Am, Bm), because the processes of synthesis of actin and myosin formation are independent of each other.[3] Factor A in myosin formation (Am) does not react to X-rays until doses over 500 r are applied. On the contrary, factor Bm is very sensitive to X-rays and is easily detectable with only 5 r doses. Factor Am is detectable by irradiations between 108 and 156 hrs. after fertilization. The most effective time of irradiation for factor Am is 156 hrs. in the above experimental conditions. Exposures between 36 and 132 hrs. after fertilization show clearly the reaction of factor Bm to X-rays. The most effective time of X-irradiation for factor Bm is 36 hrs. after fertilization.

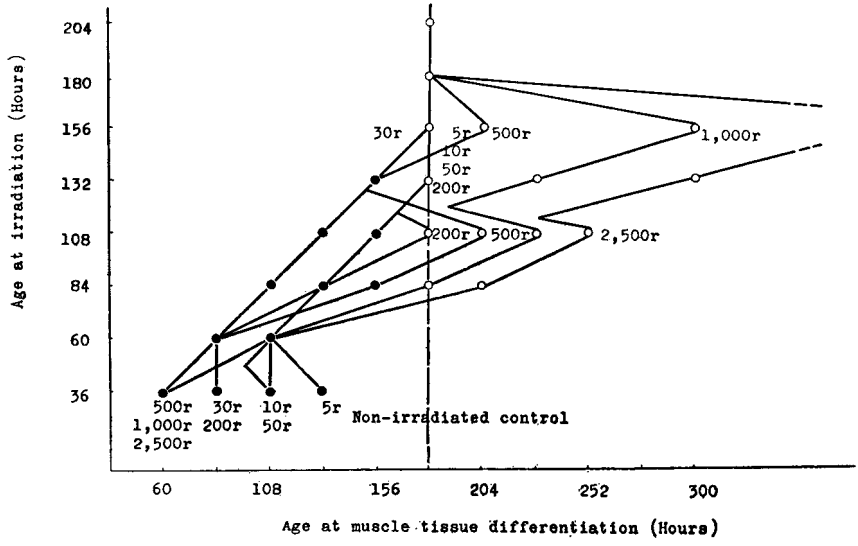


Fig. 2. Chemo-differentiation of muscle tissue in early *Triturus* embryos irradiated by X-rays applying doses from 5r to 2,500r.

The differentiation of muscle tissue is not accomplished before both

actin and myosin formations coincide. The chemo-differentiation process of muscle tissue of X-irradiated *Triturus* embryos is represented in Fig. 2.

1) OGAWA, Y. Ann. Rep. of Natl. Inst. Genet. **11** : 37. 1961
2) OGAWA, Y. Nature **182** (4645): 1312. 1958
3) OGAWA, Y. Nature **186** (4718): 77. 1960

23. *Sensitivity of a day-old chicks to low and high temperatures*

(By Takatada KAWAHARA)

The present study deals with a comparison between purebreds and their hybrids with regard to tolerance for unusually high or low temperatures. A day-old chicks of White Leghorns, Barred Plymouth Rocks and their reciprocal crossbreds were used. A number of chicks belonging to the same full-sib group from each strain were divided into two groups, one for high-temperature exposure (42.5°C, 100 minutes), and the other for low-temperature treatment (1°C, 120 minutes). After exposure to those abnormal temperatures the chicks were transferred to an incubator controlled at 37.6°C and kept for 14 hours for observation.

The results of analysis of the obtained data are summarized as follows: 1). F_1 chicks were more tolerant to low temperature than the purebreds, the lethality of the hybrid chicks being 55.5% in contrast to 71.7% for purebreds. The difference of 16.2% was statistically significant at the 1% level. 2). F_1 chicks, however, were less tolerant than the purebreds to high temperature treatment. The lethality of treated hybrid chicks was 61.8%, being 6.8% higher than that of 55.0% of the purebreds. The difference was statistically significant at the 5% level. 3). The correlation between low and high-temperature tolerance was -0.427 ± 0.110 in the purebreds, while in the crossbreds, it was $+0.185\pm0.129$.

24. *Electrophoretic haemoglobin patterns in inbred rats and chicken*

(By Kazuo MORIWAKI)

Recently several investigators have contributed valuable investigations of human haemoglobins in terms of genetics and protein chemistry. It was also revealed by means of paper electrophoresis that the haemoglobin of mice occurs in two types, single and diffuse, which are considered to be Mendelian characters.

The present study is an attempt at finding by the method of electrophoresis technique a difference in haemoglobin type among inbred rat strains. The strains used were as follows; Albany, Castle Black, Fischer, Long-Evans, Wayne pink-eyed hooded, Wistar and Wistar-King A. At an ionic strength of 0.05 of veronal buffer (pH 8.6), two bands were observed on the anodic side of the paper; a faster major one and a slower minor one. When the buffer was diluted to 0.025 of ionic strength, four bands appeared; two major bands on the anodic side and two minor bands on the cathodic side. However, at these two concentrations of the buffer no difference in the haemoglobin patterns could be observed between the strains.

Between the species some difference in the patterns was found. At an ionic strength of 0.05, *Rattus rattus* caught in a field showed one anodic band, and *Rattus norvegicus* (cf. Wistar) showed two anodic bands, as mentioned above. The mode of inheritance of these two types is not known, because these two rats could not mate.

Further, an electrophoretic analysis of haemoglobin was performed in chicken inbred strains. The haemoglobins moved to the cathodic side at an ionic strength of 0.025 of veronal buffer (pH 8.6), accompanied by one faster and one slower band, but no difference was detected between the strains. On the other hand, electrophoretical analysis has been carried out on an agar plate containing 1% agar and 0.05 M citrate buffer (pH 8.6). The presence of two components in the haemoglobin could be confirmed by this method for all the strains. For those experiments the following chicken strains were used; White Leghorn, Plymouth Rock, Rhode Island Red and Nagoya.

25. *Further study on post-axial polydactylism in the house mouse*

(By Tosihide H. YOSIDA, Hitoshi SAKAMOTO and Akira NAKAMURA)

A mutation, causing post-axial polydactylism of the front feet of the house mouse was found in this Institute and its genetics have been studied by the authors. It was found that this character depended on the interaction of several genes; one major gene, and some minor genes which probably modify the manifestation of the character.

Further genetical studies showed that the major gene was dominant and two minor genes were recessive; the latter suppressed the action of the major gene.

26. *Invasion of tetraploid cells of the Yoshida sarcoma into the lymph nodes of the rat*

(By Tosihide H. YOSIDA)

In ascites tumor cell populations of the Yoshida rat sarcoma tetraploid cells are rare. However, in previous studies, the present author found that the tetraploid cells predominantly invaded lung, kidney, liver and spleen, but did not invade to a large extent either thymus or mediastinal lymph nodes.

In the present study, another form of metastasis was investigated. Cells of the Yoshida sarcoma were injected subcutaneously under the tail. Following this procedure, regularly metastasis to the lumbar node took place. Metastatic tumor cells in the lumbar node, in this situation, revealed a distribution of tetraploid cells, similar to that found in the ascites.

27. *Karyological analysis of a metastatic tumor cells in MH134 hepatoma of the C3H strain of the mouse*

(By Naomichi INUI and Tosihide H. YOSIDA)

In 1956 Sato converted a hepatoma (MH134 of strain C3H) to the ascites form. This neoplasm readily metastasizes to lymph nodes. In the present experiment the chromosome constitution of the metastatic tumor cells in lymph nodes was compared with that found in ascites. Metastases were induced experimentally in the lymph nodes in the mouse by injecting ascites tumor cells under the skin of tails of 10 animals. Seven to ten days after transfer the tail was amputated at the base, and about 20 days later the mice were killed. The lymph node was greatly enlarged at the base of the tail in six animals and contained metastatic tumor cells. The ratio of diploid to tetraploid cells in the lymphatic glands did not differ from that found in the ascites.

28. *Ploidy in tumor cells after direct inoculation into various organs of the mouse*

(By Tosihide H. YOSIDA and Toshiharu KAMIOKA)

Following a peritoneal injection of tumor-ascites cell population of ELD, only a few (about 2 or 3%) tetraploid cells were found in ascites, but distinct metastases contained predominantly tetraploid cells. Two possible

explanations were considered. (1) Tetraploid cells have a higher ability to invade lung, liver, spleen and other organs. (2) These organs provide better conditions for the multiplication of tetraploid tumor cells.

An experimental approach to this problem was to inject tumor cells directly into the various organs. A population of the Ehrlich ascites tumor (ELD), which had a ratio 27 : 100 of tetraploid: diploid cells was used for the present study. Approximately 100 tumor cells were inoculated directly into brain, lung, liver and under the skin of mice. With the exception of the lung, tumors developed in those sites. The ratio of tetraploid to diploid tumor cells in various tumors was examined.

In the ascites population as control, the T/D ratio was 18/100, in the brain 32/100, liver 20/100, skin 22/100. Thus, it was shown that when tetraploid cells were artificially introduced into the organs, they could grow. These results indicate that at least the two above mentioned factors are involved in the selective metastasis of tetraploid cells from a mixed population, and that both greater invasiveness of tetraploid cells. and a better metastasis environment play a role.

29. *Competition of diploid and tetraploid tumor cells in the mouse*

(By Tosihide H. YOSIDA)

In the previous studies by the present author and his collaborators concerning the invasiveness of tumor cells, it was found that polyploid cells can multiply more readily in some organs, such as brain, liver, kidney, lung and spleen than the diploid ones, but they cannot increase as much as to become preponderant in the lymphatic glands. It is suggested that the metastatic tumor cells in the above mentioned organs receive better or more suitable nutrition than in the lymphatic glands.

To find out whether the tetraploid cells require better nutrition to multiply in the peritoneal cavity than do the diploid cells, the following experiment was performed: (1) Walker solution for tissue culture, which contained various amino acids and vitamins, was injected every day into the peritoneal cavity after transplantation of Ehrlich ascites tumor. (2) As control, physiological salt solution was injected into the peritoneal cavity by the same method. (3) As another control, a dilute solution of 4-NQO (a carcinostatic agent) was injected. A remarkable increase in tetraploid tumor cells occurred in the ascites tumor in mice which were injected with Walker solution (1). No remarkable change in the frequency of the tetraploid cells was found in the control (2), while a de-

crease of tetraploid cells occurred in the other control experiment (3) in which injection with a carcinostatic drug was used. These results strongly suggest that tetraploid tumor cells can multiply better in the peritoneal cavity, when the conditions are made to suit their development.

30. *Inhibitory effect of the components of Swertiae japonica on cell division in living plant tissue and on growth of rat tumors*

(By Yō TAKENAKA and Yoshito OGAWA)

The three components of *Swertia japonica*, swertiamarin, gentiopicrin and amarogenin were investigated regarding their effect on cell divisions in root tips of *Allium scorodoprasum* var. *viviparum* and on the growth of Yoshida sarcoma.

Water solutions of various concentrations were prepared from the above three chemicals, and living roots of *A. scorodoprasum* var. *viviparum* were placed in the solutions for 4 to 24 hours. The living root-tips were treated according to the hydrochloric-acetic acid-orcein method and oberved under microscope. Using water solutions of swertiamarin and

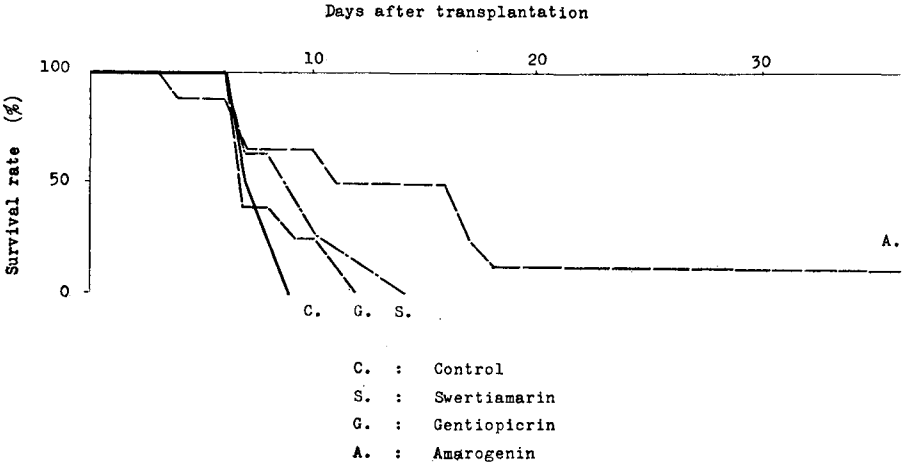


Fig. 1. The effect of three compounds extracted from Swertia japonica on life-prolongation of the host rat.

gentiopicrin, no structural change was found in the living cells, but that of amarogenin caused remarkable radiomimetic phenomena, even after 4 hour treatment with 4% solution.

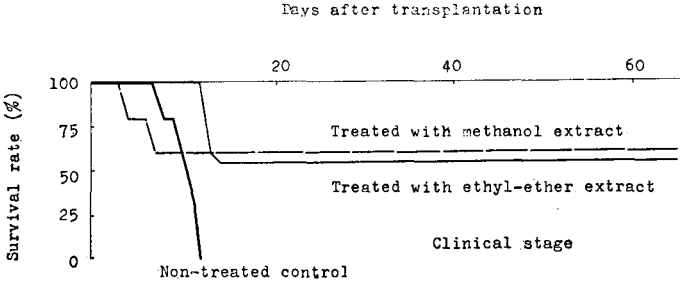
After transplantation of the ascites tumor to a Wister rat (two months old), the above three chemicals were immediately injected at the rate of 0.5 mg per 1 g body weight and the same injections were made again after 24 hours. Their toxicity, systemc symptoms and prolongation of life of the host rat were investigated.

Groups treated with swertiamarin and gentiopicrin showed no difference from the non-treated control in systemic symptoms and in the duration of host's life. But the group injected with amarogenin showed 4 days after the last injection, a remarkable recovery in systemic symptoms and 60% of the treated animals remained alive for about 16 days (Fig. 1), though the control group died out within 8 days after transplantation. These three chemicals showed no toxicity at the above-given doses.

31. *Anti-cancer activity of extract from Glycyrrhizae Radix**

(By Yoshito OGAWA)

The effect of methanol and ethyl-ether extract of *Glycyrrhizae Radix* (*Chinese Licorice*) on Yoshida-sarcoma, transplanted into a strain of rat, Wister (two months old) was examined. *Glycyrrhizae Radix*, which is obtainable in Japanese drug stores, was first treated with the same volume of methanol on the water bath. The methanol insoluble tissue was then extracted five times with the same volume of ethyl-ether at room temperature. The yields of methanol and ethyl-ether fractions were 7.8% and 0.5% in weight of original plant tissue, respectively.



Immediately after the transplantation of the ascites tumor into the abdomen of a rat, the obtained extracts were injected at the axilla sub-

* This work was supported by a Grant-in-Aid for Fundamental Scientific Research (No. 0433) from the Ministry of Education in Japan.

cutaneously two times with a 24 hour interval making up a total of 100 mg per 100 gr. body weight of the host rat, and its influence on the sarcoma was investigated in respect to the following three points: 1) toxicity of the extracts; 2) systemic symptoms of the rat bearing the sarcoma (appetite, ascites, icterus, metastasis and body weight); 3) prolongation of the host's life.

The methanol fraction showed an inflammation at the site of injection and some collapse of the regional tissue was found 7 days after treatment, though the ethyl-ether fraction showed no remarkable toxicity in the injected region and no systemic symptom at the above mentioned doses. As to the prolongation of the host's life, 40% of the animals treated with the methanol fraction died in the first seven days. This may be due to the toxicity of the injected substances. But the remaining 60% of this group, showed a remarkable recovery in systemic symptoms ten days after the injection and were alive for more than two months, though the non-treated control group died out between the 8th and 12th day after the transplantation of sarcoma. But in the group treated with the ethyl-ether fraction, no animal died before the 12th day after transplantation and about half of this group remained alive for more than 2 months after transplantation. The progress of their recovery in systemic symptoms is just the same as that found in the group treated with the methanol fraction.

These results seem to indicate that the effective substance of *Glycyrrhizae Radix* shows no toxicity to the animals and is easily separable from the toxic component by treatment with ether. Further chemical and pharmacological researches of this effective substance are now being carried out.

B. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF PLANTS

32. *Cytoplasmic male sterility of common wheat*

(By Hitoshi KIHARA)

In order to study the effect of alien cytoplasm on male fertility of common wheat, genomes of 3 hexaploid species, i.e., *Triticum vulgare*, *T. spelta* and *T. compactum* have been introduced by successive backcrosses into the cytoplasm of *Aegilops caudata*. Donor of the cytoplasm was "SB_8" (Kihara 1958), that possessed *caudata* cytoplasm and genomes of *T. vulgare*.

Pollen and seed fertilities of the successive backcross generations are summarized in Table 1.

Table 1. Pollen and seed fertilities in nucleus-substitution lines of 3 common wheat species

Strain	Year	Pollen fertility (%)	Seed fertility (%)	
			Selfing	Crossing
SB_8	1957	0.0	0.0	23.2(12.0~ 52.8)
SB_9 ×*vulg.* (SB_9)	1958	0.3(0.0~ 0.5)	0.0	41.2(2.5~ 58.8)
〃 ×*vulg.*^{2} (SB_{10})	'59	0.2(0.0~ 0.5)	0.0	56.3(42.5~ 75.0)
〃 ×*vulg.*^{3} (SB_{11})	'60	2.0(0.0~ 6.8)	0.0	50.9(5.0~100.0)
〃 ×*vulg.*^{4} (SB_{12})	'61	0.1(0.0~ 0.9)	0.6(0.0~ 1.6)	66.5(50.0~ 85.0)
SB_8 ×*spelta*	1958	0.0	0.0	27.9(3.6~ 40.0)
〃 ×*spelta*^{2}	'59	0.0	1.3(0.0~ 6.3)	39.1(5.6~ 62.5)
〃 ×*spelta*^{3}	'60	0.1(0.0~ 1.1)	0.2(0.0~ 1.5)	7.5(0.0~ 25.0)
〃 ×*spelta*^{4}	'61	0.0	0.1(0.0~ 0.2)	52.7(40.0~ 68.2)
SB_8 ×*comp.*	1958	33.1	6.2(2.4~11.4)	33.4(16.7~ 50.0)
〃 ×*comp.*^{2}	'59	39.9(6.7~52.6)	51.6(19.2~90.7)	53.6(27.5~ 67.6)
〃 ×*comp.*^{3}	'60	85.5(64.6~96.7)	23.6(0.0~67.2)	48.3(0.0~ 95.0)
〃 ×*comp.*^{4}	'61	90.1(78.0~96.2)	45.5(28.1~62.9)	92.8(86.4~100.0)
T. vulgare	1961	98.8	83.6(78.4~86.0)	78.3(65.0~ 95.0)
T. spelta	〃	97.0	63.1(47.2~71.1)	89.4(85.0~ 91.7)
T. compactum	〃	93.8	65.6(63.6~77.5)	83.6(63.3~100.0)

Apparently, the cytoplasm of *Ae. caudata* causes almost complete male-sterility of *T. vulgare* and *T. spelta*, while *T. compactum* possesses genes that restore male fertility.

In order to investigate the number of the restoring genes in *T. compactum*, "SB_{11}" plants (♀) were pollinated with the pollen of the F_1 hybrid, *T. vulgare* × *T. compactum*. In the subsequent generation completely male-sterile and partially male-fertile plants were segregated to a ratio of 37 to 12, suggesting 3 : 1 segregation ratio. The average fertility of the latter group was 4.8% ranging from 0.7–20.8%.

This fact seems to indicate that *T. compactum* carries 2 complementary genes for restoration of male fertility; based on this assumption, the genotypes, $Ms_1Ms_1Ms_2Ms_2$ and $ms_1ms_1ms_2ms_2$ are tentatively assigned to *T. compactum* and *T. vulgare*, respectively. The partially fertile plants, which were segregated in the above cross ($ms_1ms_1ms_2ms_2 \times Ms_1ms_1Ms_2ms_2$) are assumed to be of a genotype $Ms_1ms_1Ms_2ms_2$. The designation proposed is supported by a fact that "SB_8" ($ms_1ms_1ms_2ms_2$) × *T. compactum*

($Ms_1Ms_1Ms_2Ms_2$) produced partially fertile F_1 hybrids, whose fertility (6.2% on the average ranging from 2.4 to 11.4%) is well comparable with that of the partially fertile plants segregated in the cross, "SB_{11}"×(*T. vulgare* ×*T. compactum* F_1).

33. *Pistillody of Triticum durum induced by an Aegilops cytoplasm*

(By Hitoshi KIHARA and Koichiro TSUNEWAKI)

Nucleus of *Triticum durum* (2n=28) was placed into cytoplasm of *Aegilops caudata* (2n=14) by successive backcrosses. Since hybridization between *T. durum* (♂) and *Ae. caudata* (♀) was not easy, a strain that possessed *caudata* cytoplasm and genomes of *T. vulgare* (2n=42) was used as the donor of *caudata* cytoplasm.

T. durum with *caudata* cytoplasm became completely male sterile because of the pistillody of all stamens. The pistilloid stamens had no ovule and instead had only a cavity. In some pistilloids even such a cavity was not formed. In order to test the function of pistilloid stamens as female organs, a normal pistil was carefully removed from each floret and the remaining pistilloid stamens were artificially pollinated. 204 floerts were treated in this way but did not produce any seed. These results clearly indicate that the pistilloid stamens of *T. durum* induced by *caudata* cytoplasm are not functioning as female organs.

Seed fertility of *T. durum* with *caudata* cytoplasm, when cross-pollinated, was only slightly higher than 10%, being much lower than that of normal *T. durum*. An anatomical study of apparently normal pistils indicated that a majority of ovaries had abortive ovules. This seems to be a cause of the depression of female fertility.

These results indicate that effect of *caudata* cytoplasm on the manifestation of the *durum* genomes is twofold, *i.e.*, induction of pistillody and depression of female fertility.

34. *Progressive necrosis of seedlings in hexaploid wheat*

(By Koichiro TSUNEWAKI)

Tsunewaki (1960) and Tsunewaki and Kihara (1961) found that progressive necrosis of common wheat is controlled by 3 complementary genes, Ne_1 on chromosome 5B, Ne_2 on chromosome 2A and Ne_3 on chromosome 3D, and that *T. aestivum* var. Kharkov, *T. aestivum* var. Prelude and *T. macha* var. *subletschumicum* are of genotype, $ne_1Ne_2Ne_3$, $Ne_1ne_2Ne_3$

and $Ne_1Ne_2ne_3$, respectively.

In order to investigate the distribution of the 3 necrosis genes in common wheat and its ancestral species, the author crossed the above-mentioned varieties as testers to 16 varieties of common wheat (including 5 species) and 12 strains of synthesized hexaploids (including 6 species of Emmer wheat and 3 varieties of *Ae. squarrosa*).

Necrosis observed in the seedling stage of those F_1 hybrids is summarized in Table 1.

Table 1. Seedling necrosis in the F_1 hybrids between 3 tester varieties and 16 common wheats and 12 synthetic hexaploids

Tested varieties	Testers		
	Kharkov ($ne_1Ne_2Ne_3$)	Prelude ($Ne_1ne_2Ne_3$)	*T. macha sublet.* ($Ne_1Ne_2ne_3$)
Chinese Spring, Marquillo, Trumbull, American Banner, Pawnee, S-615, *T. vulg. eryth.*, *T. comp.* 44, *T. spelta Duha*, *T. sphaero.*	−	−	−
Nowinka, *T. macha paleo*, ABD-1, ABD-8, ABD-9, ABD-14, ABD-16, ABD (Pentad), ABD-22	+	−	−
Riebesel	−	+	−
ABD-3, ABD-13	−	−	+
T. macha sublet.	+	+	−
Prelude, ABD-11, ABD-23, ABD (Golden Ball)	+	−	+
Kharkov, Jones Fife, H_{11}, Fulcaster, Blackhull	−	+	+

+: necrotic, −: normal

From this result, at least, one variety representing each one of the 7 possible genotypes has been found. It is interesting to notice that all three, Ne_1, Ne_2 and Ne_3, are widely distributed in common wheat, while Ne_2 is rarely found in Emmer wheat, in which Ne_1 widely occurs. Ne_3 is also widely distributed in *Ae. squarrosa*.

35. *Relation between chlorophyll content and free amino acids in mutants of Triticum monococcum*

(By Taro FUJII and Yukio ONO)

Free amino acids in *chlorina*, *virido-albina* and their double recessives were examined with paper chromatographic method. On the two-dimensional development of extract in seedling stage, seven spots were observed

in normals and *chlorina* showing the presence of aspartic acid, glutamic acid, glycine, glutamine, phenylalanine; in addition, two unidentified spots *a* and *b* appeared. On the other hand, nine spots were observed in both *virido-albina* and double recessive plants. The two additional spots indicate alanine and leucine.

Eight spots of the same kinds were observed in normals and *chlorina* in maturing stage, but at that time ten spots were observed in *virido-albina* and double recessives. Alanine is absent only in *chlorina* but it is present in the other three strains as a diluted spot, and glutamine is absent in normals and *chlorina* though they are present in them at seedlings stage. Leucine and spot *a* were absent in four strains, though spot *a* was observed in all strains in seedlings and leucine was observed in *virido-albina* and double recessives at early seedling stage. Furthermore, spots *c*, *d* and *e* newly appeared at this stage in all strains.

The chlorophyll content in double recessive plants was recovered to the *chlorina* level and no further increase occurred (cf. Ann. Rep. No. 8). The *chlorina* gene, therefore, is epistatic to *virido-albina* gene as to the ability to recover, nevertheless the same kinds of amino acids were observed in both *virido-albina* homozygotes and double recessive plants at seedling stage or at ripening stage.

36. *On the occurrence of chlorophyll mutations in clusters in einkorn wheat*[1)]

(By Taro FUJII)

Dormant seeds of *Triticum monococcum* were subjected to 10, 20 and 30 kr of gamma-rays. All spikes from all irradiated X_1 individuals were harvested and chlorophyll mutations were scored in the X_2 generation. Number of spikes with chlorophyll mutants increased with increasing dosage. In some cases, several spikes proved to carry the same kind of mutation from the same X_1 individual. Namely, 8 spikes among 16 from one individual segregated *albina*, while in another case, only one spike among 60 spikes *xantha* was found. Thus distribution of cluster mutations showed a wide variation, and the average number of cluster mutants per X_1 individual was not related to dosage. Frequency distribution of cluster mutants of *albina* type was calculated according to KONDO (cf. KONDO, S. 1961, Jap. Jour. Genet. 36: 6-17), as given in Table 1. According to the results, the number of original cells in embryonic stage was

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

estimated as 35±3, and some of these differentiated cells seem to have furnished the initials for the spikes.

Table 1. Frequency of *albina* clusters

No. of spikes which segregated *albina* seedlings in X_2	No. of X_1 plants	Calculation	
		Observed	According to KONDO
0	151	0.795	0.795
1	24	0.126	0.122
2	8	0.042	0.055
3	4	0.021	0.019
4	3	0.016	0.005
5	0	0	0.002
6	0	0	0.0005
7	0	0	0.0001

37. *Crossing experiments with chlorophyll mutants of einkorn wheat*

(By Taro FUJII)

Crossing experiments among several viable chlorophyll mutants of *Triticum monococcum* were carried out and F_1 plants were obtained from 28 combinations. Among them, some were between different types of mutant such as *basi-viridis* or *virido-albina* and *chlorina*. And others were between different strains within the same type of mutant such as *basi-viridis*-5061×-5061 or *virido-albina*-5066×-5079, *etc.* All F_1 plants from 27 combinations showed normal green color and relatively high fertility and in the F_2 generation they segregated plants with both parental characters and double recessives. Linkage relationships between these mutants are not yet determined because of low germination rate and high mortality of some mutant strains and double recessive segregants.

The F_1 seedlings between *virido-albina*-5066 and -5079 showed *virido-albina* character in reciprocal crosses. There was a small difference between both strains concerning the recovery of chlorophyll pigment. Therefore, it is concluded that the genes for their characters are located on the same locus, being multiple alleles. A large number of lethal mutants such as *albina* and *xantha* are listed in our collection. From these facts, many genes seem to have contributed to chlorophyll development and it is suggested that the chlorophyll pigment is synthesized in several steps.

38. *Developmental anatomy of glume epidermis in the genus Oryza*[1)]

(By Hitoshi KIHARA and Tadao C. KATAYAMA)

The surface structure of the glume is an important taxonomic character in *Oryza*, as was recognized by Roschevicz. In order to examine the details, the present authors studied the glume of 18 *Oryza* species by SUMP (Suzuki's Universal Microprinting) method (1960). Histological investigations of epidermal cells of glumes were carried out this year. The distinction between the glume characters of the four sections is very conspicuous.

The cells of *O. subulata* (Section Rhynchoryza) are of equal size and are arranged like fish scales, and seldom short cells occur. In some cases such short cells develop into hairs. In all other three sections (Sections Sativa, Granulata, Coarctata) the epidermal cells are arranged more or less in regular longitudinal rows. The cells of species belonging to Section Sativa are arranged also in regular crosswise district, with the result of a checkerboard arrangement. In the case of species belonging to the latter three sections, each cell divides into two kinds of cells at the late developmental stage; one is strongly elongated and the other is short and either remains undifferentiated or develops into a hair.

The aspect of cells in the maturing stage of each section were summarized as follows:

In Section Sativa, where the short cells and the long cells are immediately adjacent to one another, the tubercles result from uneven thickening at the points of contacts of six cells which consisted of four long and two short cells. The short cells are much reduced in size.

In Section Granulata, with short cells not usually adjacent in arrangement, the tubercles are formed over the area, where two short and two long cells are in contact. They are every so often fused together and form large warts.

In Section Coarctata, species belonging to this section show a great variation in the structure of epidermal cells. One of the species, *O. ridleyi* has not tubercles. Otherwise the epidermal cells are very similar to that of the species belonging to Section Sativa.

In Section Rhynchoryza, small cells, which occurred rarely in the epidermis, have either a short hair or a protrusion. The cells are diagonally arranged. Cell walls between two cells are thin and apparently

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fused, then the dual partition between its cells is not discernible. Each epidermal cell has one tubercle on its surface.

39. *Genome analysis in the genus Oryza III*[1)]

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

Genome analysis of *Oryza* species was continued this year. 10 interspecific hybrids were newly produced, among which one hybrid was morphologically and cytologically investigated.

The F_1 hybrid between *O. grandiglumis* and *O. latifolia* showed invariably 24 bivalents at meiosis. The genome constitution of *O. grandiglumis* is apparently the same to that of *O. latifolia* and should be CCDD. However, the hybrid was highly sterile, *i.e.*, its seed fertility was only 2.7%. Those two species, therefore, seem to belong to different intersterile groups with the same genomes.

40. *Variation in blast susceptibility in wild rices closely and distantly related to cultivated rice*[1)]

(By Keizo KATSUYA)

In several wild rices, which are closely or distantly related to the cultivated rice and were collected from various countries, variation was estimated in susceptibility to P-2 strain of *Piricularia oryzae*. The susceptibility was tested by the leaf-sheath method using the leaf sheath of the next below the youngest leaf. 30 individuals were used in each strain, and each individual was tested twice.

In general, closely related species to cultivated rice, *Oryza perennis*, *O. sativa* f. *spontanea*, *O. barthii* and *O. breviligulata*, were highly heterogeneous with respect to blast susceptibility, while distantly related species, *O. officinalis*, *O. eichingeri*, *O. minuta*, *O. latifolia*, *O. alta* and *O. malabarensis*, were homogeneous compared with the former. In susceptibility of species closely and distantly related to cultivated rice highly significant differences occurred between and within strains. *O. latifolia* collected from Cuba and Guatemala, *O. minuta* from Malaya and Philippines and *O. perennis* from Cuba were highly susceptible.

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

41. *Some considerations on rice cultivation in New Guinea*[1)]

(By Tadao C. KATAYAMA)

In Territory of Papua and New Guinea and Netherlands New Guinea, several experiments on rice were started a few years ago at some experiment stations and are extensively carried on.

Some rice cultivation by the natives was found in several districts, but it was mainly limited to those where Indonesians are living. Recently those people have been routed out from their places, so that rice cultivation markedly decreased. However, in some areas rice is cultivated by a primitive shifting method. The strains used include both of "japonica type" and "indica type" from the view point of morphological and physiological characters. The former are mainly used in Territory of Papua and New Guinea and the latter are mainly used in Netherlands New Guinea.

Some diseases were found in several districts such as *Ramularia oryzae* and *Sphaerulina oryzina*. These diseases covered a large area in some district. Two insects were found in New Guinea. One is *Cnaphalocrocis medinalis* and the other is *Nephotettix bipunctatus cincticeps*. These insects occupy a large area and seem to become a serious problem in near future. The natives usually leave rice fields, which have been severely infected by insects or diseases, lying idle for several years.

As to the future of rice cultivation in New Guinea, an extension of rice cultivation at the sea-side seems to be promising, because of abundant rain fall and high temperature; guidance and efforts of the government, especially in employing more efficient methods for upland cultivation and irrigation systems for lowland rice fields seem to be successful. However, popularization of rice cultivation among the natives planned by the government seems to be very difficult, because the natives hate hard work and the laboring force is small, although rice is their favored food.

42. *Some observations on the blooming of Oryza species*[1)]

(By Tadao C. KATAYAMA)

An investigation has been made on the mode of flowering of 21 *Oryza* species, including date of flowering, order of blooming among the spikelets

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of a panicle, number of flowers that bloom on successive days, time of blooming, *etc.*, because these characters are important for plant breeding as well as ecological and phylogenetic studies of rice.

The blooming of spikelets in a panicle takes place in a regular sequence, that is, it is little influenced by environmental conditions such as temperature and relative humidity. In all species blooming starts from the uppermost branch of a panicle and proceeds to the lower branches in succession. However, some differences in the flowering order of spikelets on a branch are found among several species. In many species, the blooming occurs first in the uppermost spikelet of a branch, followed by the lowest one and then proceeds toward the upper spikelets in consecutive order. In 4 species, namely, *O. glaberrima*, *O. breviligulata*, *O. stapfii* and *O. coarctata*, the blooming occurs first in the uppermost spikelet, followed by a spikelet slightly nearer the base than the middle of the branch and from there proceeds both upward and downward. In 2 other species, *O. brachyantha* and *O. subulata*, blooming starts from the top of a branch, proceeding straight downward.

In all species, but *O. ridleyi* and *O. subulata*, flowering of all spikelets occurs continuously. In *O. ridleyi*, however, a male sterile spikelet on the top of each branch flowers several days earlier than the other spikelets. In *O. subulata* it takes several days between the flowering of a branch and that of the following one.

The number of spikelets, which flower on each of successive days, is almost constant for each species. The average number of spikelets, which flower every day on a branch, is much smaller in the species of Sections Granulata, Coarctata and Rhynchoryza than in those of Section Sativa.

43. *Photoperiodic responses of Oryza species IV*[1]

(By Tadao C. KATAYAMA)

Using 23 *Oryza* species, intra-specific differentiation of photoperiodic sensitivity and its relation to latitude were investigated.

Intra-specific differentiation. All species belonging to Section Sativa contained both photoperiodically sensitive and insensitive strains, indicating intra-specific differentiation with respect to photoperiodic sensitivity. Percentages of sensitive strains were 79.6, 99.2 and 79.9%, respectively in the cultivated species (*O. sativa* and *O. glaberrima*), their

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close wild relatives (*O. sativa* var. *spontanea*, *O. perennis*, *O. barthii*, *O. cubensis*, *O. breviligulata*, and *O. stapfii*), and non-related species (*O. australiensis*, *O. officinalis*, *O. minuta*, *O. malampuzhaensis*, *O. eichingeri*, *O. punctata*, *O. latifolia*, *O. alta* and *O. grandiglumis*). The heigher proportion of insensitive strains in the cultivated species than in the closely related wild species seems to indicate that photoperiodic sensitivity is essential for the latter but its lack in the former is counterbalanced to some extent by other economic characters.

The species belonging to the 3 other sections did not show any indication of intra-specific differentiation. All strains of *O. granulata*, *O. meyeriana* and *O. abromeitiana* of the Section Granulata and *O. subulata* of the Section Rhynchoryza were photoperiodically insensitive, while those of *O. coarctata*, *O. ridleyi* and *O. longiglumis* of Coarctata section were all sensitive.

Relation between the latitude of habitats and the critical day length. Among photoperiodically sensitive strains, a high negative correlation was found between the latitude of their natural habitats and the critical day length. The correlation coefficient obtained for all wild species was -0.6817 (d.f.$=166$). Strains collected in Assam showed some significant deviation of their critical day length from that of the strains collected at other localities in the same latitude, probably due to the high altitude of Assam (ca. 5,000 ft.). The correlation coefficient became -0.8426 (d.f.$=111$), when Assam's strains were omitted from the calculation.

The correlation coefficient for the cultivated rice, including both *O. sativa* and *O. glaberrima*, was -0.4171 (d.f.$=115$), the correlation being much smaller than that obtained for the wild rices. This fact also suggests that photoperiodic adaptation is more important for the wild rices than for the cultivated ones.

44. *Intra-plant variation in seed size in rice*

(By Kan-Ichi SAKAI and Akio SUZUKI)

In the biometrical study of length and breadth of seeds in wild and cultivated rice strains, it was noticed that intra-plant variability was less in the former than in the latter. This variability is the measure of the developmental stability in seed size. Among wild species, *Oryza perennis* and *O. rufipogon* were much different from each other in respect of the intra-plant variability, though there seemed to be some difference between geographical strains within the same species.

No evidence, supporting either less variability in the heterozygotes or intra-plant variability due to genic segregation, was obtained in a hy-

bridization experiment of Japanese rice varieties. Thus, it seems rather reasonable to assume that higher stability in seed size in wild rice forms than in the cultivated ones could be attributed to the effect of long-term natural selections. (See also No. 90 on page 107)

45. *Variation in susceptibility to blast disease in wild and cultivated rice*

(By Iwasaburo GOTO and Kan-Ichi SAKAI)

The degree of susceptibility to blast disease in various strains of wild and cultivated rice was determined by the leaf-sheath inoculation method using strain 53-33 for *Piricularia oryzae*, kindly supplied by the National Institute for Agricultural Sciences, Tokyo.

Among six geographical groups of cultivated varieties, a marked difference was observed in the degree of susceptibility. The six groups can be arranged in the order of their susceptibility as follows: Africa > Japan > South America > Java, Thailand and Ceylon. African varieties belong to *Oryza glaberrima*, while those of Japan represent *O. sativa japonica*, the remainders belonging to *O. sativa indica*.

The progeny of three wild populations formerly collected in Ceylon in 1959 and grown for one generation in Formosa in 1960, were tested for their within- and between-population variability. Partition of variance was made into three components, *i.e.* variance due to environmental causes, V_E, variance between plants within line within variety, V_I, and variance between varieties or lines, V_S. Variability between plants within line is measured by $V_I/(V_E+V_I)$, and variability between lines or varieties by $V_S/(V_S+V_I+V_E)$. There was no distinguishable difference in the degree of susceptibility between cultivated and wild rices. It was found that the within-line variability was higher in wild strains than in cultivated ones while the between lines within strain variability was in the reverse order. This may mean that wild rice is more heterozygous with regard to blast susceptibility than cultivated rice. It is widely accepted that the susceptibility of rice to blast disease considerably varies depending upon the level of nitrogenous fertilization, the higher the dose of N-fertilizers the more susceptible become the plants.

Let the susceptibility of a given plant to a certain disease be expressed by the following model:

$$S = c + b$$

where S is the susceptibility of the plant growing in a given condition while c and b stand for the susceptibility of the plant determined

by resistant genes, if any, and that produced by environmental or cultivation conditions, respectively. Then, the inter-strain variance of the observed susceptibility could be partitioned as follows:

$$V_S = V_c + V_b + 2W_{cb}.$$

Thus, we may be able to define

V_c/V_S as degree of inherent susceptibility (D_c)

V_b/V_S as degree of environment-respondent susceptibility (D_b)

$2W_{cb}/V_S$ as degree of covariation between the two (D_{bc}).

On the basis of this principle of partition, it has been concluded that in case of blast-disease, the degree of inherent susceptibility was very small while the degree of environment-respondent susceptibility was large, in other words, susceptibility of rice to *Piricularia* fungus would be mostly dependent upon the pathological response of genotypes to fertilizer application. Wild rice seemed different from cultivated rice in that the former had a lower D_c but a higher D_b than the latter.

46. *Rice varieties intermediate between wild and cultivated forms and the origin of the Japonica type*

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

Investigations of rice varieties collected from the Jeypore Tract, India (partly reported by the writers in Ann. Rep. 9, 1958) were completed in 1961. Variations in the character complex of the strains were studied with three discriminant formulas, for classifying *perennis vs. spontanea* types of wild rice, wild *vs.* cultivated rice forms, and the Indica *vs.* Japonica types of cultivated rice, respectively. A greater part of the strains were intermediate between wild and cultivated forms, and those approaching wild forms were of the *perennis* type. This indicates that cultivated rice might have evolved from the *perennis* type of wild rice. It was also found that those strains would probably become differentiated into Indica and Japonica types as they approach cultivated forms, indicating that the two types are monophyletic. Most of the Jeypore strains showed high F_1 fertilities with test-strains of different types whose mutual F_1 hybrids were partly sterile, in the same manner as wild strains of *O. perennis*. Their hybrid-sterility relationships did not show correlation with variations in other characters. (Publ. in Bot. Bull. Acad. Sinica 3, in press).

47. *Responses to various growing conditions of wild and cultivated rice forms*

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

A set of ten rice varieties ranging from wild (*spontanea* type of *O. perennis*) to improved cultivated forms (*O. sativa*), in which intermediate wild-cultivated forms from the Jeypore Tract, India, and native varieties of tropical Asian countries were included, were tested in different growing conditions in the experimental field of Taiwan Provincial Agricultural College, Taichung. It was found in general that the nearer the genotype approached improved cultivated varieties, the higher would become the response to fertilizer application, weeding and transplanting, which may be regarded as elements of intensive rice culture in Asia. It was suggested that the evolution of genotype and that of environment might proceed in parallel.

48. *Intra-specific variations of Oryza breviligulata in West Africa*

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

Oryza breviligulata A. Cheval. et Roehr. is a wild species genetically in close relation with the cultivated rice species, *O. glaberrima* Steud., and is distributed in West Africa. From the results of variation studies in various characters of this species, the directions of intra-specific differentiation were investigated by using the technique of factor analysis. Only the first factor had an important contribution, which represented the variation of those wild forms in the direction of cultivated type. Actually, some strains were in various characters approaching cultivated varieties of *O. glaberrima* (c.f. Morishima and Oka, 1960, Ann. Rep. 11). It was pointed out that *O. stapfii* Roschev. was synonimous with *O. breviligulata*. It was found further that *O. breviligulata* had a smaller amount of genetic variability in its populations than *O. perennis*, but varied in a wider range among populations. The results of analysis of intra-populational variations suggest that this annual wild species might have a relatively high amount of self-pollination.

49. *Differences in breeding behavior between wild and cultivated rice species*

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

It is known that *O. perennis* is partially allogamous, while *O. sativa*

is nearly autogamous. With regard to this difference in breeding behavior, the pollinating systems of the two species were compared. It was found that *O. perennis* had 1) longer anthers (more pollen-grains), 2) longer duration of life of pollen-grains emitted from the anthers, 3) longer time interval from flower openning to pollen emission, and 4) more extruded stigmas, than *O. sativa*. The former two conditions might be related to the capacity of plants to pollinate other plants, and the latter two to the probability of being pollinated by others. Thus, in both respects, *O. perennis* seems to be suited for outcrossing. Further, regarding the longevity of seeds, it was found that seeds of *O. perennis* did not germinate when immersed in water, but could be kept alive in water or in moist soil for more than two years. This behavior would bring about overlapping of generations in the natural habitat.

50. *Tetraploid F_1 hybrid between Oryza sativa and O. glaberrima*

(By Kokichi HINATA and Hiko-Ichi OKA)

Two tetraploid strains induced by colchicine treatment of seedlings, one belonging to *O. glaberrima* and the other to *O. sativa*, were crossed. The F_1 plants showed partial fertility, namely about 45% good pollen-grains and 25% seeds set. The number of quadrivalent chromosomes per P.M.C. was 4.37 on the average, ranging from 3 to 10; it was smaller by about two than that found in the parental autotetraploid strains. However, the same decrease in the number of quadrivalent chromosomes is also found in tetraploid F_1 hybrids between varieties of *O. sativa*. It then appears difficult to evaluate by this method how the two species differ in chromosome structure. Observation of segregation ratios in tetraploid F_2 hybrids is under way.

51. *Hybrid-sterility relationships among Asian strains of Oryza perennis and O. sativa*

(By Kokichi HINATA and Hiko-Ichi OKA)

A number of strains of *O. perennis* Moench. (including those of *O. sativa* f. *spontanea* Roschev.), collected from India, Thailand, Malaya and other Asian countries, were each crossed with a set of test-strains belonging to *O. sativa* and *O. perennis*, and the F_1 fertility data were compared with those formerly obtained by the second writer for *sativa* strains. As pointed out by the writers before (Ann. Rep. 11, 1960), the *perennis* strains mostly

showed high F_1 fertilities with the test-strains. It was suggested, from the results of multi-variate analysis of the data, that the F_1 sterility found between *sativa* varieties might be on the increase with the approach to cultivated forms. However, as Oka and Chang (1959, Ann. Rep. 10) showed, populations of *O. perennis* were found to contain variations of sterility factors, mainly in the form heterozygotes. These features are well explained by the hypothesis of *gametic-development genes* for the F_1 sterility between *sativa* varieties.

52. *Investigation on the measuring method of the intercellular spaces on several plants*

(By Tadao C. KATAYAMA)

On the aerating system of roots and leaves and its physiological significance in several plants many works have been published. In the case of rice plants, possibility of oxygen transfer from the top to the roots has been discussed with a view to understanding root respiration under paddy field conditions, which may be closely related to their resistance to excessive soil moisture.

As a quantitative determination of the total intercellular air space in whole part of plant is lacking, the author investigated the measuring method of its volume using several plants, as *Oryza sativa*, *Ligustrum japonicum*, *Daphne odora*, *etc.*

The volumes of plant organs and their intercellular spaces can be simply measured by means of measuring both gravity and buoyancy methods. The latter method surpasses the former one in accuracy of measurement. The buoyancy method is achieved by the following way; materials are first weighed in air (G_1), secondly weighed in the condition submerged in fluid such as water or liquid paraffin (G_2), and once again weighed in fluid after having been infiltrated with fluid using an electric vacuum pump (G_3); in the case of gravity method, materials are once more weighed in air (G_4) in addition to the weighing procedure of three times mentioned above.

Computations were made by the following formulae:

$$V_1=\frac{G_1-G_2}{\rho-\rho_a}$$

$$V_2=\frac{G_4-G_3}{\rho-\rho_a}$$

$$V_a=\frac{G_3-G_2}{\rho-\rho_a}$$

$$V_g=\frac{G_4-G_1}{\rho-\rho_a}$$

V_1 and V_2: volume of organs in the case of buoyancy and gravity methods,

V_a and V_g: volume of intercellular spaces in the case of buoyancy and gravity methods,

ρ and ρ_a: density of fluid and air, respectively.

At the measurement of organ volume, it was confirmed that the measuring error was less than 1%. By these measuring methods, it is possible to measure not only in the case of turgescent organ but also wilted one. Using vacuum pump, precaution should be taken that air should not be remained in the organ. However, it was also confirmed that the measuring error owing to the above cause did not exceed 2% at 20°C. In addition, some conceivable theoretical errors caused by shrinking, deformation, infiltration of the organs were discussed. But it was certified that they were quite negligible.

53. *Arisaema triphyllum, Jack-in-the-Pulpit, in Minnesota, especially at the Cedar Creek Natural History Area*

(Sadao SAKAMOTO)

In Minnesota two species of *Arisaema*, *A. triphyllum* (L.) Schott. with two subspecies: subsp. *triphyllum* Huttleston and subsp. *Stewardsonii* Huttleston and *A. Dracontium* (L.) Schott., are found. The distribution map of these species based upon the herbarium specimens was studied. *Arisaema triphyllum* subsp. *triphyllum* is most common in Minnesota; *A. Dracontium* occurs only in the southeast corner of the state. In specimens of subsp. *triphyllum*, three color phase variations of the spathe were observed; pale green with no stripes (type A), pale green with light purple stripes (type B) and pale green with deep purple stripes (type C). Type B is most common in Minnesota. Type A is distributed at random. Type C is only found in the northeast part of the state.

The following observations were carried out at three observation-stations in the Cedar Creek Natural History Area, Minnesota.

Color variations in inflorescence and flower parts were very characteristic. Generally, the plant having purple color in spathe, in spadix and in anthers (male plant), and no purple color in stigmata (female plant), are most common in this Area.

Flowers in the male inflorescence start to bloom from the middle of

inflorescence toward both upper and lower extremities. Range and average number of flowers per inflorescence are greater in female than in male. Three types of inflorescence, male, intermediate (monoecious) and female, were found. Proportions of these types differed among the stations according to the environmental conditions. In total, the proportion of male plants was 71.4%, of intermediate 5.9%, and female 22.7% in the populations studied. The plants with a single foliage leaf were usually males, and those with two foliage leaves usually females.

Of 12 quantitative characters measured, all are greater in female than in male except length of the peduncle.

Fruit- and seed-fertility of each plant were decidedly variable and low, and the average of 12 plants showed 52.5% and 17.6%, respectively. On the contrary, average number of seeds per fruit of each plant was very uniform, ranging from 1.0 to 2.2 and with 1.6 as the average. The ovary contained 3 to 7 ovules and 4.7 ovules per ovary was the average. Number of seeds per fruit was very different from the number of ovules per ovary. It is assumed that one of the factors causing such prominent differences would be something like developmental competition for food among the fertilized ovules during the young stage of fruit development.

54. *Fertility and crossability of certain wild brambles of Minnesota and Wisconsin*

(By Sadao SAKAMOTO and Arthur N. WILCOX*)

The identification of 48 strains of *Rubus* collected in Minnesota and in Wisconsin was studied. In this collection, 7 species were identified: *R. strigosus*, *R. occidentalis*, *R. allegheniensis*, *R. flagellaris*, *R. minnesotanus*, *R. Groutianus* and *R. vermontanus*.

From their records of performance under cultivation at the Fruit Breeding Farm during 1958–1961, 9 strains were recognized as relatively winter-hardy.

Seventeen strains of blackberries and dewberries were selected for further examination. Chromosome numbers of each strain were determined. In *R. allegheniensis*, diploid and triploid strains were found. In *R. minnesotanus*, tetraploid (?) and pentaploid were observed. *R. flagellaris* was pentaploid. *R. Groutianus* and *R. vermontanus* were all tetraploid. The origin of different ploidy forms was discussed.

Several horticultural characters, such as tip-rooting, seed weight, *etc.*, were observed.

* Department of Horticulture, Institute of Agriculture, University of Minnesota, St. Paul, Minnesota, U.S.A.

Pollen fertility and pollen germination on culture media were variable from strain to strain.

Fruit fertility was also very variable. The highest self- and open-pollinated fruit fertility obtained were 28 and 38 per cent, respectively, in a strain of *R. minnesotanus*. The ratio between self- and open-pollinated fertility was calculated. Three strains of *R. allegheniensis* and one of *R. minnesotanus* showed low values of the ratio.

Crosses among blackberries and dewberries, and between the blackberry and dewberry were made and studied. Cross-pollinated fruit fertility ranged from 0 to 12.4 per cent. Crossability was higher in the crosses within sections of the genus than in those of intersectional combinations.

Crosses between the blackberry or the dewberry and the cultivated red raspberry were made in an exploratory way, looking toward breeding for new combinations of desirable characters. When crosses were made between the red raspberry as the female parent and the blackberry or the dewberry as the male parent, they were much more successful in the production of seed than in the reverse combination.

55. *The origin of Prunus yedoensis*

(By Yō TAKENAKA)

Prunus yedoensis Matsumura (Somei-Yoshino) is the most famous flowering cherry tree grown in Japan. Its origin has been unknown, however. This species has abundant beautiful flowers, but sets only a few seeds. Accordingly, it can be propagated only by grafting. However, it grows more rapidly than any other cherry tree. From these facts, I have assumed that it might be a hybrid. I gathered seeds from many trees of this species in 1952. The seeds were sown in 1953, and the seedlings were observed from 1954 to 1962. From these observations, *P. yedoensis* was considered to be a hybrid between *P. lannesiana* var. *speciosa* (Ōshima-Zakura) and *P. subhirtella* var. *pendula* forma *ascendens* (Edo-Higan), whose characteristics are as follows: *speciosa* is distinguished from *ascendens* by underneath glabrous and larger leaves, and vigorously growing stems. The seedlings of *yedoensis* showed in these respects a series of intergrades ranging from *speciosa* to *ascendens* types. Some of the seedlings bloomed in the spring of 1959, and since then, 25 trees had flowers. In many flower characters, viz., size, color, and hairiness at peduncle, receptacle, calyx, style and ovule, the trees showed a wide range of variation from *speciosa* to *ascendens*.

In further experiments started in 1957, I made reciprocal crosses between *speciosa* and *ascendens*. In 1961, 8 hybrid plants bloomed. They

were intermediate between the parents in the characters of stem, leaf and flower, and appeared as a whole to be similar to one another, though they showed some minor differences. Two of them had hairs on the style and ovule like *P. yedoensis*, and the other two had a few hairs, while the rest were hairless. In other respects, they were similar to *P. yedoensis*, though the size of leaves and flowers was about 15% to 20% larger.

It seems that the parental trees used for this crossing experiment, which were taken from populations of the respective species, have been heterozygous. Accordingly, the hybrid plants might have segregated as to hairiness and some other minor characters. It may be concluded that *P. yedoensis* is a hybrid between *speciosa* and *ascendens*.

The question arises from where *P. yedoensis* was introduced. Tradition says that it was brought over from Ōshima, one of the Izu islands. But on the island the only flowering cherry growing wild is *P. lannesiana* var. *speciosa*.

Dr. E. Koehne (1912) named a flowering cherry tree collected by Taquat (a missionary) from Quelpart island of Korea *P. yedonisis* var. *nudiflora*. Later, Dr. G. Koizumi believed that *P. yedoensis* was brought from Quelpart island. On the other hand, Dr. E. H. Wilson (1916) stated: "To me *P. yedoensis* Matsumura strongly suggests a hybrid between *P. subhirtella* var. *ascendens* Wilson and the wild form *P. lannesiana* Wilson. It has many characters of the latter and in its venation, pubescens and shape of the cupula resembles the former." Dr. Koizumi went to Quelpart island in 1932 and found one tree which he identified with *P. yedoensis*. Since his identification was questionable, I visited the island in 1933 and observed the tree. The tree, which was growing wild, showed difference from *P. yedoensis*; the amount of hairs on calyx lobes and back side of leaves was less, and the peduncles were shorter than those of the latter. At any rate, it was not *P. yedoensis*. I assume that it has been a hybrid between *P. subhirtella* var. *pendula* f. *ascendens* and *P. quelpartensis* (Tanna-Yamazakura; perhaps a form of *P. verecunda*) or other cherry species.

In Japan, the putative parental species of *P. yedoensis*, *P. speciosa* and *P. ascendens*, were reported to grow together in Bōsō-peninsula and Izu-peninsula. I surveyed wild cherry trees in these two districts. Though both species were found in Izu-peninsula, only *speciosa* was found in Bōsō-peninsula. In Izu, further, I found trees which were presumably the offspring of *P. yedoensis*, and a tree which appeared to be a new hybrid between *speciosa* and *ascendens*. That tree was identical with one of my synthesized trees. It then seems most probable that *P. yedoensis* has originated in Izu-peninsula.

56. *Cytogenetic studies in the genus Nicotiana XIV*

(By Yō TAKENAKA)

The reduction division in PMC's was studied in 4 hybrids between *N. paniculata* and 4 other species of section Alatae, namely *N. paniculata*×*N. alata*, *N. paniculata*×*N. langsdorffii*, *N. paniculata*×*N. plumbaginifolia* and *N. paniculata*×*N. longiflora*.

1) F_1 of *N. paniculata* (n=12)×*N. alata* (n=9).

At MI of PMC's of this hybrid, the number of bivalents ranged from 0 to 6, with the mode at 3. The frequency of PMC's with 4 and 2 bivalents followed that of PMC's with 3 bivalents. PMC's with 1 and 5 bivalents were occasionally found but those with 6 bivalents or without bivalents were very rare.

In the same hybrid, Kostoff (1943) found that the number of bivalents ranged from 2 to 7 and observed occasionally a few trivalents. His observation of trivalents is doubtful, because the trivalent-like chromosomes in my preparations were heteromorphic bivalents. Otherwise Kostoff's results generally agree with mine.

2) F_1 of *N. paniculata* (n=12)×*N. langsdorffii* (n=9).

At MI of PMC's of this hybrid, bivalent range was from 0 to 4 with the mode at 1 and 2. The frequency of PMC's with 0 bivalents followed that of PMC's with 1 or 2 bivalents. PMC's with 3 bivalents were occasionally found but those with 4 bivalents were very rare.

In the same hybrid, Goodspeed (1954) found that the number of bivalents ranged from 0 to 4, with the mode at 2. His results agree with mine. According to Kostoff's description (1943), he and also Dremlug observed in the same hybrid that the number of bivalents ranged from 0 to 7 and some trivalents were present. The difference between the results observed by Goodspeed and also myself and those of Kostoff and Dremlug is too large to be attributed to different strains used by the authors.

3) F_1 of *N. paniculata* (n=12)×*N. plumbaginifolia* (n=10).

So far as I know, no investigation of this hybrid has been published.

At the MI of PMC's, the bivalent range was from 0 to 3, with the mode at 0. Accordingly *N. paniculata* is assumed to be less related to *N. plumbaginifolia* than to *N. alata* and *N. langsdorffii*.

4) F_1 of *N. paniculata* (n=12)×*N. longiflora* (n=10)

No findings on this hybrid have been reported till now, so far as I know.

At MI of the PMC's of this hybrid, the number of bivalents ranged

from 0 to 2, with the mode at 0. *N. longiflora* is very closely related to *N. plumbaginifolia* and the chromosome pairing in the hybrids *N. paniculata* × *N. longiflora* and *N. paniculata* × *N. plumbaginifolia* was similar.

From the results of the chromosome pairing in the four hybrids mentioned above, it is assumed that *N. paniculata* is not related to the above four species belonging to section Alatae.

57. *Alkaloid decomposition by tobacco extract*

(By Masao TANAKA)

The citric acid extract of pulverized tobacco leaves developed on a chromatogram with 50% acetone contains a substance which decomposes tobacco alkaloid.

On the paper strip on which the extract has been developed, a yellowish orange spot of alkaloid appears at the position of *Rf* 0.85 when it is exposed to the vapour of cyanogen bromide after spraying with an ethanolic solution of *p*-aminobenzoic acid. When the developed chromatogram is treated in the same way as that of the cited above after spraying with a dilute solution of alkaloid, two spots of lighter hue resulted from the lack of reaction appear adjacent to the anterior and posterior ends of the original spot of alkaloid on the yellowish orange background of the paper. The posterior spot is larger than that of the anterior one.

Decomposition of alkaloid can be demonstrated numerically by nephelometric analysis—by measureing the density of precipitation caused by adding a solution of silicotungustic acid—of alkaloid solutions in which divided chromatograms have been dipped and incubated for several hours.

Alkaloid decomposition by tobacco extract is detectable in majority of tobacco varieties. It is activated by the exposure of ultraviolet light and prevented by the spray with a dilute solution of pepsin. Nornicotine seems to be more easily decomposed than nicotine. This phenomenon corresponds with the facts that leaves of the tobacco varieties which possess nicotine-nornicotine converting factors have a lower level of alkaloids in comparison with those of the varieties without it, and that majority of the low alkaloid species of *Nicotiana* are nornicotine types.

58. *Inheritance of anthocyanin concentrations in Torenia flowers*

(By Toru ENDO)

Torenia fournieri Lind. is known to occur in flower colors, deep blue-purple and white. Two plants with pale blue-purple flowers appeared in the sample of the deep blue-purple form. The flowers of deep and pale blue-purple forms and those of six F_1 hybrids among all three forms were determined to contain the same three anthocyanins, namely, malvidin-, petunidin- and delphinidin-glycosides. The white form is slightly purplish on the lower lip, where traces of the anthocyanins were detected. Malvidin-glycoside is the major component in all materials; it was isolated in crystalline form and identified with malvin. The other two are minor components.

It was concluded from F_2 segregations that the genotypes of the three forms were *AABB*, *AAbb* and *aaBB* for deep blue-purple, pale blue-purple and white, respectively, and that *A* and *B* are basic complementary genes controlling anthocyanin formation. The genotype *aabb* may be ascribed to the acyanic segregants with pure-white flowers. Further, the effects of genes *A* and *B* on anthocyanin formation were estimated for six different genotypes, as shown in Table 1. In the table, the relative concentrations of anthocyanins are optical density at absorption maximum, 524 mμ, of 1% aqueous hydrochloric acid extracts from fresh flowers.

Thus the concentrations of the anthocyanins show the following re-

Table 1. Relative concentrations of anthocyanins in *Torenia* flowers of different genotypes

Parentage	Flower color	Genotype	No. of plants	Variation in O.D.	O.D./flower
BP	deep blue-purple	*AABB*	25	0.737~1.140	0.869±0.254
pP	pale blue-purple	*AAbb*	25	0.037~0.242	0.096±0.013
W	white	*aaBB*	5	0.013~0.032	0.024±0.007
BP×W	deep blue-purple	*AaBB*	25	0.464~1.060	0.898±0.215
W×BP	deep blue-purple	*AaBB*	25	0.710~1.130	0.902±0.288
BP×pP	deep blue-purple	*AABb*	25	0.592~1.125	0.824±0.128
pP×BP	deep blue-purple	*AABb*	25	0.472~0.862	0.703±0.103*
pP×W	blue-purple	*AaBb*	25	0.441~0.760	0.596±0.091*
W×pP	blue-purple	*AaBb*	25	0.264~0.772	0.416±0.157*

* significant at the 5% level as compared with *AABB*.

lations,

$$AABB = AaBB > AABb > AaBb > AAbb > aaBB > aabb = 0.$$

It was found that dominance of *A* over *a* is complete and that of *B* is incomplete under the present environmental conditions.

59. *Cytological studies on the nuclei of yeasts*

(By Yoshiaki YONEDA)

The nuclear structures and the division process of five species of yeasts, namely a strain of *Saccharomyces*, *Schizosaccharomyces pombe*, *Lipomyces starkeyi*, *Torula utilis* and *Torula rubra* were studied.

The nucleus of all species contained chromatin and a fairly large nucleolus. The chromatin was Feulgen-positive and stained with aceto-orcein and Giemsa. The chromatin of *Saccharomyces*, *Schiz. pombe* and *T. utilis* also stained with methyl green. The nucleolus was Feulgen-negative and stained densely with haematoxylin and aceto-carmine. Giemsa staining revealed both the chromatin and the nucleolus, but the latter took a more reddish color than the former. The nucleolus of *Saccharomyces* also stained with pyronine. Besides the chromatin and the nucleolus, a nuclear membrane and karyoplasm were also recognized in the nucleus.

The nuclear division of yeast proceeded in two stages, namely in the first stage the division of the chromatin (prophase to telophase) took place (dividing stage), and in the second stage followed the separation of the divided chromatin (separation stage). The homogeneous chromatin at resting stage granulated at prophase and chromosomal bodies appeared at metaphase. Six or more such bodies were recognized in *Saccharomyces*, about four in *Lip. starkeyi* and four in *T. utilis*. In *T. rubra*, such bodies were also recognized, but their number was not determined. In *Schiz. pombe*, chromosomal bodies were not clearly distinguished. Anaphase and telophase were carried out on one side of the nucleoli. In these stages, the nucleoli of four species except *Saccharomyces* kept their spherical shape. The nucleolus of *Saccharomyces* changed the shape in these stages. The divided chromatin condensed at telophase.

In the separation stage, the nucleoli of *Saccharomyces* and *Schiz. pombe* elongated, became constricted at the midregion and separated into daughter nuclei, while those of *Lip. starkeyi*, *T. utilis* and *T. rubra* were left behind in the mother cells and disintegrated there. In the latter species, the daughter nucleoli seem to be synthesized *de novo* in the daughter nuclei. Thus, two types of nucleoli were found with regard to their

behavior during nuclear division.

In *T. utilis* and *Lip. starkeyi*, a slender structure was found, which connected two masses of separating chromatin. The chromatin was assumed to be separated by elongation of this structure. This finding of the spindle-like structure suggests a possibility that the spindle mechanism also operates during the dividiug stage of *T. utilis* and *Lip. starkeyi*.

It was confirmed by staining with Feulgen-carmine method that the nucleus of *Saccharomyces* is a small body lying outside of the central vacuole. A central granule was found at the center of metaphase chromatin ring in *Saccharomyces*. It seems to divide at anaphase.

The vegetative nuclei of yeasts are assumed to multiply by mitosis. However, the behavior of the nucleoli during nuclear division seems to be different from those of higher organisms.

C. MATHEMATICAL GENETICS

60. *A general formula for the probability of fixation of mutant genes in a population*

(By Motoo KIMURA)

In this report I will consider the probability of fixation of genes in a random mating population of effective size N. Let $u(p, t)$ be the probability that a mutant gene becomes fixed in the population (i.e. its relative frequency becomes 1) by the t^{th} generation, given that its frequency is p at $t=0$. If $M_{\delta p}$ and $V_{\delta p}$ are respectively the mean and the variance of the rate of change in p per generation, then $u(p, t)$ satisfies the following equation,

$$\frac{\partial u(p,t)}{\partial t}=\frac{1}{2}V_{\delta p}\frac{\partial^2 u(p,t)}{\partial p^2}+M_{\delta p}\frac{\partial u(p,t)}{\partial p}, \tag{1}$$

with boundary conditions of

$$u(0,t)=0\,,\qquad u(1,t)=1\,. \tag{2}$$

The probability of ultimate fixation denoted by $u(p)$, i.e.

$$u(p)=\lim_{t\to\infty}u(p,t)$$

may be obtained from (1) by putting $\partial u/\partial t=0$:

$$\begin{cases}\dfrac{1}{2}V_{\delta p}\dfrac{d^2u(p)}{dp^2}+M_{\delta p}\dfrac{du(p)}{dp}=0\,,\\ u(0)=0\,,\quad u(1)=1\,.\end{cases} \tag{3}$$

Thus we obtain

$$u(p)=\frac{\int_0^p G(x)dx}{\int_0^1 G(x)dx}, \tag{4}$$

where

$$G(x)=e^{-\int\frac{2M\delta x}{V\delta x}dx}.$$

The above formula (4), which gives the probability of ultimate fixation of a mutant gene with initial frequency p, has simplicity and generality comparable to Wright's well-known formula for the gene frequency distribution at equilibrium. It includes previous results as special cases and can be used to solve problems where there is random fluctuation in selection intensity.

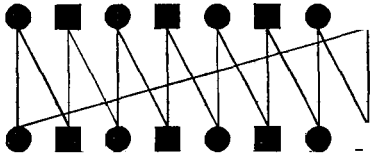
61. *On circular mating systems*

(By Motoo KIMURA)

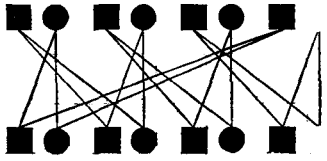
It is well known that in a random mating population of N monoecious individuals, the frequency of heterozygotes decreases at the rate of $1/(2N)$ per generation, as first shown by Wright. It was also found by him that in a population consisting of n males and n females ($N=2n$) the heterozygosity decreases asymptotically at the rate of $1/(2N)$ under random mating, while it decreases at the rate of $1/(4N)$ under what he calls maximum avoidance of consanguineous mating.

The purpose of this note is to report an unexpected finding that in mating systems which I would like to call circular mating systems, the asymptotic rate of decrease of heterozygosity is much smaller.

In the simplest case of what may be called the individual circular mating (Fig. 1a) n males and n females are arranged alternately and each individual is mated to adjacent individuals to form, so to speak, a circle. For this mating system, the ratio λ by which heterozy-



1a



1b

Figs. 1a and 1b. Examples of circular mating systems involving 8 individuals. 1a: circular individual mating. 1b: circular pair mating.

gosity decreases each generation satisfies the equation

$$(1) \qquad \begin{cases} \lambda=\dfrac{1}{2}+\dfrac{1}{2}\cos\theta \\ \sin\theta=\cot n\theta\,, \end{cases}$$

from which we can obtain the ultimate rate of decrease in heterozygosity:

$$(2) \qquad \varepsilon=1-\lambda=\frac{\pi^2}{16(n+1)^2}-\frac{\pi^4}{2^5 4!(n+1)^4}\cdots\,,$$

where λ is the largest root of the equation (1). Thus for a large N,

$$(3) \qquad e\sim\frac{\pi^2}{4N^2}=\frac{5}{2N^2}\,.$$

This may be compared with the corresponding value in the case of "maximum avoidance" of consanguineous mating, which is

$$(4) \qquad \varepsilon\sim\frac{1}{4N}\,.$$

For example, 100 individuals in (3) is approximately equivalent to 1,000 individuals in (4).

Circular mating may also be carried out between subpopulations in such a way that they are arranged on a circle and males in each subpopulation are mated to females in a neighboring subpopulation. The simplest case of this type of mating is circular pair mating illustrated in Fig. 1b. In this case, it can be shown that the ultimate rate of decrease in heterozygosity is asymptotically

$$(5) \qquad \varepsilon\sim\frac{\pi^2}{N^2}\,,$$

i.e. only 1/4 as slow as in the case of the circular individual mating, though it can still be much better than the "maximum avoidance" system in keeping heterozygosity if N is large.

Investigation on the properties of the circular mating systems is in progress in collaboration with Dr. J. F. Crow.

62. *The maintenance of supernumerary chromosome in wild populations of Lilium callosum by preferential segregation*

(By Motoo KIMURA)

In *Lilium callosum*, a supernumerary chromosome called f_l (meaning long fragment) by Kayano shows meiotic drive and tends to increase

its number through maternal line transmission. It markedly reduces both pollen and seed fertility when more than one is present. The frequency distribution of f_l in natural populations was investigated. The analysis has shown that individuals with f_l's are not only less fertile but also less viable. A mathematical theory was developed to analyse the mechanism of distribution of f_l in the populations. The concept of distortional load (or load due to meiotic drive) was applied to the maintenance of f_l in the natural populations and it was estimated that this load amounts to about 17 percent. The detail of the present investigation has been published recently in GENETICS in collaboration with H. Kayano.

Results of this investigation seem to suggest a general mechanism by which a relatively large but inert supernumerary chromosome is evolved in the history of a species.

For any supernumerary chromosome to be permanently maintained in a population, probably it must be endowed with meiotic drive (i.e. segregation distortion) mechanism *from the outset* so that it is protected from loss either by selection or random genetic drift. Such a supernumerary chromosome which is derived originally from the proper set of chromosomes must be deleterious for the possessor because of the trisomic condition that it creates and the segregation distortion has to be sufficiently strong to counterbalance the selection pressure. The spontaneous origin of the meiotic drive mechanism might be due to mutation in the centromeric region or by some other unknown cause, but recent discovery of SD factor in *Drosophila melanogater* by Hiraizumi seems to suggest that such event is quite probable in the long history of at least some species. Once infected by such mechanism, the genetic load will immediately be imposed by such segregation distortion. Thus for the species to survive, it must either get rid of the supernumerary chromosome by suppressing the distortion or to reduce the load by making the effect of the supernumerary chromosome less deleterious. In the latter case, which is of interest in the present context, both evolutionary modification of the proper genotype by natural selection and loss in function of the supernumerary chromosome due to the accumulation of amorphic mutations in it must be effective. The relative importance of these two factors may be evaluated by finding two completely isolated but closely related races, one having a large supernumerary chromosome at a very high frequency and the other entirely lacking of it and by crossing them to see if introduction of the supernumerary chromosome from the former race into the latter will greatly influence the fitness of the latter or not. If the apparent inertness of the supernumerary chromosome is really due to accumulation of amorphic mutations, no exces-

sively strong deleterious effect will be found upon introduction into the other race.

63. *Distorted segregation and substitutional load*

(By Yuichiro HIRAIZUMI)

Let p_t and q_t be respectively the frequencies of a driven (A_2) and its normal element (A_1) in the gamates of t-th generation. The fitnesses of three genotypes and their frequencies are listed below. Here we assume random mating.

Genotype	A_1A_1	A_1A_2	A_2A_2
Fitness	$1-s$	$1-s(1-h)$	1
Frequency	q_t^2	$2p_tq_t$	p_t^2

The average fitness of this population is

$$\bar{w}=1-s(1-p_t)\{p_t(1-2h)+1\},$$

and the load in the t-th generation is given by

$$L_t=s(1-p_t)\{p_t(1-2h)+1\}.$$

Let k be the segregation ratio of A_2 from a heterozygote A_1A_2 in the next generation ($1\geq k>0.5$), then we have the following equation for the frequency change of A_2.

$$\frac{dp_t}{dt}=\frac{p_t(1-p_t)}{\bar{w}}\{p_ts(1-2h)+2k(1-s+sh)-(1-s)\}.$$

The total substitutional load is then computed to be

$$L_s\fallingdotseq\int_0^\infty L_t dt=\int_{P_0}^1 \frac{s\{p_t(1-2h)+1\}[1-s(1-p_t)\{p_t(1-2h)+1\}]}{p_t\{p_ts(1-2h)+(1-s)(2k-1)+2ksh\}}dp_t.$$

If $s\ll 1$, this is approximated by

$$\begin{aligned}L_s&=s\int_{P_0}^1\frac{p_t(1-2h)+1}{p_t\{p_ts(1-2h)+(1-s)(2k-1)+2ksh\}}dp_t\\&=-\Bigg[s\log p_0+\{2ks(1-h)-(2k-1)\}\\&\quad\times\log\left\{\frac{s(1-2h)+(1-s)(2k-1)+2ksh}{p_0s(1-2h)+(1-s)(2k-1)+2ksh}\right\}\Bigg]\Big/\{(1-s)(2k-1)+2ksh\},\end{aligned}$$

where p_0 is the initial frequency of A_2. When $2k-1=0$ (segregation normal), the above solution can be reduced to a simple form obtained by Kimura (1959). The numerical examples of L_s are given in Table 1.

When $2k-1=0$, L_s is independent of s but with drive it is no more independent; a larger value of s involves a larger substitutional load. It is worth noting that $h=1$, i.e., complete dominance of A_2 over A_1, reduces L_s from 103.6 to 4.6 when $k=0.5000$. On the other hand, $k=0.5100$ (only 0.01 in excess) reduces the load to the same, or less extent. This shows how effectively a slight drive works to accelerate the accumulation of a beneficial element in a population.

Table 1. Substitutional load, L_s, under various values of k, h, and s. The initial frequency of p is 0.01 for all the cases

	k	L_s		
		$s=0.005$	0.01	0.02
0	0.5000	103.6	103.6	103.6
	0.5001	47.0	57.1	71.4
	0.5010	9.7	16.2	25.5
	0.5100	1.3	2.5	4.6
	0.5500	0.3	0.6	1.1
0.001	0.5000	99.0	99.0	99.0
	0.5001	42.0	55.2	67.4
	0.5010	9.7	16.2	25.5
	0.5100	1.3	2.5	4.6
	0.5500	0.3	0.6	1.1
0.01	0.5000	73.2	73.2	73.2
	0.5001	37.9	48.9	58.5
	0.5010	9.6	15.7	23.8
	0.5100	1.3	2.5	4.6
	0.5500	0.3	0.6	1.1
0.1	0.5000	27.0	27.0	27.0
	0.5001	21.6	23.9	25.4
	0.5010	8.9	11.8	17.0
	0.5100	1.2	2.4	4.3
	0.5500	0.3	0.5	1.0
1	0.5000	4.6	4.6	4.6
	0.5001	4.3	4.3	4.5
	0.5010	2.9	3.2	4.2
	0.5100	0.7	1.3	2.0
	0.5500	0.2	0.3	0.9

64. *Population dynamics of sex-linked recessive lethals*

(By Terumi MUKAI)

In general, the heterogametic sex has a mechanism working toward elimination of detrimental genes from the sex chromosomes, contributing a great deal to the formation of their specific genic systems (MUKAI 1961). A study was carried out with respect to a single locus in order to understand the process of elimination from the population of the recessive lethals, the most detrimental genes of the sex chromosomes.

In order to set up mathematical models in the homogametic sex, the following parameters are defined:

Q_n = relative frequency of lethal heterozygotes (which are described as Aa) in the n-th generation.
$1-Q_n$ = relative frequency of AA individuals in the n-th generation.
m = relative mating ability of Aa males, that of AA being 1.
f = relative fecundity of Aa females, that of AA being 1.
v = relative zygotic viability of Aa individuals, that of AA being 1.
s = relative competitive ability of a sperm, that of A being 1.
n = the number of generations.

In the case of *Drosophila*-type sex-linked recessive lethal genes (females are homogametic), the following general formula can be obtained:

$$Q_n=\frac{Q_1(2-fv)(fv)^{n-1}}{2^{n-1}[(1-Q_1)(2-fv)+fQ_1]+Q_1[2-f(1+v)](fv)^{n-1}} \qquad (1)$$

Although the lethal gene can be maintained in a population under the condition of $fv>2$ (the equilibrium frequency Q is given as formula (2)), it is practically impossible to satisfy this condition.

$$Q=\frac{2-fv}{2-f(1+v)}. \qquad (2)$$

In the case of *Bombyx*- or chicken-type sex-linked recessive lethals, which might be assumed to be completely lethal in the heterogametic sex (female), the following general formula can be obtained:

$$Q_n=\frac{Q_1(1+s-smv)(smv)^{n-1}}{\Delta} \qquad (3)$$

where $\Delta=(1+s-smv)[(1-Q_1)(1+s)^{n-1}+Q_1(smv)^{n-1}]+Q_1m[(1+s)^{n-1}-(smv)^{n-1}]$.

Under the condition of $1+s<smv$, the lethal gene is maintained in the population. The equilibrium frequency is as follows:

$$Q=\frac{1+s-smv}{1+s-m(1+sv)}. \qquad (4)$$

However, it is too difficult to satisfy this condition, and all lethal genes

will be eliminated from the population.

Details of this work will be published elsewhere.

65. *Equation for polygenic mutation rate per locus per unit dose*

(By Sohei KONDO)

Let us assume that an individual of an arbitrary isogenic line has n polygene loci with gene g_i at locus i ($i=1, \cdots, n$), that g_i mutates from the wild type g_{io} to an allele g_{ir} with the probability p_{ir} ($r=1, \cdots, \nu_i$; ν_i being the total number of the polygene alleles at locus i) and with a contribution of amount x_{ir} to change the quantitative character X under consideration and finally that x_{ir} is small enough to assume the validity of additivity law for change in X from the contribution of simultaneous mutations in g_i's in an individual. Then, we obtain the probability $f(x)$, that an irradiated organism gives rise to an overall genetic change x in X, as the coefficient of z^x in the expansion of the generating function:

$$G(z) \simeq \exp\left[-\sum_{i,r} p_{ir} + \sum_{i,r} p_{ir} z^{x_{ir}}\right] = \sum_x f(x) z^x , \qquad (1)$$

where we have neglected higher order terms of p_{ir}. Assuming $p_{ir}=c_{ir}D^k$ (D: radiation dose, k: a contant, c_{ir}: proportionality constant), from equation (1) we have equations for j-th moment of x as follows:

$$\mu_j = A_j D^k ; \; A_j = \sum_{i,r} c_{ir}(x_{ir})^j \qquad (j=1, 2, 3,) . \qquad (2)$$

This means that the mean, the variance and the third moment of x have the same relationship with dose except for the difference in proportionality constant A_j. If $k=1$ as the case of major genes, then variance μ_2 must increase linearly with dose.

To obtain an explicite formula for average mutation rate per locus per unit dose, we assume that $k=1$, $c_{ir}=c$, $\nu_i=\nu$, $x_{ir}=x_i$ and that x_i is distributed about mean a in a normal distribution with standard deviation σ_a. Then, from equation (2) we obtain

$$\left.\begin{aligned} \mu_1 &= n\nu cDa , \\ \mu_2 &= n\nu cD(a^2+\sigma_a{}^2) , \\ \mu_3 &= n\nu cD(3a\sigma_a{}^2+a^3) . \end{aligned}\right\} \qquad (3)$$

That is, mutation rate per locus per unit dose νc is given by

$$\nu c = \mu_1/nDa , \qquad (4)$$

where

$$a = \frac{3}{4}\frac{\mu_2}{\mu_1}\left[1+\left\{1-\frac{8}{9}\left(\frac{\mu_3}{\mu_1}\right)\left(\frac{\mu_1}{\mu_2}\right)^2\right\}^{1/2}\right] . \qquad (5)$$

D. GENETICS AND BIOCHEMISTRY OF MICROORGANISMS

66. *A temperature independent flagellation mutant in Salmonella**

(By Tetsuo IINO)

A strain of *Salmonella typhimurium*, TM2, produces peritrichate flagella (six to ten flagella per bacterium) when it is cultivated in the nutrient broth at temperatures below 40°C. The optimal temperature for flagellar production is for this strain around 30°C. At 44°C the cell can divide but they lose the ability to produce flagella whose number per bacterium decreases as the cells multiply though the preformed flagella are not destroyed at that temperature. In ten to fifteen cell generations after the culture of flagellated cells was transferred to 44°C, more than 99% of the cells become non-flagellated and are therefore non-motile. The remaining cells carry only a few flagella and rotate in the broth culture.

A mutant which produces flagella at 44°C was isolated from the strain TM2 cultivated on a semisolid nutrient agar plate at 44C°. The mutant cells can produce flagella at 44°C as well as at 37°C, 30°C and 20°C, in either antigenic phase-1 or phase-2. No differences in the specificity of H-antigen between the flagella of TM2 and the mutant or between those produced at 30°C and 44°C by the mutant could be detected by a cross absorption experiment. The mutant marker is transduced independently of any known genes which control flagellation or motility in Salmonella. The physico-chemical comparative study of the flagella produced by the wild type and the mutant is on the way.

67. *A modified selective medium for the isolation of motility mutants of Salmonella***

(By Masatoshi ENOMOTO and Tetsuo IINO)

With the exception of some specific serotypes (Sasaki, Annual Report, No. 11, 1960), chi-phage infects motile cells of Salmonella. The non-motile cells are resistant against chi-phage, regardless of the presence of

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paralyzed flagella. Therefore, a nutrient agar plate spread with chi-phage (X-NA) has been used as a selective medium for the isolation of non-motile mutants. On X-NA, however, the cells of many motile strains tend to lose temporarily their motilty, and grow to minute colonies. Moreover, some motile clones, which may be temporary carriers of chi-phage, as well as the non-motile mutants can form colonies. A modified selective medium, X-NGA, was devised to remove these defects of X-NA and to improve the procedure of isolation of non-motile mutants. In X-NGA, the nutrient agar of X-NA was replaced by semisolid nutrient gelatine agar which has been used for the isolation of motile mutants from non-motile bacterial cultures (Iino, Annual Report, No. 9, 1958). When the motile cells of Salmonella are plated on X-NGA and incubated 4 hours at 37°C and then the colonies are allowed to develop at 24°C for 18 hours, a non-motile mutant clone will form a compact colony, while the clone of a temporary phage carrier spreads as a swarm and the development of non-heritable chi-resistant minute colonies is suppressed remarkably. In certain salmonella strains, *e.g. S. typhimurium* TM2, *S. abony* SW 1391 and *S. abortus-equi* SL 23, it was also found that the colonies formed on the plate by paralyzed and non-flagellated mutants are different, the former being large, opaque and yellowish-white, while the latter are small, translucent and white. By the use of NGA and X-NGA as selective media, it is now possible to select with high efficiency back and forth occurring motility mutations in Salmonella.

68. *H-antigen of heterozygous hybrids between Salmonella abony and Salmonella typhimurium**

(By Hideo HIROKAWA** and Tetsuo IINO)

An Hfr strain of *S. abony*, SW 1391 ($M^-Sh^-H_1{}^bH_2{}^{enz}$), was crossed with *S. typhimurium* SW 1292 ($P^-L^-Ara^-Gal^-Xyl^-Rha^-H_1{}^iH_2{}^{1,2}$). The auxotrophic markers of the parental strains were adopted as the selective markers. Each prototrophic clone isolated from the minimal selective media was transferred successively to nutrient agar plates (NA). H-antigens of the colonies grown on NA were typed by slide agglutination test. The nutritional requirements and sugar fermentation of the colonies were examined by the replica plating method.

* 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.

** Present address: Biochemical Institute, Faculty of Science, University of Tokyo, Tokyo.

Among the hybrids isolated, considerable numbers of persistent heterozygotes were obtained in respect to the selective and the unselective markers. The frequency of segregation of these persistent heterozygotes was less than 1%. When a suspension of the heterozygous cells, which contains 0.7% segregants, was irradiated by ultra-violet germicidal lamp (340 erg/mm^2), and plated on the indicator media, 20% of the colonies grown on them segregated.

The heterozygous cells carried the same H-antigens, *i* or *1.2*, as the female parental strain when isolated from the selective medium, and most of them continued to express *i* and *1.2* antigens alternatively in successive subcultures. By selection with anti-*i* or anti-*i.1.2* serum, the subclones which express alternatively either *b* and *1.2* or *b* and *e.n.x* were isolated. Two antigen types of the same H-phase had never been expressed simultaneously by a heterozygous clone. These subclones were usually still heterozygotic and dissociated cells expressing the hidden antigen *i* at a low frequency.

69. *Chi-phage resistance of the salmonella serotypes having g-antigen**

(By Itiro SASAKI)

It has been known that the salmonella serotypes having *g*-antigen are resistant against chi-phage, and that the essential genetic factor of the resistance is associated with the determinant of flagellar antigen *g*. So far, the attempt at isolating host range mutants of chi-phage against these serotypes has been unsuccessful by the standard method of selection, namely plating resistant bacterial cells with an excess of phage particles. However, some host range mutants (h^G) have been isolated from chi-page by serial mixed culture method, multiplying the phage in a mixed broth culture of both sensitive and resistant strains. A chi-phage resistant mutant SJ 100 (*i* : *1, 2*) was isolated from a sensitive strain, of *S. typhimurium*, TM 2. The strain forms smaller and thinner colonies than TM 2, and in antigenic *1, 2* phase it is resistant to chi-phage. In contrast to h^G, the host range mutants (h^T) against SJ 100 is easily isolated by the standard method of selection. Among 105 h^T mutants, only two can attack *g*-type Salmonella, while all of the h^G mutants, so far tested, can attack SJ 100 in *1, 2* phase as well as in *i* phase.

From these results and the result reported last year (Annual Report,

* 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. to T. Iino

No. 11, 1960) it is inferred that chi-phase resistant factors in *g*-type Salmonella consist of more than two blocking steps of phage multiplication, and that at least one factor is involved with the resistance factor of SJ 100. The following result further supports the above conclusion and suggests that in TM 2 there is a factor which blocks the infection of hG 12 in cooperation with a resistance factor associated with the determinant of *g*-antigen. A clone of hG mutant, hG 12, can attack both TM 2 and a strain of *S. dublin* (*gp*:-), NTCT 4197, while the *gp* : *1, 2* type recombinant, SJ 26, obtained by transduction of the *gp*-determinant from NTCT 4197 to TM 2, is resistant against hG 12.

The salmonella strains tested fall into four groups in regard to sensitivity to the host range mutants hG and hT.

(1) A type from which a higher yield of phage is obtained.
(2) A type from which a lower yield of phage is obtained.
(3) A type from which 'lysis from without' occurs only by the phages.
(4) A type which is completely resistant to the phages.

E. RADIATION GENETICS IN ANIMALS

70. *Radiation-induced mutation rate of polygenes controlling the number of sternopleural bristles in Drosophila melanogaster*

(Terumi MUKAI and Sadao CHIGUSA)

In order to obtain a fundamental information on the influence of radiation on human populations, the mutation rates of polygenes controlling the number of sternopleural bristle numbers have been estimated.

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

One of the most important results shown in Table 1 is that the variances of irradiated groups have increased in females but decreased in males as compared with the controls. We may attribute this difference to mutations (some radiation-induced changes) in hetero-chromatic parts of Y chromosomes, although this hypothesis should be re-examined. Thus, the mutation rates were estimated by using the data for females only.

Table 1. Means, variances, and third moments about the means of the sternopleural bristle numbers

	250r Experiment				500r Experiment			
	Control		Treated		Control		Treated	
	♀	♂	♀	♂	♀	♂	♀	♂
Mean	16.6244	16.0355	16.6778	15.9579	16.7538	16.1350	16.8980*	16.1881
Variance	1.4408	1.5872	1.6479**	1.4814	1.5907	1.7173	1.7340*	1.2629**
Third moment about the mean	0.4406	—	0.5228	—	0.5989	—	0.6620	—
No. of genomes tested	1592	1156	1704	1307	975	674	1088	914

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

1) The differences of the third moments about the means between the control and treated groups were not tested statistically.

Table 2. Estimation of radiation-induced mutation rates of polygenes controlling the number of sternopleural bristles

	250r Experiment	500r Experiment
Heterozygous effect of each mutation	1.240	0.710
Number of positive mutations per genome	0.089	0.243
Number of negative mutations per genome	0.046	0.041
Mutation rate	1.08×10^{-6}/locus/r	1.14×10^{-6}/locus/r

A method for the estimation of polygenic mutation rates developed by MUKAI (1961) with reference to BATEMAN's work (1959) was employed. In this method, the means, variances, and the third moments about the means of the distribution pattern have been used. The results are shown in Table 2.

The mutation rates were estimated on the assumption of 500 loci which control the number of sternopleural bristles. The estimated polygenic mutation rates were of the order of 10^{-6}/locus/r, and this is very close to the estimate of the mutation rates of polygenes controlling the viability (ca. 1.26×10^{-6}/locus/r) (MUKAI 1961).

Indeed, the assumption of 500 loci may be not an underestimate when FALCONER's (1960) estimation that the number of loci controlling the

abdominal bristles is 100 is considered. BATEMAN (1959) and KONDO (unpublished) have mentioned that the mutation rates estimated by this kind of method are underestimates, while the effect of each mutation, which is assumed to be a constant, is an overestimate. Thus, it may be concluded that the radiation-induced polygenic mutation rates are substantially higher than those for major genes, and are at least of the order of 10^{-6}/locus/r.

71. *Further radiation-genetical consideration concerning the genetic structure of natural populations*

(Terumi MUKAI)

Following the balance hypothesis (DOBZHANSKY 1955), we can say that heterosis (overdominance with respect to fitness) is a product of natural selection within populations.

Suppose that there are two isogenic lines, AA and BB extracted from different natural populations. A and B indicate the genomes. After irradiation (indicated by superscript [$'$]), heterozygotes AA' and AB' are produced. In expectation of radiation-induced mutations in A' and B', we compare the viability of AA' and AB' with AA and AB, respectively.

On the basis of the classical hypothesis, a reduction of viability in AA' and AB' can be expected in comparison with AA and AB, respectively.

On the contrary, according to the balance hypothesis, the results should be different, *i. e.*, the viability of AA' would increase as compared with AA, because a genic system, which will manifest heterosis when it becomes heterozygous by mutation, has been produced by natural selection. However, we can not expect increased viability in AB' as compared with AB, because A and B have independently experienced natural selection in different populations. Consequently, the genotype AB would not be a genic system that could reveal heterosis.

Thus, if AA' and AB' would manifest lower viabilities than AA and AB respectively, this might be one evidence in the favor of the classical hypothesis. However, if AA' would reveal better viability than AA, and AB' would show lower viability than AB, this would support the balance hypothesis.

BURDICK and MUKAI (unpublished) irradiated the males of the two isogenic lines with X-rays at doses of 30, 60, 90, 120, 150, and 180r, and tested the heterozygous viabilities in F_2 with respect to the radiation-induced mutations. As a standard of the viability estimation, *Xa*

heterozygotes with one of the isogenic lines (AA), $\frac{X_a}{+_A +_A}$ were employed. In every radiation treatment, the viability of AA' increased in comparison with AA, while that of AB' was decreased as compared with that of AB. These experimental results indicate that the balance hypothesis might be valid on the polygene level.

To follow up this argument, an experiment on a larger scale is now in progress.

72. *Further studies on two types of dose-rate dependence of radiation-induced mutation rates in spermatogonia and oögonia of the silkworm*

(by Yataro TAZIMA and Sohei KONDO)

Two types of dose-rate dependence of radiation-induced mutation frequency have been found in early gonial cells of the silkworm. In

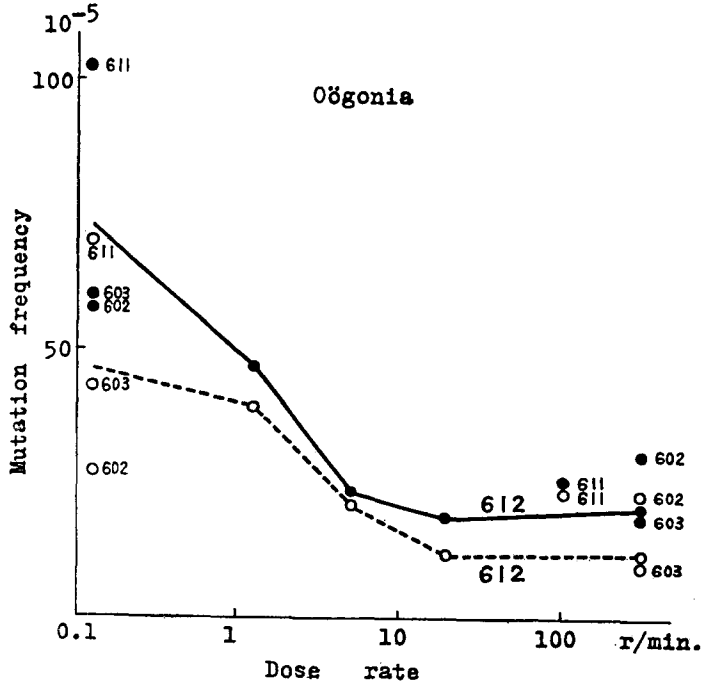

Fig. 1. Dose-rate dependence of radiation-induced mutation rate for late gonia. Solid line represents the changes in mutation frequency for $+^{pe}$ locus and broken line those for $+^{re}$ locus.

one type mutagenic effectiveness of chronic irradiation is lower than that of acute irradiation, and in the other the relation is reversed. The occurrence of either type depends upon the stage of germ cells. These findings have been interpreted by assuming, 1) differential repair for the former and 2) selective killing for the latter type (Genetics, 1961).

Further evidences compatible with the hypothesis of selective killing have been obtained. Variation in induced mutation frequency was studied along with the development of germ cells both for acute and chronic irradiation. It varied drastically after acute exposure, being highest at the time of hatching and decreasing rapidly toward later stages, while it was almost constant after the chronic treatment. Irradiation with various dose-rates in later stages revealed that mutagenic effectiveness of radiation decreases gradually with the increase in dose-rate within the range from 0.14*r*/min to 320*r*/min (Fig 1). In this stage mutation frequency for the acute treatment did not deviate, even at 150*r*, from the linear relation extapolated from higher doses, suggesting that the delivered dose was too high to realize the expectation that a small dose of acute irradiation might give a higher frequency than expected from the linear relation.

Although directly supporting evidences are still meagre, beyond doubt two distinct types of dose-rate dependence occur, one of which can not be interpreted by the repair hypothesis.

73. *Comparison of acute and chronic irradiations in respect to radiation-induced lethal mutation rates in the silkworm*

(by Yataro TAZIMA and Takao KOBAYASHI)

By using the lethal-free strain described in the preceding issue of this Annual Report (No. 11, 1961), experiments have been continued to compare radiation-induced lethal mutation rates between acute and chronic irradiations. Egg layings from a lethal-free strain, which had been checked again in P_1 generation, were divided into three groups, two for irradiation and one for control. The experiment was run in two series, with two parental lines. Both acute (320/min) and chronic (0.139r/min) γ-irradiations were administered to very young larvae with the same doses, 1000*r*. The dose-rates ratio was ca. 2300 : 1. Acute irradiation was given in one exposure of 3.12 minutes just after hatching, while the chronically irradiated group was exposed for 120 hours, starting two days before and continuing three days after hatching. During this period the germ cells are known to be at the primordial stage, or the most advanced ones at the stage of primary gonia. After the emer-

gence of irradiated individuals the females were crossed to males of the lethal-free strain and about 600 eggs were extracted at random from 30 layings for each group and were treated with hot hydrochloric acid to obtain the next generation, G_2.

By backcrossing G_2 females to males of the lethal-free strain about 200 G_3 layings were produced. Among them 100 G_3 layings were raised separately after the application of artificial hatching treatment. By sibmating G_3 moths within each batch, more than 100 layings of G_4 eggs were produced for each batch, to determine the frequency of radiation-induced lethals in G_1. The results are given in Table 1.

Table 1. Data obtained after the test for lethals in G_4 of the irradiated groups and scored on G_3 line basis.

Expt.	Irradiation	Number of G_3 lines				Total No. of lethals detected	Freq. of. lethals per G_3 line
		Detcted lethals per line			Total observed		
		0	1	2			
No. 211	Acute	63	33	4	100	41	0.4100
No. 221	Chronic	78	19	3	100	25	0.2500

The detected lethals in Table 1 comprise radiation-induced and spontaneous lethals contained in the gametes which were transmitted from G_2 females of the irradiated strain and spontaneous lethals contained in the gametes which were transmitted from G_2 males of the lethal-free strain (marked by D in Fig. 1). Therefore, to calculate radiation-induced mutation rates we have to subtract all those background spontaneous lethals from the observed data.

With regard to spontaneous lethals, their accumulation during three generations, P_2, P_1 and G_1, in the irradiated line and in P_2 and P_1 of the lethal-free strain (D), should be taken into account, because all lethals accumulated down to P_3 had already been eliminated and those newly arisen in G_2 and G_3 could not be detected in the present test. In the irradiated line, two kinds of gametes are transmitted in each batch from G_1 to G_2, one coming from the irradiated mother and the other from the non-irradiated lethal-free father (C).

Since we have found that the probability of the occurrence of spontaneous lethals is 0.004046 per gamete per generation (Annual Report No. 11), the number of accumulated lethals in each gamete transmitted to G_2 could be calculated as follows:

in the lethal-free strain (D)..... 0.016184 (for two gametes, P_2 and P_1)

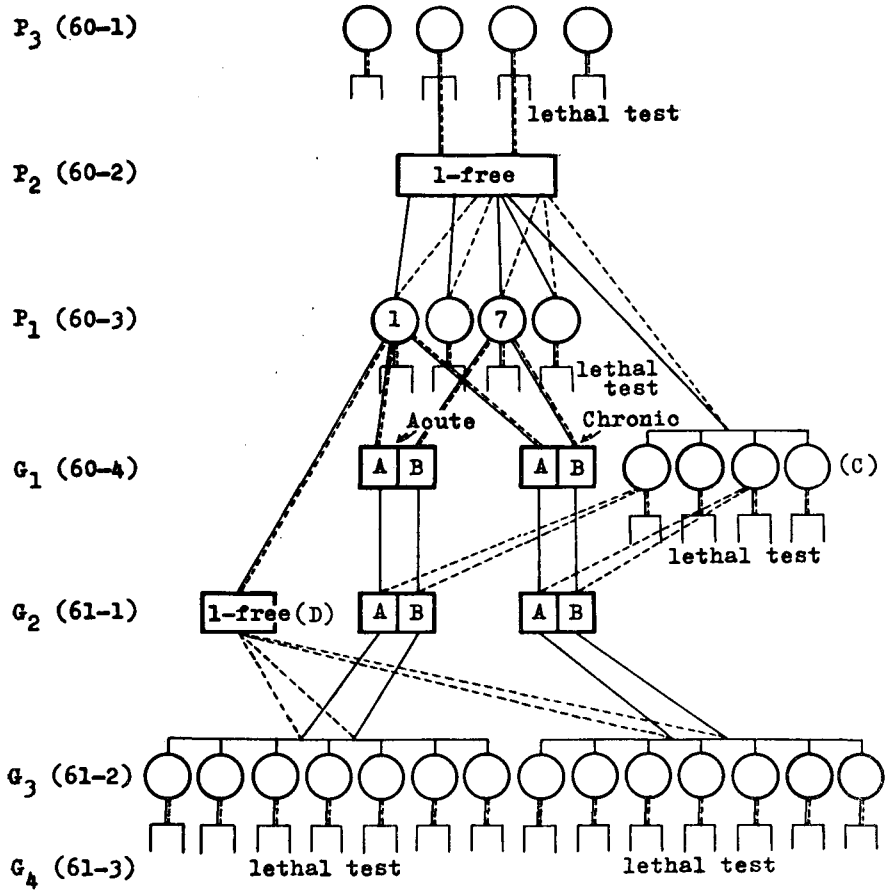

Fig. 1. Breeding scheme employed in the experiment. Single-pair culture is represented by a circle. Mass culture of several layings is indicated by a rectangle. Irradiation was given in G_1 generation.

in the irradiated strain one gamete from non-irradiated (C) .....0.008042 (in P_2 and G_1), the other one gamete from irradiated G_1....0.012138 (in P_2, P_1 and G_1). The total number of spontaneous lethals in the genetic background of this experiment, which can be detected in G_4, should therefore be 0.036418.

By subtracting the above figure from the observed values for both irradiated groups, the induced mutation rates were obtained (Table 2).

As may be clearly seen from the table, the calculated induced mutation-rate is higher in the acute group than in the chronic group, showing

Table 2. Calculated induced lethal mutation rates for both irradiated groups.

Expt.	Irradiation	Freq. of lethals detected	Background lethals	Induced lethals	Induced rate per gamete per 1000 r
No. 211	Acute	0.4100	0.0364	0.3736	0.3736
No. 221	Chronic	0.2500	0.0364	0.2136	0.2136

a good agreement with the results obtained by specific loci method (TAZIMA et al. 1961).

This work has been originally carried out in 1960 under the contract between I. A. E. A. and this Department. The results reported now were obtained in 1961 after the discontinuation of the contract.

74. *Differences in response of silkworm gonia to acute and chronic γ-irradiation*

(by Toshihiko SADO)

Two types of dose-rate dependence of radiation-induced mutation rates have been revealed both for the spermatogonia and the oögonia of the silkworm. In one type, mutagenic effectiveness is lower at chronic than at acute irradiation, and in the other type it is higher at chronic than at acute irradiation. The second type is found in the later stages of gonial development (Tazima *et al.* 1960, 1961). As a possible explanation it was assumed that germ cells at highly sensitive stages are selectively killed by acute but not by chronic irradiation, under the same dose. In order to obtain cytological evidence supporting this assumption, the present experiment was undertaken.

Both sexes of a wild type silkworm strain, C108, were exposed to Cs-137 γ-rays of high dose-rate (320r/min.) or to Co-60 γ-rays of low dose-rate (0.128r/min.) in early larval stages. Total doses given were 1000r in both groups. In the acute group, the insects were irradiated at early third instar, while in the chronic group, the irradiation time was so adjusted that it was terminated at the same time as for the acute group.

Acutely treated cells. Under acute irradiation the results obtained for male germ cell were quite similar to those of the earlier experiments (Sado 1958, 1961). High incidence of necrosis of the secondary spermatogonia was observed 24 and 48 hours after the exposure and the necrotic cells rapidly disappeared from the testes. Five days after the

exposure, regeneration of spermatogonia was observed to set in. In the female, no corresponding stages are found with cells mitotically as active as the secondary spermatogonia. No necrotic cells were observed in the ovary 24 and 48 hours after the treatment. But on the third day after the exposure, a few degenerating cells were observed in the proximal part of the ovarioles where the oöcytes and nurse cells were differentiating. However, the damage in the cells was much less severe in the female than in the male. Acutely irradiated oögonia, if not all, appear to degenerate around the critical stage when differentiation into oöcytes and nurse cells would occur if they had not been exposed to radiation.

Chronically treated cells. In the case of chronic irradiation, the insects were examined every 24 hours after the onset of the treatment. At 24 and 48 hours a few degenerating secondary spermatogonia were observed in the irradiated testes. Mitoses of spermatogonia, but not many, were observed at this time. 24 and 48 hours after the treatment was started the total doses given reached 200 r and 400 r, respectively. During the first 3 to 5 days, a reduction in the number of spermatogonia was remarkable. An increase in the number of secondary spermatogonia was not observed, mainly due to mitotic inhibition of the primary and perhaps of very early secondary spermatogonia. At the time of termination of the exposure the testes contained a few primary spermatocytes but very few, or none, secondary spermatogonia. Thus, it seems that a part of the secondary spermatogonia present at the time of the onset of the treatment were killed but others differentiated into primary spermatocytes although they were less numerous than in the non-irradiated testes. In the chronically irradiated females, degeneration of irradiated oögonia was not observed even at the termination of the treatment. But the development of the germ cells was retarded due to mitotic inhibition of the oögonia.

Thus, the experiment failed to furnish a conclusive evidence supporting the validity of the selective killing hypothesis which has been proposed by Tazima *et al.* (1961) for the explanation of the second type of dose-rate dependence.

75. *Number of gonial cells of the silkworm at early stages around hatching*

(by Takao KOBAYASHI)

Tazima, Kondo and Sado (1960) obtained many cluster mutations in their experiments on dose-rate dependence of radiation-induced mutations. In these experiments, the earlier was the irradiated stage, the larger

became the cluster size. This finding suggests that the cluster appearance of mutation is intimately correlated to the number of gonial cells present at the irradiated stage. That is to say, the mutation-bearing gonial cells multiply with the development of the insect.

In order to provide a cytological evidence for the theoretical considerations of the above authors, a cytological investigation has been carried out before and after hatching, in which special attention was paid to the number of germ cells in the gonads.

There are scarcely any follicular septa in the gonads before hatching. They appear first on the hatching day, and are completed on the next day. When septa are formed, the gonial cells seem to gather on the periphery of each follicle. From cytological observation, it was difficult to determine the sex of the gonads at those stages.

The number of gonial cells was counted carefully in each gonad from two days before to one day after hatching. The results are shown in Table 1.

Table 1. Number of gonial cells before and after hatching

Stage	Number of gonads examined	Number of germ cells per gonad		
		Minimum	Maximum	Mean
Two days before hatching	17	29	57	47.5
One day before hatching	28	37	79	59.1
Hatching day	11	82	162	119.5
One day after hatching	15	102	236	171.4

From these results, it is evident that the number of gonial cells increases rapidly after hatching.

The observed numbers agree in general with the theoretical calculation of Kondo (1961).

76. *Induction of a presumably somatic mutuation with 5-bromouracil in the silkworm*

(by Makoto NAKAJIMA)

It has been known in bacteria that certain base analogues incorporate into deoxyribonucleic acid resulting in a high incidence of mutations. So far, in higher organisms such investigations have been scarcely attempted.

In order to examine whether or not a similar situation may be found

in higher organisms, the present study has been undertaken with the silkworm. We confined ourselves to the detection of the mutagenic action of two base analogues in producing spots due to somatic mutation of the larval skin. For this purpose, heterozygotes for opaque (+) and translucent (*od*) served as a convenient material. The detection of mutation was carried out on the epidermis of the fifth instar larvae which had been raised during the first instar by feeding mulberry leaves supplemented with a solution of either of the two base analogues. The results obtained are as follows:

(1) With the administered doses from 4 to 38 μg. per individual, 5-bromodoexyuridine induced a large number of translucent spots, each comprising a number of cells. The supplementation of aminopterin, known as an inhibitor of thymidine synthesis, showed no significant effect on the production of the spots. The effect of 5-bromodeoxyuridine on producing mutations was about 8 times as high as that of 5-bromouracil for equimolar administration.

(2) The strongest effect of 5-bromodeoxyuridine was observed when it was administered on the second day of the first instar, showing the lowest viability among those treated on the first or third day.

(3) The translucent spots induced by 5-bromouracil as well as by 5-bromodeoxyuridine were similar to those induced by γ-irradiation at the same stage. But the mean size of the spots produced by the base analogues was about half as large as of those produced by γ-rays.

Although the mechanism of production of such spots is at present not clear, at least two possibilities may be considered. First a mutation could have occurred in some of the epidermal cells during development as a result of the replacement of the normal base in DNA with 5-bromouracil. Second, it may be that somatic crossing over was induced in some cells by the stimulating action of the base analogues.

77. *Theoretical estimation of "survival cell number versus dose" curve from experimental frequency distribution of the number of mutants*

(by Sohei KONDO)

Assuming that the initial mutative events in germ cells are distributed in a Poisson distribution, the probability distribution function $f(m)$ of m mutants to be recovered later in the offspring from each mating pair has been theoretically derived in terms of the number of the offspring per mating pair, the number n of germ cells at the time of occurrence of the initial mutative events and the frequency of the initial mutation

per germ cell (Kondo: 1961). It has been previously found that by adjusting n, the theoretical frequency distribution $f(m)$ can be fitted very well the observed $f(m)$ for the case of silkworm exposed to acute γ-rays at the gonial stage.

Conversely, from experimental $f(m)$ distribution curves for various values of radiation dose D we can construct the curve of the number of viable germ cells, n, versus dose. Such a curve is useful for evaluating the contribution of killing effects on germ cells to mutation mechanism, especially, the dose-rate dependence of radiation-induced mutation.

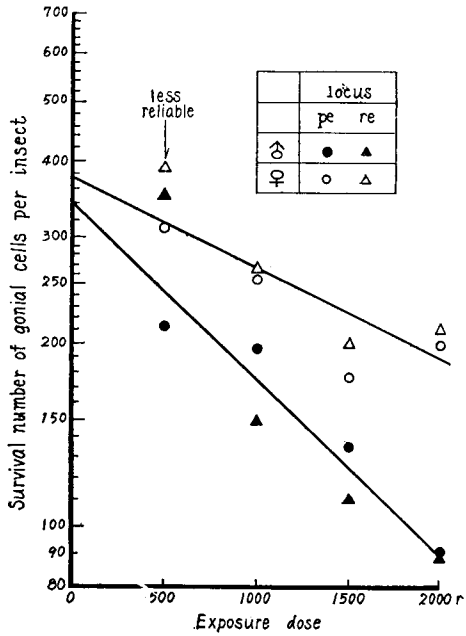

Fig. 1. Theoretical estimation of "survival cell number versus dose" curve from experimental frequency distribution of the number of mutants.

Fig. 1 shows two n-versus-dose curves constructed in the above-mentioned way from the experimental data[1] of silkworm exposed to acute γ rays at the early gonial stage (time of hatching). The LD_{50} is about 1000 and 2000 r for spermatogonia and oögonia, respectively.

1) Y. Tazima (unpublished).

78. *Deformities in chick embryos caused by γ-irradiation*

(by Takatada KAWAHARA)

After a few preliminary experiments, it has been concluded that induction of deformities by γ-irradiation in chicks was most effective when 48 hours old embryos were treated after incubation. It was also found that in that stage embryonic lethality was rather low in comparison with treatments started earlier. On the basis of these findings, chick embryos of 48 hours were irradiated by γ-rays, the does applied being 600r by ^{137}Cs. The materials used were chick embryos of White Leghorns (WL), Barred Plymouth Rocks (BPR), Nagoyas (NG) and hybrids between WL and the two others.

Deformities noticed were mainly toe defects, though a few individuals having slightly or strongly defective legs or wings were found. The frequency of deformities in purebred and crossbred chick embryos is presented in Table 1.

The results of statistical analysis of the data obtained are summarized as follows: The difference between the races in respect of the incidence of deformities was remarkable, BPR showing a very low frequency in comparison with WL or NG. The frequency in White Leghorns was 5.25% higher than that in Nagoyas though it was statistically not significant. A considerable difference was observed between the reciprocal hybrids. Hybrid chicks born from White Leghorn dams showed a much higher frequency of deformities than the reciprocals. There was no observable relation between sex and frequency of deformities.

Those results suggest that the frequency of deformities due to γ-irradiation in chick embryos is largely controlled by maternal factors.

Table 1. Deformities due to γ-irradiation of chick embryos

Breed or cross	Treated (600r)		Non-treated (control)	
	No. of tested embryos	Incidence of deformities (%)	No. of tested embryos	Percent of abnormal embryos
WL	799	32.79	421	0.48
BPR	296	5.07	78	0
NG	138	27.54	12	0
WL ♀ × BPR ♂	317	19.56	618	0
BPR ♀ × WL ♂	269	9.29	150	0.67
WL ♀ × NG ♂	261	38.38	158	0
NG ♀ × WL ♂	213	22.53	47	0
Total	2293		1484	

79. *Genetic effects of chronic low dose irradiation on mice*

(Susumu MURAMATSU, Tsutomu SUGAHARA and Yoshiko OKAZAWA)

The genetic effects of radiation on mice were studied by the treatment of gamma-irradiation from conception to reproduction for three successive generations. Progenies of mice of a multiple recessive stock, "wavy" stock, irradiated chronically with 34.4 *r* (the dose rate of 0.43*r*/22 hr.-day) of ^{60}Co-gamma-rays for three successive generations, as described in the previous paper (Sugahara *et al.* 1961), were tested for recessive lethal mutations on the basis of four different criteria (Haldane's method (1956), litter size, rate of early death and sex ratio) by mating them with the wild CBA strain and studying their F_1 and F_2 progenies.

Table 1. Number of animals used for the analysis

	Male line	Female line	Total
Irradiated series			
P_1	100	100	200
F_1	1,170	1,362	2,532
F_2	8,337	8,450	16,787
Non-irradiated series			
P_1	100	100	200
F_1	1,158	1,071	2,229
F_2	8,011	7,739	15,750

The numbers of animals used for analysis are given in Table 1 with regard to generations and lines. Male line or female line means F_1 and F_2 of recessive P_1 males or females, respectively.

Table 2. Mutation rates calculated from four different data on the same materials

Method	Lethal effective in the period	Mutation rate
Haldane's method	from conception to weaning	$4.8\times10^{-3}/r$/total autosomes
Litter size in F_2	from conception to birth	0.5×10^{-3}
Early death rate	from birth to weaning	2.1×10^{-3}
Sex ratio	from conception to birth	$2\times10^{-3}/r$/X-chromosomes

Results obtained are summarized in Table 2.

The calculated values seem to be practically in good accordance with

each other (0.5~4.8×10^{-3}/*r*/total autosomes) except for that from sex ratio (2×10^{-3}/*r*/X-chromosome). But, unfortunately, neither linearlity nor dose rate dependency can be tested from the present data. Thus, it can be said that the genetic effects of radiation on mice were demonstrated at a low dose with a low dose rate. The results may be very significant for the assessment of human radiation hazards. Detailed analysis is now in progress and the results will be published elsewhere.

F. RADIATION GENETICS IN PLANTS

Boron effects upon gamma-ray and thermal neutron irradiations of einkorn wheat; RBE of heavy particles from $^{10}B(n, \alpha)^7Li$ reaction[1]

80. I. *Effects on seedling growth, fertility and chromosome aberrations*

(By Seiji MATSUMURA and Tomoo MABUCHI)

As an approach to the investigation of boron effects, seeds of *Triticum monococcum flavescens* soaked in water and 0.1, 0.5 and 1.0% aqueous solutions of borax for 2 days were exposed to gamma-rays in our Institute and to thermal neutrons in the Japan Atomic Energy Research Institute's Nuclear Reactor, JRR-1. Gamma-irradiation with ^{137}Cs was applied at the dosages of 0.5, 1, 2 and 3 kr. The thermal neutron flux was calculated to be 5.2×$10^9 n_{th}/cm^2 \cdot sec$ when the reactor was operated at 500 watts. The thermal neutron integrated flux ranged from 1.3 to 10.4× $10^{11} n_{th}/cm^2$ for 25~200 seconds. Gamma contamination dose rate was 117 r/min. The measurements of thermal neutron integrated fluxes and gamma contamination dosages were made with glass plates of different $AgPO_3$ concentrations.

There was no marked difference in the germination rate among gamma-irradiations with various dosages and various borax concentrations. Only a slight decrease in the germination rate was found in thermal neutron treatment at higher dosages, especially with higher concentrations of borax. The higher were the dosage of gamma-rays and thermal neutrons and the concentration of borax in the thermal neutron treatments, the more reduced was the growth of seedlings and seed fertility and the more increased were mitotic disturbances in the root tips immediately after germination.

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

The frequency of chromosome aberrations in PMC's increased markedly with the increase of the dosage of gamma-rays and thermal neutrons, and especially with increasing borax concentration for the neutron treatments. Assuming that the chromosome aberration frequency *versus* gamma-ray dose curve is linear and independent of dose rate as well as of borax concentrations, the overall effective doses of thermal neutrons of 1.3×10^{11} and $5.2\times10^{11}n_{th}/cm^2$ can be converted to equivalent gamma-ray doses for the various borax concentrations as given in line 4 of Table 1. Subtraction of the equivalent gamma-ray dose for 0% borax concentration from that for 0.1 or 1.0% borax concentration at the same integrated neutron flux gives the biological response to thermal neutron in units of gamma-ray dose due to the added borax (see line 5 of Table 1).

Table 1. Estimated RBE values of the heavy particles from $^{10}B(n, \alpha)^7Li$ and the relevant data

1. Borax concentration (%)	0		0.1		1.0	
2. Content of borax aqueous solution (weight % relative to dry seed)	91		85		79	
3. Integrated thermal neutron flux ($10^{11}/cm^2$)	1.3	5.2	1.3	5.2	1.3	5.2
4. Biological dose of thermal neutron equivalent to gamma-ray dose (krad)	0.25	1.6	0.9	3	2	5
5. Biological response to thermal neutrons due to added borax in units of gamma-ray dose (krad)	—	—	0.65	1.4	1.75	3.4
6. Dose calculated from $^{10}B(n, \alpha)^7Li$ reaction (krad)	—	—	0.011	0.044	0.105	0.42
7. RBE of $(\alpha+^7Li)$ (5÷6)	—		60	32	17	8

The physical absorbed doses in rads due to heavy particles from $^{10}B(n, \alpha)^7Li$ reaction were calculated by Dr. KONDO, as given in line 6 of Table 1. The RBE (relative biological effectiveness) of $(\alpha+^7Li)$ relative to gamma-rays is shown in line 7 of Table 1. That the higher borax concentration results in a lower RBE value will mean that the boron atoms cannot penetrate the surface covering of the seeds as freely as water molecules. The lower RBE values for higher neutron integrated flux means that the linear assumption of chromosome aberration *versus* gamma-ray dose curve does not hold true.

81. II. *Effects on chlorophyll mutations*

(By Tomoo MABUCHI and Seiji MATSUMURA)

In order to study RBE for gene mutations of $(\alpha+{}^{7}Li)$ from the thermal neutron capture reaction ${}^{10}B(n, \alpha){}^{7}Li$, compared with gamma-rays, chlorophyll mutations in the X_2 seedlings were investigated. Their frequency increased roughly in a linear relation to the dose of gamma-rays and thermal neutrons and also markedly with increasing boron concentration only for thermal neutron treatments, as could have been expected. From these data, chlorophyll mutation rates per $10^{11} n_{th}/cm^2$ of thermal neutrons are plotted in Figure 1, for the various borax concentrations. The mutation rate per kr of gamma-rays is 1.3%. An absorbed dose due to ${}^{10}B(n, \alpha){}^{7}Li$ reaction is calculated as 82.6 rad per 10^{11} n_{th}/cm^2 in 1.0% borax solution by Dr. KONDO (see Part III of this series). The mutation rate per rad due to ${}^{10}B(n, \alpha){}^{7}Li$ reaction is about 4×10^{-4}. Therefore, we may conclude that the RBE value of the heavy particles from ${}^{10}B(n, \alpha){}^{7}Li$ relative to gamma-rays is about 30 for gene mutations.

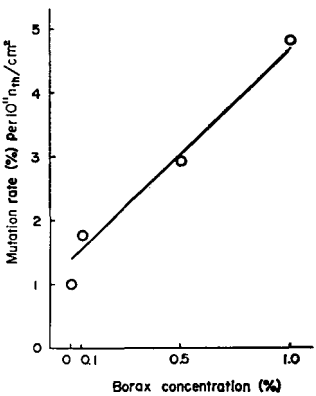

Fig. 1. Relationship between mutation rate and borax concentration.

82. III. *Calculation of absorbed dose due to* ${}^{10}B(n, \alpha){}^{7}Li$ *reaction*

(By Sohei KONDO)

The physical absorbed dose in rad units due to heavy particles from ${}^{10}B(n, \alpha){}^{7}Li$ reaction has been calculated by the first collision dose: $d_B = 1.602\times10^{-8}\sigma EN\Phi$(rad) where 1.602×10^{-3} is the conversion factor from MeV/gram to rad, $\sigma(=755\times10^{-24}\ cm^2)$ the thermal neutron capture cross section for boron, E(2.34 MeV) the sum of energies of α and ${}^{7}Li$ particles emitted by the capture reaction, N the number of boron atoms per gram of seeds and Φ the integrated flux of thermal neutrons. The values of N have been estimated by assuming that boron atoms can enter seeds as freely as water molecules. That is

$$N=\frac{4N_0 x Y}{100A_{borax}(1+Y)},$$

where N_0 is AVOGADRO's number, A_{borax} the molecular weight of borax,

factor 4 the number of boron atoms per borax molecule, $x(\%)$ the concentration of the borax solution used and Y the increase in weight of the soaked seeds relative to dry weight.

Table 1 shows the calculated absorbed dose per 10^{11} thermal neutrons per cm^2.

Table 1. Calculated absorbed dose d_B due to $^{10}B(n, \alpha)^7Li$ reaction and relevant experimental data

x (%)	Y	N (B atoms/g)	d_B (rad per $10^{11} n_{th}\,cm^{-2}$)
0.1	0.85	3.05×10^{18}	8.6
0.5	0.83	1.50×10^{19}	42.5
1.0	0.79	2.93×10^{19}	82.6

Let us plot a biological response of treated seeds at a given dose of thermal neutrons $\Phi(n_{th}/cm^2)$ mixed with γ rays against the values of borax concentration x together with the response of pure γ ray treatment against the dose. Then, the overall effective dose of the thermal neutron field at the integrated flux of Φ can be converted to equivalent gamma-ray dose for various borax concentrations. Subtraction of the equivalent gamma-ray dose for 0% borax concentration from that for 0.1, 0.5 or 1.0% borax concentration gives the biological response to the reaction of thermal neutrons with the added borax in rad units of gamma-ray dose. Division of this value by the absorded dose due to heavy particles from $^{10}B(n, \alpha)^7Li$ reaction calculable from Table 1 gives the RBE of the heavy particles relative to γ rays. This method does not require the knowledge of the γ ray and fast neutron contamination in the thermal neutron field nor the contribution of the thermal neutron reaction with other elements contained in the seeds.

83. *Radiological study of wheat monosomics*

(By Koichiro TSUNEWAKI)

The experiment reported here was designed to study the effects on radiosensitivity of wheat produced by elimination of a specific chromosome in each of 21 monosomic lines of *Triticum aestivum* var. Chinese Spring.

Dormant seeds were treated with γ-rays from Co^{60}. The γ-ray dosage per hour was 250r. The experimental design used was a split plot in a randomized block design with 3 replications and 10 γ-ray dosages, *i.e.*, 0, 10, 20, 25, 30, 35, 40, 45, 50 and 60 Kr, for units, and 22 genetic

strains, *i.e.*, the disomic and 21 monosomics for subunits. About 50 seeds from each treatment were planted per strain per replication.

At the moment data on survival rate and plant height at the 5th leaf stage of the R_1 generation have been analyzed. There is a differential radiosensitivity between the disomic and the monosomics, the former being more resistant than any of the latter. A differential radiosensitivity also exists among the monosomics. Comparing with the survival rate of the disomic, Mono-3B showed a distinctive drop in survival at a dosage of 30 Kr, Mono-5B at 35 Kr, Mono-2A, 2B, 2D, 4A, 6A and 6D at 40 Kr, and Mono-1A, 1B, 1D, 4D, 5D and 6B at 45 Kr. Mono-3A, 3D, 4B, 5A, 7A, 7B and 7D did not show any significant drop.

Three monosomic lines belonging to each of the homoeologous groups 2 and 7 showed a very similar pattern of radiological response. In the homoeologous groups 1, 3 and 6, Mono-1A and 1D, 3A and 3D, and 6A and 6D, respectively, showed a similar radiological response but the third member of each group differed distinctly from the others. Among all 3 monosomics of the homoeologous groups 4 and 5 the responses were distinctly different one from another.

These facts seem to indicate that functional differentiation of chromosomes has taken place to a different extent in the 7 homoeologous groups. It is also interesting to notice that monosomics of homoeologous chromosomes in A and D genomes showed, in general, a similar response to irradiation, while those of B genomes differed from the others.

84. *Changes in rust susceptibility due to partial irradiation of wheat seedlings and its dose dependence*

(By Keizo KATSUYA)

The stems and leaves of seedlings at leaf stage of *Triticum vulgare* (Norin No. 16) were irradiated by X-rays at 5 kr. The seedlings were inoculated 10 days after irradiation by spraying with an aqueous uredospore suspension of *Puccinia triticina*. The first leaves of the seedlings were removed 10 days after inoculation and the size of uredosori was measured. They were on plants whose whole bodies or stems were irradiated about 1.7 times larger than on the control and plants whose leaves only were irradiated.

Seedlings at 2 leaf stage of *T. vulgare* (Norin No. 50) were irradiated by gamma-rays, at 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 kr. The seedlings were inoculated 6 days after irradiation by spraying with the spore suspension. Seedling length was measured 7 days after irradiation, and then the

size of the sori was measured 12 days after inoculation. Inhibition of seedling growth increased with the increase of dosage and the seedling length at dosage above 1 kr remained unchanged. Also, the size of the sori increased with the increase of the dosage, however it did not further increase at dosages above 5 kr.

85. *A study on the relation between dose rates of gamma-rays and chromosomal aberrations*[1)]

(By Noriko SAKURAI* and Seiji MATSUMURA)

The frequency of root tip cells showing the first mitotic division and the time of its occurrence after germination in various temperatures were preliminarily investigated in non-irradiated seeds of two-rowed barley (*Hordeum sativum* JESS. var. *distichum* HOCK.). In relatively high temperature (12°~16°C), the first mitosis began 20~24 hours after sowing, and then the root elongated to 1.2~1.6 mm.

Further, a study on the relation between chromosomal aberrations in root tip cells and various dosages was carried out. Dormant seeds were subjected to gamma-rays (^{137}Cs) of 2, 5, 10, 20 and 50 kr. After irradiation the seeds were kept in a dark room at 16°C and their root tips were fixed 34, 42, 58 and 60 hours later. The observation of chromosomal aberrations was arbitrarily limited to cells in early telophase I just before the formation of nuclear membrane. The frequency of chromosomal aberrations involving fragments and chromatin bridges increased with the increase of dosage, but it decreased in the later stages. These results indicate that some of the cells which showed abnormal division recover their normal behavior in stages later than 1st division.

In addition a study was carried out for a detailed comparative study of dose rate and storage effects on growth and chromosomal aberrations. Two lots, each of 100 dormant seeds, whose water content was 12.3%, were subjected to gamma-rays of 5 and 10 kr. For acute and chronic exposure to gamma-radiation two dose rates, namely 312.5 r/min with ^{137}Cs and 20 r/hr with ^{60}Co were adopted respectively. The acute treatments were given in three ways; namely, at the beginning or at the end of the chronic irradiation and in two fractionations at both.

Irradiated seeds were sown in the greenhouse and the seedlings were

* Kihara Institute for Biological Research, Yokohama.

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

measured 14 days after sowing. There was no striking difference between 5 and 10 kr, in so far as the germination is concerned, but growth of seedlings was slightly delayed at 10 kr as compared with 5 kr. At the two above dosages, chronic irradiation and the acute one which was applied at the start of the former were more effective in inhibiting growth than the others. This may indicate that storage of dormant barley seeds after irradiation increases radiation damage.

For the observation of chromosomal aberrations, root tips were fixed 58 hours after sowing. At 10 kr the frequency of abnormal cells and breakages per cell at high dose rate was higher than at low dose rate, especially when acute irradiation was applied at the start of the chronic one. It is assumed that the frequency of "two-hit" aberrations was relatively high and dependent upon the intensity of radiation. Unexpectedly, chronic irradiation with 5 kr was more effective than acute irradiation given in fractions. It is supposed that storage effect were involved.

86. *Polyploids and aneuploids of Triticum dicoccum produced by N_2O-treatment*

(By Hitoshi KIHARA and Koichiro TSUNEWAKI)

As previously reported, the present authors treated an Emmer wheat, *T. dicoccum* var. Khapli ($2n=28$) with nitrous oxide and obtained polyploids and aneuploids from almost all florets treated under the optimum condition. In this report a result of progeny test of those plants will be described with a consideration on the prospect of aneuploid studies in Emmer wheat.

All tetra- and hypotetraploids ($2n=54\sim56$) produced offspring which were tetra-, hypertetra- or hypotetraploids ($2n=51\sim61$). In those groups, there was on the average a strict correlation between the chromosome number of the parents and that of the offspring.

Progenies of 2 monosomics consisted of 12 disomics and 1 trisomic but no monosomics were recovered. The trisomic seemed to be produced from an aberrant meiosis in the monosomic parent as is known in monosomics of common wheat. A plant, whose roots were a mosaic of mono- and trisomic cells, gave in its offspring 4 plants, one of which was monosomic. Since the seed-fertility of those monosomics including the mosaic plant was about 25%, the very low transmission rate of the monosomic condition in this Emmer variety was seemingly due to strong selection against chromosome-deficient gametes and/or zygotes. A

trisomic plant produced 17 descendants among which 2 trisomics were recovered. The transmission rate of the trisomic condition is also significantly lower than that known in common wheat.

These facts suggest that it will be very difficult to establish an aneuploid series in Emmer wheat and that both monosomic and trisomic analyses will mean hard work.

87. *Comparison of ^{32}P β ray and ^{137}Cs γ ray effects on seedling height of wheat*[1)]

(By Sohei KONDO and Hiromi ISHIWA)

The previous expriments turned out that ^{32}P β rays were less effective on wheat seeds than expected from the calculated dose by assuming that seeds are in an infinite ^{32}P homogeneous solution. This discrepancy may be attributable to the partial shielding of β rays by the proximity of seeds touching each other when they were soaked in the ^{32}P solution in batches of 20 seeds per a gauze pouch.

The present experiment has been improved by minimizing such a partial shielding. Seeds of *Triticum monococcum flavescens* have been soaked in 2 inch petri dishes (100 seeds/dish), with blotting papers elevated by thin glass fibers about 2 mm from the glass bottoms so that

Table 1. Comparison of radiation effects on seedling height between seeds soaked in ^{32}P solution of 0.154 mc/m*l* and those exposed to ^{137}Cs γ rays.

Time after treatment (days)	γ ray dose equivalent to ^{32}P treatment (kr)	Assumed contribution of ^{32}P inside embryos[1)] (kr-equiv.)	Calculated dose to soaked embryos due to external ^{32}P β rays (kr-equiv.)	Total embryo dose due to ^{32}P (kr-equiv.)
16	4.1	1.15	2.0	3.2
33	4.8	1.85	2.0	3.9

1) The increase (0.7 kr-equivalent per 17 days) in effectiveness of ^{32}P treatment shown in column 2 has been assumed to be due to disintegration (34%) of ^{32}P inside the soaked seeds during the 17 day period, and the effect of such disintegration during the first 16 days has been calculated by multiplying 0.7 kr by the ratio of disintegration during the first 16 days to that during the latter 17 days.

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

seeds can be assumed to have been approximately in homogeneous infinite solution. The seeds have been soaked for 48 hrs but exposure to ^{32}P solution or to Cs-137 γ rays has been limited within the interval from 12 to 36 hour after the initiation of the soaking.

Table 1 shows the comparison of effectiveness of ^{32}P β rays with ^{137}Cs γ rays on seedling height. The higher effectiveness of ^{32}P β rays than expected from calculation is partly attributable to the ^{32}P which penetrated into the embryos, as suggested by the observed increase in the relative effectiveness of ^{32}P β rays to γ rays with increase in time after the soaking period (see column 2, Table 1). Furthermore in our previous calculation of the effective β ray dose of embryo the curvature of seeds was not taken into account. Improvement of the dose calculation is now under way.

88. *Effect of surface dose change on small seeds exposed to Co-60 γ rays*

(By Sohei KONDO and Hiromi ISHIWA)

It is often the case that when small seeds are exposed to Co-60 γ rays, seeds placed in the surface layer show a difference in biological responses compared with those in the interior. This is mainly due to the well-known fact that the absorbed dose varies appreciably with depth in the surface region of the materials exposed to high energy photons.

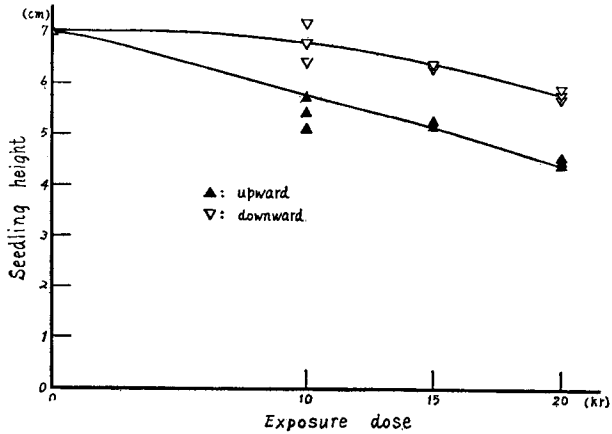

Fig. 1. Effect of surface dose change on small seeds exposed to Co-60 γ rays.

Since the genetic system of a seed is confined to the embryo the effective dose of an irradiated seed will be equivalent to that of the embryo. From this reasoning, we have irradiated rice seeds arranged in a monolayer, one group of seeds with the embryo facing upward toward the ^{60}Co source and the other downward.

The irradiation has been carried out by using our Co-60 γ ray facility whose depth dose curve is known; the surface dose is higher about 50% than the interior dose because of the high contamination with external secondary electrons. The experimental results are shown in Fig. 1. Radiation effects on seedling heights for rice seeds with embryos facing upward have been about 50% more effective than those for the seeds with embryos facing downward as expected from the physical measurement of the depth dose curve.

89. *Relation between chlorophyll mutations and dose rates of gamma-radiation in rice*

(By Seiji MATSUMURA and Tomoo MABUCHI)

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, for the first 3 doses. These chronic, moderate and acute irradiations were terminated almost simultaneously before sowing. The germination rate, seedling height, seed fertility and chlorophyll mutations were investigated. Chronic irradiation, which was applied from the start, was the most effective in inhibiting the growth of seedlings and decreasing the fertility, especially at 20 kr, indicating an intensification of radiation damage due to storage (cf. Ann. Rep. No. 11 : 100).

The spectrum and frequency of chlorophyll mutations in the X_2 generation are shown in Table 1. In the untreated lot were found 36 panicle progenies which segregated *albina*. They originated from the 4 panicles of each of 9 X_1-plants, which must have been heterozygous for *albina*. This was due to have obtained a wrong sample for the control seeds. Therefore, similar panicle progenies from treated lots, which segregated *albina*, shown in brackets in Table 1, and also those segregating *striata*, were excluded from the calculation of mutation rates. As expected, the frequency of chlorophyll mutations increased roughly in a linear relation to dose, except at chronic 10 kr. There was no marked difference between chronic and moderate irradiation, while the acute one

Table 1. Spectrum and frequency of chlorophyll mutations in the X_2-generation

Dosage (kr)	Dose rate (r/hr)	No. of X_1-plants	No. of X_2 panicle progenies	*Albina**	*Viridis*	*Xantha*	*Tigrina*	*Albo-viridis*	*Striata*	Chlorophyll mutation rate (%)
0	—	307	1,085	36 (36)	0	0	0	0	0	0.00
5	10	288	995	26 (19)	4	3	0	3	9	1.71
5	300	279	950	46 (34)	3	0	0	0	9	1.58
5	10,000	287	999	28 (15)	8	1	4	1	9	2.70
10	10	297	1,039	63 (43)	16	0	0	5	8	3.95
10	300	236	871	16 (11)	14	0	0	0	10	2.18
10	10,000	292	1,034	27 (6)	7	4	2	3	9	3.58
20	10	219	686	25 (18)	17	2	4	1	4	4.81
20	300	321	1,016	52 (32)	6	7	1	12	5	4.53
20	10,000	268	819	44 (12)	25	3	2	0	9	7.57
30	300	300	988	64 (28)	20	5	2	2	7	6.58
30	10,000	230	644	43 (10)	20	1	1	1	12	8.70

* See text.

showed clearly a higher mutation rate than the other two indicating a dose rate dependency in the frequency of chlorophyll mutations.

90. *Occurrence of variants in developmental stability in seed size in the X-rayed progeny of rice*

(By Kan-Ichi SAKAI and Akio SUZUKI)

Two groups of plants derived from the same variety of Japanese rice, but different from each other in that one group was X-irradiated (20 kr) in 1959 while the other remained untreated, were propagated year after year by one plant-one offspring method in order to minimize the effect of natural selection.

Each group included approximately 1500 plants each year. In the X_3 generation, 400 plants selected at random among the X-irradiated progeny were compared with the same number of plants taken from the control plot. It was then found that the former X-treatment has significantly increased intra-plant variability in seed size.

The intra-plant variabilities for seed length and for seed breadth caused by X-rays were positively correlated, $r=+0.4345$, suggesting that

a common genic system is, to an extent, responsible for both variabilities. Intra-plant variability was not correlated with the absolute values of length or breadth of seed.

91. *Mutations in Pharbitis Nil induced by gamma rays*

(By Hitoshi KIHARA and F. A. LILIENFELD)

Further observations on the progeny of γ-irradiated wild and cultivated Morning Glory are reported.

Five new mutants, all of them recessive, could be added to those previously reported (Annual Report, No. 9, 1958). All hitherto observed are given in Table 1.

The great majority of deviating forms are found in the cultivated strain P7. They all represent known types of cultivated Morning Glory which have arisen by spontaneous mutation in the course of many years of cultivation.

It is noteworthy that no albinotics were found which occur very often in the offspring of irradiated cereals.

Table 1. Mutants found in 3 strains of *Pharbitis Nil*

Material	Mutations	Remarks
Wild strain from Nepal with standard leaves and blue flowers	1) maple-like leaves 2) chlorina	viability good weak, highly sterile
Wild strain Tendan (North China) with standard leaves and blue flowers	3) deep purple flowers* (lost in a typhoon)	viability good
Japanese cultivated strain (P7) with standard leaves and blue flowers	4) delicate leaf (Sasa)* 5) maple leaves (Tatsuta)* 6) dragonfly leaves (Tombo) 7) white flowers, black seeds 8) white flowers, white seeds	dwarf, sterile viability good

* mutants reported in 1958.

92. *Irradiation experiments with cultivated chrysanthemum*[1)]

(By Taro FUJII and Tomoo MABUCHI)

Rooted cuttings of two varieties of chrysanthemum were irradiated

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

with 2, 4 and 8 kr of gamma-rays. Per cent of survivals was slightly decreased at 2 kr irradiation and at 4 and 8 kr it was very low in both varieties. About 25 per cent of planted cuttings died at 8 kr and the plant height of survivals was depressed to about one half of that of the non-irradiated control. 8 kr irradiation seems to be too high for mutation experiments with chrysanthemum cuttings. All flowers from irradiated cuttings of the variety Kinrei showed the original yellow color like those of non-irradiated ones. Bud mutation occurred in the variety Hakuei; flower color changed, sometimes sectorially, from white to yellow. In some cases, all heads on the same branch were yellow, while in other cases a part of a head was yellow and the remaining part was white. In the calculation of mutation rate, these branches with mutated flower heads were scored as one mutation and the linear relationship between mutation rate and dosage was observed. At 2, 4 and 8 kr the frequency of branches with somatic mutation was 0.24, 0.61 and 4.95 per cent of all branches, respectively.

Cuttings and rooted shoots from the mutated plants of Hakuei were planted. The plants from the mutated branch showed the same yellow color and those from non-mutated branches within the same individual had white heads. This case therefore represents a typical bud mutation.

G. HUMAN GENETICS

93. *Prezygotic selection in ABO blood groups*

(By Ei MATSUNAGA and Yuichiro HIRAIZUMI)

There are three possible selective mechanisms at prezygotic stages: 1. meiotic drive or unequal production of gametes carrying different alleles in heteorozygous parents: 2. sperm competition which may occur independently of the female genotype: 3. sperm competition which may occur as a result of serological incompatibility between the A or B antigen of the sperm and the anti-A or anti-B antibody in the secretion of female genital organs.

Although by statistical methods a distinction cannot be made at present between the first two possibilities, it is possible to test whether or not prezygotic selection is operating to an appreciable extent. In order to compare different family sizes of various matings in terms of the number of children of a specific genotype and thus to test the possibility of prezygotic selection in males, a concept of "family size equivalent" for children of a given genotype has been introduced. The family size

equivalent for O children of O×O matings is of course the mean number of observed children for such matings. The corresponding value for ♀O × ♂A matings may be given by the following formula:

$$O_{eq}(♀O \times ♂A) = m \times \frac{1}{n} \times \frac{1}{P_0}$$

where n is the observed number of ♀O×♂A matings, m is the observed number of O children from such matings, and P_0 is the probability that an O child will be produced in such matings. This value represents the mean number of children produced if sperm carrying A gene in AO fathers were all eliminated and only sperm carrying O gene participated in the fertilization. If there were no prezygotic selection, then no difference would be seen between O_{eq}(♀O×♂A) and O_{eq}(♀O×♂O). But if there were such selection, say, for O-sperm and against A-sperm, then the value for O_{eq}(♀O×♂A) would be larger than O_{eq}(♀O×♂O). It is important to realize that zygotic selection would not affect the comparison between the two equivalent values, since they were calculated respectively for O children in O mothers. Prezygotic selection in the BO father can be tested by comparing O_{eq}(♀O×♂O) with O_{eq}(♀O ×♂B). Many other comparisons between two mating types in which the genotypes of mothers and children are constant but only fathers' genotypes differ may be used to check the above hypothesis.

Using Japanese family data, it has been demonstrated that the sperm carrying O gene are at an advantage over those carrying A and B genes. The ratio of O sperm in heterozygous male has been estimated at about 0.55. Moreover, it seems that this selection is due either to meiotic drive or to sperm competition without interaction with the maternal genotypes.

A preliminary report of this work together with a table will be published in Science 135: 432–433, 1962.

94. *Selection in ABO blood groups: Viability and its relation to the proportion of prenatal deaths*[1]

(By Yuichiro HIRAIZUMI and Ei MATSUNAGA)

Based on a total of approximately 2,200 Japanese family data, Matsunaga and Hiraizumi (in press) found an evidence of pre-zygotic selection in ABO blood groups; namely, about 55% ($=k$), instead of the expected 50%, O-bearing sperm from AO and BO fathers are transmitted to their children.

1. This work was supported by Grant RF 61113 from The Rockefeller Foundation.

We now study the post-zygotic selection (viability) operating on this system.

1. Estimation of viability.

The relative viability of each genotype can be estimated by comparing the family size equivalent value for the children of the genotype in question with that of a standard genotype (=OO)[2] in the same mating. The equivalent values must be corrected in an appropriate way (substitution of k value) when the mating involves either AO or BO fathers. For convenience we let the relative viability of the standard O children be unity. The viabilities thus estimated are listed in Table 1.

Table 1. Viabilities of genotypes estimated on the basis of $k \doteqdot 0.55$

	Viability					
	OO	AA	BB	AB	BO	AO
Compatible	1.0000	1.0674	1.4466	1.3845	1.0475	1.0672
Incompatible	—	—	—	0.8598	0.9413	0.9792 (in O♀) 1.4319 (in B♀)*

* A surprisingly high viability of incompatible AO children in B mothers is observed in both matings B♀×A♂ and B♀×AB♂. The incompatible AO children also show very high sex-ratio (c.f. paragraph 3 in this report). Perhaps this high viability is real but the reason for it is not yet known.

It is interesting to examine whether there is any evidence of heterosis in viability. The figures in Table 1 were rearranged for this purpose and are presented in Table 2.

In Table 2 we see no sign of heterosis. However, if we do not take account of pre-zygotic selection, we shall be led to quite an opposite conclusion. Table 3 represents the same comparisons as Table 2, but this time the viabilities are computed assuming that the proportion of O-bearing sperm from the AO and BO fathers is 50%.

In Table 3 heterosis is clearly seen in all the three pairs. This shows

2. Even a mating which does not produce standard O children can be used for estimating viability. For example, no O children will be produced from the AB♀×AO♂ mating, but we may compute V^e_{AB} by comparing the equivalent value of AB children with that of BO children whose viability is estimated from other independent matings (AB♀×O♂, B♀×O♂). However, the reliability of V^e_{AB} thus estimated will be much lower than that V^e_{BO}. The reliability of V^e_{AA} (and V^e_{BB}) may be also very low, because for this estimation we must substitute the estimate of V^e_{AO} (V^e_{BO} ets.).

Table 2. Comparison in viability of homozygote with heterozygote. V_{ij} represents the viability of the compatiable genotype ij. $k \fallingdotseq 0.55$

Pair No.	Viability		
1	$V_{OO}=1.0000$	$V_{AO}=1.0672$	$V_{AA}=1.0674$
2	$V_{OO}=1.0000$	$V_{BO}=1.0475$	$V_{BB}=1.4466$
3	$V_{AA}=1.0674$	$V_{AB}=1.3845$	$V_{BB}=1.4466$

how important it is to examine first pre-zygotic selection when we discuss the phenomenon of heterosis.

Table 3. Comparison in viability of homozygote with heterozygote. $k=0.50$

Pair No.	Viability		
1	$V_{OO}=1.0000$	$V_{AO}=1.0672$	$V_{AA}=0.7420$
2	$V_{OO}=1.0000$	$V_{BO}=1.0475$	$V_{BB}=1.0269$
3	$V_{AA}=0.7420$	$V_{AB}=1.0334$	$V_{BB}=1.0269$

2. Relation between viability and proportion of prenatal deaths.

Substituting the estimates of viabilities and the value of k, the average viability of children can be computed for each mating. This makes it possible to study the relation between the average viabilities and the proportions of prenatal deaths ($=r$). Two reports by Matsunaga and Itoh (1958) and Haga (1959) are available for estimating r in each mating. From their data the average of r's, $\bar{r}$, in the compatible matings is computed to be 0.077 while it is 0.116 in the incompatible matings. On the other hand the expected value of $\bar{r}$ (this can be computed by substituting the estimates of viabilities and of k) in the incompatible matings is computed to be 0.158. Thus the two values, observed ($=0.116$) and expected ($=0.158$), agree reasonably well suggesting that our viability estimates are fairly reliable.

If we compute the expected value of $\bar{r}$ in the incompatible matings assuming $k=0.50$, we get $\bar{r}=0.209$. Thus the value of $\bar{r}$ on the basis of $k=0.55$ agrees with the observed value better than the one which is computed by assuming $k=0.50$.

3. Relation between viability and sex-ratio.

It is generally accepted that the viability of males is, on the average, lower than that of females (r is larger in male than in female) and therefore males will suffer more than females from reduced viabilities. Admitting this is true, we may expect a positive correlation between

sex-ratios (proportion of males) and viabilities of genotypes. Hence we can make one more check of our viability estimates for reliability.

For simplicity we classify the genotypes of children into two groups according to their viabilities; group 1 includes genotypes with the relative viabilities larger than, or equal to, unity, and group 2 includes those with viabilities less than unity. In group 1 the average sex-ratio is 0.5324 while it is 0.5196 in group 2. Thus there is an indication that the difference in viability is reflected reasonably in the sex-ratio, although the difference in sex-ratio between the two groups is statistically insignificant[3]. Further accumulations of data are required to confirm this point.

95. *Selection in P blood groups in man: A probable case of pre-zygotic selection operating in both sexes*[1]

(By Yuichiro HIRAIZUMI)

A number of instances of pre-zygotic selection have been reported for various organisms, but in cases so far investigated it operates only in either one of the two sexes. Recently Matsunaga and Hiraizumi found for ABO blood groups that more than 50% (approximately 55%) O-bearing sperm from heterozygous AO and BO fathers were transmitted to their children, while the segregation ratio from heterozygous mothers was normal.

The purpose of the present investigation was to find out whether pre-zygotic selection is operating on the P blood groups in man. P locus consists of two alleles, P and its recessive allele p. The Japanese family data used in this study are those published during the period from 1937 to 1959. They consist of 7 publications, dealing with 401 families with 997 children in total. The frequency of p allele ($=0.8142$) is estimated from these data (frequency of $pp \fallingdotseq 0.663$). In addition, there are several published data available but they are omitted from this analysis because they seem to be biased as to gene frequency distributions, perhaps due to biased sampling procedures. Since a complete analysis will be presented in detail elsewhere, only the perliminary results are reported here.

3. The sex of children was not indicated in many of the family data used in this study so that the number of families available for analizing sex-ratio was relatively small. This may be the reason why the clear difference between the two groups is not statistically significant.

1) This work was supported by Grant RF 61113 from the Rockefeller Foundation.

1. Pre-zygotic selection in the father.

A method of comparison in terms of the "family size equivalent for children of a given genotype" was a powerful tool in studying the pre-zygotic selection in ABO system, so this method was again used in this study. For family size equivalent, see Matsunaga and Hiraizumi, 1962, Science.

The pairs of equivalent values are listed in Table 1.

Table 1. Comparisons of family size equivalents for p-children

Pair No.	Family size equivalents for p-children		Difference
1.	(p♀×p♂): 2.119	(p♀×P♂): 2.405	+0.286
2.	(P♀×p♂): 2.240	(P♀×P♂): 3.964	+1.724
Total	: 2.144	: 2.689	+0.544±0.36

The value of k_1 (segregation ratio of p from heterozygous Pp fathers) is estimated to be 0.6269 (0.10>Pr.>0.05). Thus, instead of the expected 50%, about 60% p-bearing sperms from Pp fathers are transmitted to their children, although the difference between the estimated k_1 and the expected 0.50 is not statistically significant.

2. Pre-zygotic selection in the mother.

Principally the same method may be applied to examine the segregation ratio from heterozygous mothers. In this case, however, we must first check the female fertility difference between the two genotypes, $P-$ and pp. The average number of children from $P-$ and pp mothers is 2.2027 and 2.1211, respectively. This suggests that there is no, or little if any, difference in female fertility between $P-$ and pp mothers. This view is also supported by Matsunaga and Itoh's observation (1958). We therefore proceed assuming that there is no female fertility difference. Table 2 represents similar comparisons as those in Table 1, but this time the genotype of the children and of the fathers is the same in each pair.

Table 2 suggests that pre-zygotic selection is also operating in the heterozygous mothers, and that it does so in the same manner as in the fathers (k_2, the segregation ratio of p from Pp mothers, is 0.5870), although again the value of k_2 does not differ significantly from the expected value of 0.50.

However, if we combine k_1 and k_2 together, then the deviation from 0.50 becomes significant. From those observations it is suggested that the pre-zygotic selection favouring p allele is operating on the two sexes in the same way.

Table 2. Comparisons of family size equivalents for p-children

Pair No.	Family size equivalents for p-children		Difference
1.	(p♀×p♂): 2.119	(P♀×p♂): 2.240	+0.121
2.	(p♀×P♂): 2.405	(P♀×P♂): 3.964	+1.559
Total	: 2.179	: 2.558	+0.379±0.42

3. Viabilities of three genotypes

Viabilities of the three genotypes, V_{pp}, V^c_{Pp} (compatible), V^i_{Pp} (incompatible) and V_{PP}, can be easily estimated from the present data, *i.e.*, $V_{pp}=0.6940$, $V^c_{Pp}=0.8204$, $V^i_{Pp}=0.8223$, and $V_{Pp}=1.0000$. Matsunaga and Itoh (1958) reported that there was no demonstrable difference in the mean number of children, proportion of natural abortions, etc., between P-compatible and P-incompatible matings. In the present study, again there is no sign of difference between V^c_{Pp} and V^i_{Pp}. We therefore use the average of the two estimates, 0.8214, as a measure of viability of *Pp* heterozygotes.

It is worth noting that the estimates of viabilities of three genotypes are in favour of the so-called classical hypothesis founded on the superiority of homozygotes in fitness.

4. Conditions for the co-existence of two alleles

The fitness of an organism is the resultant of many components, and therefore the informations on viability alone are not always sufficient to understand the total fitness. But since viability is one of the most important components of fitness, we assume, for the time being, that viability is a good measure of the total fitness.

Let the viability of *aa*, *Aa* (compatible and incompatible) and *AA* be V_{aa}, V^c_{Aa}, V^i_{Aa} and 1 respectively, and let k_1 and k_2 be the segregation ratio for *a* from *Aa* heterozygous males and females, respectively. After some calculations we get the following sufficient conditions for the co-existence[2] of the two alleles, although it is extremely difficult to obtain the necessary conditions.

$$(k_1+k_2)V^c_{Aa}-1>0 \text{ and } (1-k_1)V^i_{Aa}+(1-k_2)V^c_{Aa}-V_{aa}>0.$$

The viabilities of the three genotypes and the value of k estimated in paragraph 3 may be substituted into these conditions: clearly the

2) Conditions for co-existence are not always the same as those for the stable equilibrium; two alleles can co-exist without maintaining stable equilibrium if there are regular periodical changes in frequencies. For this see, for example, Kimura 1958, Wright 1955, etc.

estimates do not satisfy the two conditions, or the p allele would replace P when the frequency of p is sufficiently close to 1. This, of course, does not always imply that P and p cannot co-exist, because the above two are sufficient conditions, but may not always be the necessary ones.

It is, perhaps, worth noting that by trial and error procedure, we can find the following set of coefficients which meets the above two conditions: $V_{PP}=1.0000$, $V_{Pp}=0.8387$, $V_{pp}=0.6498$ and $k=0.6100$. With these coefficients P and p can maintain an equilibrium state with the zygotic frequency of 0.63 for pp and 0.37 for P–. Note that these coefficients and frequencies are very close to the estimated and actually observed ones. However, further studies must be made on this point because the equilibrium frequencies, and their stabilities, are very sensitive to even a slight change in any one of the coefficients.

Finally, it is very interesting that there are some similarities in selection mechanisms between ABO and P blood groups, *i. e.*, in both systems the recessive alleles (O in ABO and p in P systems), which are less viable when homozygous, are favoured by pre-zygotic selection.

96. *Genetic study on sporadic retinoblastoma in Japan**

(By Ei MATSUNAGA)

In our previous endeavor (1959) to establish as complete a roster as possible of retinoblastoma cases occurring in Hokkaido in the period from 1945 to 1957, 69 cases were located, of which 68 were sporadic while there was only one familial case. The incidence of the disease was calculated to be approximately 1 for each 23,829 live births or 4.2×10^{-5} for one live birth. This figure is rather close to that found for European populations. However, the question remained open what fraction of sporadic cases was actually due to fresh mutations.

In order to answer the above question, a follow-up study of patients with retinoblastoma treated at three University Hospitals in the period from 1900 to 1935 has been carried out. In parallel with this investigation, questionaires were sent to 76 institutions for blind persons asking about the outcome of marriages of persons whose eye balls had been enucleated because of retinoblastoma in childhood.

Up to present, altogether 43 cases of sporadic retinoblastoma that had survived the disease could have been followed up, of which 37 were of unilateral and 6 were of bilateral retinoblastoma. Among the 37 unilateral cases, 28 have married, of which 22 have got one or more children. Out

* This study was supported by a Grant-in-Aid from the Ministry of Education.

of these 22, however, there was only one single case, a man who transmitted the disease to one of his two children. Assuming that the penetrance of retinoblastoma gene is approximately 0.75, as was calculated in the previous study, and also considering the distribution of the numbers of children born to the affected parents as well as the mean age at the onset of the disease, the fraction of sporadic unilateral retinoblastoma actually due to germinal mutation was estimated to be only about 8%, the remaining 92% being regarded as phenocopies.

As for cases of bilateral sporadic retinoblastoma, it was found that there was only one case married out of the 6 whose ages were over 20 years. Surprisingly enough, however, this man transmitted the disease to his single child, a son who was affected with bilateral retinoblastoma like his father.

Although the number of available cases is still very scarce, the discrepancy between unilateral and bilateral retinoblastoma with regard to the probability of transmission of the disease to children seems to be very striking. Thus, it appears that the theory advanced by Vogel (1957) that about 17-18% of unilateral sporadic cases and 50-100% of bilateral cases are due to fresh mutation, may also be applied to Japanese material. The rate of mutation of the gene for retinoblastoma in Japan is calculated to be about 8×10^{-6}, which is very close to the figure of $6-7\times10^{-6}$ obtained by Vogel for Caucasians.

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97. *Dimorphism in human normal cerumen*

(By Ei MATSUNAGA)

Among a number of polymorphic traits which have been discovered in man, the dimorphism in human normal cerumen or ear-wax has been studied almost exclusively in Japan, while European and American workers have hitherto paid very little attention to this phenomenon. This may perhaps be due to the fact that the variation in cerumen is not so marked among Caucasians and Negroes as in Mongolian race.

In a paper by the auther which will be published in the Annals of

Human Genetics, Vol. 26: 273-286, 1962, various aspects of this dimorphism have been reviewed on the basis of the author's own observations in addition to the results collected by other investigators. It was shown that the ear-wax types are apparently determined by a simple genetic mechanism, and can be used as a polymorphic system for both genetics and anthropology. Frequencies of the alleles for the ear-wax types have been estimated for different ethnic groups. It was pointed out that the marked difference in the distribution of ear-wax types among the major races is probably a consequence of natural selection having acted in a long course of evolution, and there may be some differences in the selective value between individuals with wet cerumen and those with dry. In view of the apparent association with axillary odor, it was suggested that the alleles for ear-wax types may control certain metabolic processes, through which individuals with different types of cerumen may display different reactions to environmental changes. The need for future studies along this line is stressed.

98. *The frequency distribution of the so-called "drumsticks" in the polymorphonuclear neutrophil leucocytes in Japanese female blood films*[1)]

(By Yasuko TONOMURA (TOYOFUKU) and Akira TONOMURA)

The neutrophil method proposed in 1954 by Davidson and Smith is a cytological test for the detection of the genetic sex in man. The basis of this test is that 2 to 3 per cent of polymorphonuclear neutrophil leucocytes of females have a solitary chromatin mass— generally referred to as "drumstick"·— attached to a segment of the nucleus by a thin strand of chromatin, while this characteristic nuclear appendage does not occur in neutrophil leucocytes of males. The fact of nuclear sexual dimorphism in neutrophils has firmly been confirmed by many independent studies. However, some investigators have questioned the accuracy of this method in detecting the genetic sex in certain patients (Briggs and Kupperman 1956, Ashley 1958).

In order to scrutinize the validity of this neutrophil method, the authors examined the frequency distribution of the neutrophils showing the typical drumstick in Japanese females, aged 1 to 79 years. For the present investigation five hundred neutrophil leucocytes were examined in each of 250 female blood films. The first cell having one drumstick was found on the average in 42.74 neutrophils. Six drumsticks were observed on the average in 255.37 ± 8.17 neutrophils. The correctness of

1. This work was supported by Grant RF 61113 from the Rockefeller Foundation.

these average figures was readily corroborated by the results of Davidson and Smith (1954), Tenczar and Streitmatter (1956) and others. The number of drumsticks found in 500 neutrophils was, on the average, 11.84 ± 0.38 in 250 females, aged 1 to 79 years. However, the negative correlation between age and the frequency of drumsticks indicate indicates that the incidence of drumsticks decreases significantly with age ($r = -0.258$, $p < 0.01$), although the presence of drumsticks was demonstrated for all ages here examined.

On the other hand, five hundred neutrophils were examined in each of 50 male blood films, and no characteristic drumstick could be observed. At the present, the origin of the drumsticks still remains an unsolved problem, but it is suggested that the determination of the genetic sex in neutrophil leucocytes is highly accurate and much easier than skin biopsy or oral smear methods.

99. *Chromosome conditions in twenty patients*

(By Akira TONOMURA)

Case no.	Conditions	Sex	Age	Chrom. no.
1	Turner's syndrome	F	27	45
2	Sister of a mongol boy	F	23	46
3	Mother of two children, one with cleft palate and the other with cerebral palsy	F	36	46
4	Husband of case 3	M	37	46
5	Down's syndrome	M	10 months	47
6	Bourneville-Pringle's disease	M	11	46
7	〃	F	10	46
8	Microcephaly	F	27	46
9	〃 (sister of case 8)	F	20	46
10	Adrenogenital syndrome	F	6	46
11	Klinefelter's syndrome	M	19	47
12	Congenital anhidrosis	F	4 months	46
13	Testicular feminization	F	20	46
14	Boy with cleft palate	M	—	46
15	Girl with cleft palate, hair lip and heart disease	F	—	46
16	Boy with hypoplastic penis	M	8	46
17	Ullrich syndrome	M	5	46
18	Down's syndrome	M	7	47
19	〃	M	—	47
20	〃	M	1	47

The chromosome complement of twenty selected patients was examined using short term culture of either bone marrow or peripheral blood. In all cases a minimum of three cells were analysed in detail. The chromosome conditions of each case are summarized in the above table, together with brief clinical conditions.

H. TECHNICAL NOTE

100. *Measurements of γ ray contamination in the reactor of JRR-1 and response of silver-activated phosphate glass containing lithium and boron*

(By Sohei KONDO and Hiromi ISHIWA)

Two kinds of silver-activated glass dosimeters 6 mm long and 1 mm in diameter are commercially available from Toshiba Electric Company (see Table 1).

Table 1. Compositions of glass dosimeters used

Name of glass (used only for this paper)	Base composition (weight %)	Added component (weight %)
K glass	KPO_3(25), $Ba(PO_3)_2$(25) $Al(PO_3)_3$(50)	$AgPO_3$(8)
Li glass	LiPO(50), $Al(PO_3)_3$(50)	$Ag(PO_3)$(8), B_2O_3(3)

Let us expose these two kinds of glass rods encased in aluminum container 1mm thick to Φ thermal neutrons per cm^2 mixed with γ rays of Γ r and denote the readings of K and L glass by K and L r-equivalent, respectively (1 r-equivalent means the output of fluorescence of glass equal to that induced by ^{60}Co γ rays of 1 r). Then we have

$$k\Phi+\Gamma=K, \tag{1}$$

$$l\Phi+\Gamma=L, \tag{2}$$

where k and l are, respectively, readings (in units of r-equivalent) of K and L glass rods per one thermal neutron per cm^2. Using the value $k=1/(8.55\times10^9)$ (r-equiv./n.cm^{-2}) previously determined, we have the following

$$\Gamma=K-\frac{\Phi}{8.55\times10^9}, \tag{3}$$

$$l=\frac{1}{\Phi}\left[L-\Gamma\right]. \tag{4}$$

Experimental measurements of Φ/t and Γ/t are given in Table 2, where t is the exposure time. The values of Φ/t have been determined by gold foils (Ichikawa and Amano, 1961).

Table 2. Thermal neutron flux Φ/t and γ contamination dose-rate Γ/t in thermal column of JRR-1

Position and power level	No. 16 Hole; 0.5 kw	No. 7 Hole; 40 kw	
Φ/t(n/cm^2. sec)	$5.6(5.2^*)\times10^7$	$1.33\times10^{7**}$	$1.7(2.0^*)\times10^8$
Γ/t(r/sec)	$1.4(1.5^*)$	0.02**	$0.03(0.18^*)$
Γ/Φ(r/n. cm^{-2})	$2.5(2.8^*)\times10^{-10}$	$1.2\times10^{-9**}$	$1.8(6.2^*)\times10^{-10}$
excess Φ(n/cm^2)	6.6×10^{10}		
excess Γ(r)	11		
$1/l$(n. cm^{-2}/r)	$3.6(2.5^*)\times10^8$	2.3×10^8	

* Values obtained by glass plate dosimeters of different Ag concentration in 1960.
** The dosimeters presumably did not reach the ordinary position (11.8 cm from graphite surface of the core of JRR-1).

Table 2 shows that the γ contamination is in the same order both in No. 16 Hole, which passes through the center of the reactor, and No. 7 Hole which has been designed as the biological research hole.

101. *Dose-rate distribution in the Cs-137 gamma ray room*

(By Sohei KONDO and Hiromi ISHIWA)

The facility for the 2 kc ^{137}Cs γ ray irradiation was built in March, 1960. The dosimetry of the γ field was carried out by Victoreen condenser chambers and the Fricke dosimeter ($FeSO_4(NH_4)_2SO_4.6H_2O$ 2 g, NaCl 0.3 g, H_2SO_4(95-98%) 110 cc, total 5000 cc). The measured values of the effective γ-ray intensity of the source were 708.7 rhm, 747.5 rhm and 754.8 rhm for the Fricke, Victoreen 100 r (γ) and Victoreen 250 r (X) dosimeters, respectively. The curve of the dose-rate-versus-distance from the source along the vertical line passing through the center of source is given in Fig. 1 by using the Fricke dosimeter readings.

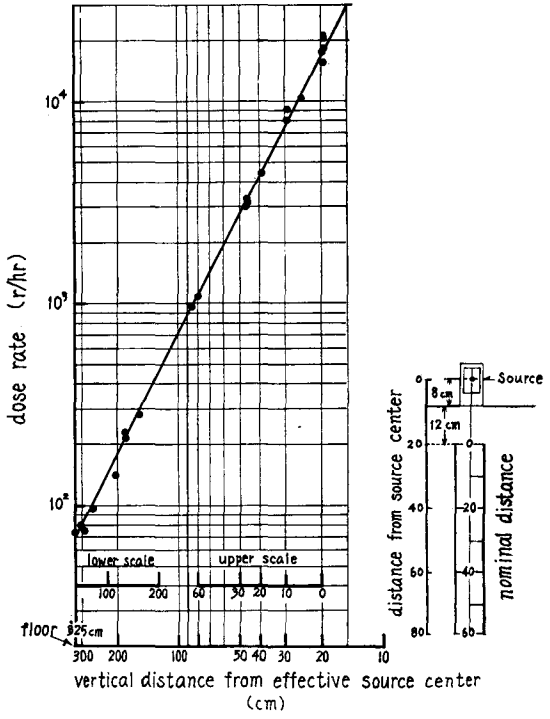

Fig. 1. Dose-rate distribution in the ^{137}Cs γ field with 2 kc (Oct. 1961).

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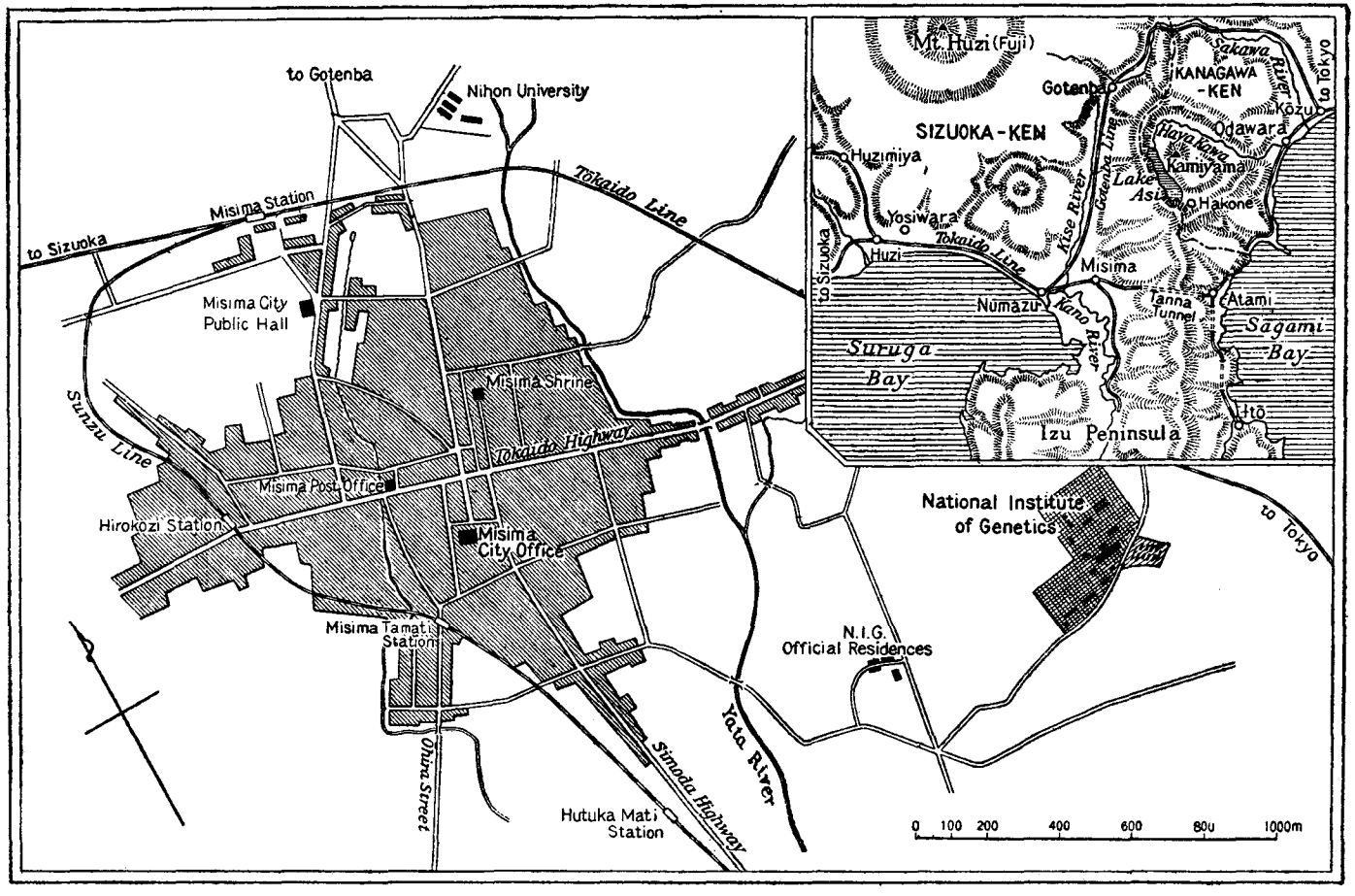
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