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GENERAL STATEMENT

In 1956 we have been very busy owing to the International Genetics Symposia in Tokyo and Kyoto. This year we could return to our normal laboratory life. Thanks to the kind support by The Rockefeller Foundation, we have started two new projects, one on the origin of cultivated rice and the other on the effects of radiation on animals. One of the main objects of rice researches is to collect wild species and cultivated varieties of *Oryza* all the world over, so that we can reexamine the classification of the whole genus which may sheet light on the origin of rice.

The first collecting party headed by Dr. OKA was sent to Thailand, India and Ceylon, where they have succeeded in collecting many seeds and specimens of wild as well as cultivated rice. To our delight our projects have been received enthusioustically by many colleagues abroad, who showed admirable cooperation spirit sending us many seeds. So far we could obtain 16 species of *Oryza*. We are now confident that the remaining some 10 species will be collected in near future.

Studies on animal genetics started at the end of this year. We will refrain from reporting until next year.

A group of our members initiated a study on the genetics and chemistry of bitter substances of *Citrullus colocynthis*. So far a bitter substance was isolated which is called Citbittol. Taste blindness for the bitter substances was sporadically found among the populations near Misima and Yokohama.

We had 3 visiting guest workers in Misima. Mr. RAJAN from New Delhi stayed 8 months and studied the effects of *X*-rays on self-incompatibility of *Brassica* in the Laboratory of Dr. MATSUMURA. Prof. CROW worked with Dr. KIMURA during the summer. Prof. WARID has visited us in the summer to get informations from various departments of the Institute. However his main interests were concentrated in the methods of plant breeding in Dr. SAKAI's Laboratory.

Both Dr. YOSIDA and Dr. OSHIMA went to America for one year in order to study in their respective fields. Dr. YOSIDA is working on cytology of tumor cells in Children's Cancer Research Laboratory in Boston. Dr. OSHIMA, a *Drosophila* specialist, is in the Carnegie Institution, Cold Spring Harbor.

Prior to his trip to India, Dr. OKA went to U. S. A., Philippines and Formosa for 3 months and met specialists in rice plants. Dr. TAZIMA went to India to visit sericultural experiment stations by the invitation of Ministry of Agriculture of India. He travelled up to Kashimir and

Assam in the North, and to Madras and Coimbatore in the South.

The first summer seminar on genetics for the senior high school teachers was held this summer. 72 teachers from 43 prefectures were gathered. The object of this seminar was to give sound informations on genetic principles and to demonstrate the segregation and linkage of genes using plant and animal materials. The lectures on recent advances in genetics were given by the members of the Institute. The seminar was received favorably by the participants.

ABSTRACTS OF DIARY FOR 1957

Jan. 25. 54th meeting of Misima Geneticists' Club.
Feb. 4. Meeting of Tobacco Research Workers.
10. 8th series of Public Lectures on Genetics.
24. 16th meeting of Board of Councillors.
Mar. 13. Meeting of Tobacco Research Workers.
14. 55th meeting of Misima Geneticists' Club.
30. The laboratory for Microbiology and Tissue Culture, under construction since last year, was completed.
Apr. 25. 56th meeting of Misima Geneticists' Club.
May 1. Grant from The Rockefeller Foundation for the study on the origin of cultivated rice was received.
4. 17th meeting of Biological Symposia.
21. 18th meeting of Biological Symposia.
23. Board meeting of Association for the Propagation of the Knowledge of Genetics.
25. 57th meeting of Misima Geneticists' Club.
June 7. 58th meeting of Misima Geneticists' Club.
14. 59th meeting of Misima Geneticists' Club.
21. Board meeting of Japan Association of Poultry Genetics.
22. General meeting of Japan Association of Poultry Genetics.
July 19. 60th meeting of Misima Geneticists' Club.
25–27. 1st series of Summer Seminar on Genetics
29. 61st meeting of Misima Geneticists' Club.
Aug. 6. 19th meeting of Biological Symposia.
10. Meeting of Editorial Board of "The Heredity" (Iden).
Sep. 27. 62nd meeting of Misima Geneticists' Club.
Oct. 18. 63rd meeting of Misima Geneticists' Club.
Dec. 1. Grant from The Rockefeller Foundation for the study on the genetic effects of radiation in animals was received.

STAFF

Director

Hitoshi KIHARA, D. Sc.

Members

1. *Department of Morphological Genetics*:
   Yataro TAZIMA, D. Ag., Head of Department
   Motoo KIMURA, Ph. D., D. Sc.
   Kimiharu ONIMARU
2. *Department of Cytogenetics*:
   Yo TAKENAKA, D. Sc., Head of Department
   Tosihide H. YOSIDA, D. Sc., Assistant Head
   Tsuguo TATEOKA, and Kiyoshi TUTIKAWA
3. *Department of Physiological Genetics*:
   Chozo OSHIMA, D. Sc., Head of Department
   Hitoshi KIHARA, D. Sc.
   Toshifumi TAIRA, and Sadao SAKAMOTO
4. *Department of Biochemical Genetics*:
   Mitsuo TSUJITA, D. Ag., Head of Department
   Yoshito OGAWA, M. D., Saburo NAWA, Bungo SAKAGUCHI, Seizo TSUDA, D. Ag.
   Tôru ENDO, and Tetsuo IINO (on leave of absence)
5. *Department of Applied Genetics*:
   Kan-Ichi SAKAI, D. Ag,, Head of Department
   Hiko-Ichi OKA, D. Ag., Assistant Head
   Yukio YAMADA, Akira MIYAZAWA, and Takatada KAWAHARA
6. *Department of Induced Mutation*:
   Seiji MATSUMURA, D. Ag., Head of Department
   Tsutomu SUGAHARA, M. D., Assistant Head
   Sohei KONDO, Taro FUJII, and Yoshihiko SUGIURA.

Part-time Staff and Research Associate

Kan OGUMA, D. Ag., Ex-Director, Emeritus Professor of Hokkaido Univ.
Yoshimaro TANAKA, D. Ag., Emeritus Prof. of Kyushu Univ.
Taku KOMAI, D. Sc., Emeritus Prof. of Kyoto Univ.
Yoshinari KUWADA, D. Sc., Emeritus Prof. of Kyoto Univ.
Sajiro MAKINO, D. Sc., Professor of Hokkaido Univ.

Hideo ETO, M. D., Assistant Professor of Tokyo Univ.
Kazuo FURUSATO,
Flora A. LILIENFELD, Ph. D.

Department of Administration

Kan-Ichi OTOFUJI, Head of Department
Sumiyoshi SUGIO, Head of General Affairs Section
Masao MIYAZAWA, Head of Finance Section
Naomi MATSUBARA, Hiroko NAKANO, and Junzo KADOWAKI
Clerks, Typists, Telephone operators, Chauffer, Field laborers and Janitors. 24

Misima Branch of Hatano Tobacco Experiment Station

Masao TANAKA, Head
Flora A. LILIENFELD
Seiji IMAI
Assistants. 4

Whole-Japan Association of Poultry Genetics

Hitoshi KIHARA, President
Kan-Ichi SAKAI, Vice-president

Association for Propagation of the Knowledge of Genetics

Hitoshi KIHARA, President
Yo TAKENAKA, Managing Director
Seiji MATSUMURA, Managing Director

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Hideo MATSUKUMA, President of Japan Monopoly Corporation
Toshio SAITO, Governor of Sizuoka Prefecture
Yakichi NOGUCHI, Professor of Tokyo University
Riichi KAWAKAMI, Head of Department of Biometry, National Institute of Public Health

Kin-ichiro SAKAGUCHI, Professor of Tokyo Uuniversity
Masatada TERADA, Professor of Tokyo Jikeikai Medical College
Takeo SAKATA, President of T. Sakata Company,
Ichiro ISHIKAWA, Commissioner of Atomic Energy Commission
Daigoro MORIWAKI, Professor of Tokyo Metropolitan University

RESEARCH PROGRAM FOR 1957

Department of Morphological Genetics

Genetic analysis and linkage study in silkworm (TAZIMA)
Studies on a food preference mutant in silkworm (TAZIMA)
Structure and function of pleiotropic genes in silkworm (TAZIMA)
Genetic effect of radiation upon silkworm (TAZIMA)
Theoretical studies of population genetics (KIMURA)

Department of Cytogenetics

Cytology and genetics of tumors (YOSIDA)
Determination of sex and sex-chromosomes in animals (YOSIDA)
Cytogenetics with tissue method (YOSIDA)
Determination and differentiation of sex in plants (TAKENAKA)
Induction of abnormal mitosis and inhibitation of growth by substances extracted from certain plants (TAKENAKA)
Interspecific hybrization in *Nicotiana* (TAKENAKA, FURUSATO & LILIENFELD)
Genetics of *Pharbitis nil* (TAKENAKA et. al.)
Karyo-systematic studies in *Poaceae* (TATEOKA)

Department of Physiological Genetics

Genetical analyses on insecticide-resistance of *Drosophila*. (OSHIMA)
Genetical and biochemical studies on the metabolisms relating to the procedure of the eye pigment-formation in *Drosophila* (OSHIMA & TAIRA)
Fundamental studies on the effectiveness of ionizing irradiation on the metabolic processes in *Drosophila* (OSHIMA & TAIRA)
Developmental genetic studies of tissue-cultured eye discs of *Drosophila melanogaster*. (OSHIMA)
Studies on the origin of wheat (KIHARA)
Studies on substitution of nucleus in what (KIHARA)
Studies on *Agropyron* (MATSUMURA & SAKAMOTO)

Physiological genetics of the reaction of Japanese morning glory to day-length (SAKAMOTO)

Department of Biochemical Genetics

Biochemical genetics of insects and microorganism (TSUJITA, NAWA & SAKAGUCHI)
Embryological and biochemical studies of silkworm (TSUJITA & SAKAGUCHI)
Biochemical studies of some mutants in silkworm and *Drosophila* (NAWA & TAIRA)
Biochemistry of the mechanism underlying variations in flower colors in plants (ENDO)
Biochemical studies on the differentiation of muscle protein in animals (OGAWA et al.)
Biochemical studies on the mechanism of cell division in animals (OGAWA et al.)
Biochemistry of the bitter substance in *Citrullus colocynthis* (OGAWA et. al).

Department of Applied Genetics

Fundamental studies on breeding and genetics in poultry (YAMADA & KAWAHARA)
Comparative Studies on poultry breeding using experimental animals (YAMADA & KAWAHARA)
Studies on polygenic inheritance (SAKAI et al.)
Studies on competition between genotypes in plants (SAKAI et al.)
Population-genetic studies of "Red-Rice" growing among upland rice (SAKAI et al.)
Studies on the technique of breeding in plants (SAKAI)
Genetic studies on "Cherry-red leaf" in tobacco plants (SAKAI et al.)
Genetic studies on the migration activity in *Drosophila* (SAKAI et al.)
Polyploidy and sterility in fruit plants (FURUSATO & MIYAEAWA)
Genetics of bitter-substance in *Citrullus* (FURUSATO)
Analysis of genes responsible for hybrid sterility and hybrid break-down in rice (OKA)
Genetic studies of some physiological and agronomic characters in rice (OKA)

Department of Induced Mutation

Measurement of X-, β- and γ-ray dosage for induced mutations (KONDO)
Relation between the quality of radiations and mutations (MATSUMURA, NEZU & KONDO)

Radiation genetics of wheat and barley (MATSUMURA & FUJII)
Studies on chlorophyll mutations induced by radiation (FUJII)
Mutation induced by irradiation of tobacco plants (MATSUMURA & FUJII)
Radiation genetics and its practical application (MATSUMURA et al.)
Studies on radiation-induced mutations in mice (SUGAHARA et al.)
Studies on the physicochemical mechanisms of radiation effects on living organisms (SUGAHARA et al.)
Studies on radiation protections in animals (SUGAHARA et al.)
X-ray diffraction studies on muscle (SUGAHARA et al.)
Improvement of sugar beets by means of induced triploidy (MATSUMURA & NEZU)

Research Students and Research Items

Hisataka ITO: Population genetics in animal breeding
Toshiteru MORITA: Biochemical genetics of amino acids and pterine derivatives.
Yoshiyuki AMANO: Effects of antibiotics on cell division.
Tadao HAMADA: Experimental studies on the incidence of leucemia in mice.
Toshihide TABATA: Tissue culture studies on human chromosomes.
Sakinya FUJITA: Histological studies on genetic anomalies in mice.
Jiro SUZUKI: Studies on chromosomes of normal ciliar cells.
Hiroyuki HIRUMI: Developmental genetics by tissue culture.
Masakatsu HORIKAWA: Tissue culture and radiation.
Shinya IYAMA: Population genetics in crops.
Hiroko IKEDA: Statistical treatment in medicine.
Narito TAKAHASHI: Population genetics in rice.
Takashi OTA: Studies on plant improvement by radiation.
Yoichi YOKOTA: Radiation dosimetry.
Shoji KAWASHIMA: Applications of radio-isotopes in agriculture.
Masaaki ONOUE: Radiation genetics in mice.
Goro YAMAMOTO: Effects of radiation on nucleic acid metabolism.
Tetsuaki HASHIMOTO: Radiation biology in mice.

FOREIGN VISITORS IN 1957

Jan. 29. Dr. R. F. CHANDLER. Jr. (The Rockefeller Foundation).

Feb. 1 to Sept. 20. Mr. S. S. RAJAN (Indian Institute of Agriculture). Did research work supported by the Colombo Plan.

May 1. Dr. R. K. ANDERSON (The Rockefeller Foundation).

3. Dr. W. A. WARID (University of Cairo, Egypt).

21. Dr. K. C. ATWOOD (Oak Ridge National Laboratory). Gave lecture on "Lethal mutation in *Neurospora*"

July 1 to Sept. 10. Prof. J. F. CROW (University of Wisconsin, U.S.A.). Colaborated with Dr. KIMURA in a joint work on population genetics. Gave lecture on "Genetic load of a population".

July 1 to 31. Assistant Prof. W. F. WARID (University of Cairo, Egypt). Studied cytogenetics of vegetable. Gave lecture on "Present status of plant breeding in Egypt"

July 19. Dr. H. L. EVERETT (Cornell University, U.S.A.).

24. Mr. D. M. SUMMENER (American Consul-General, Yokohama) and Mr. M. E. LEE (Director of American Cultural Center).

26. Mr. and Mrs. E. WILSON (Chairman of the committee for Pasadena-Misima city affiliation).

Aug. 15. Prof. Abbas ARRUSHDI (University College of Arts & Sciences Baghdad, Iraq).

Oct. 31. Dr. S. A. HOLMBERG (Algot Holmberg & Sons Ltd., Sweden).

Dec. 9. Prof. A. T. SANYAL (State College of Agriculture, Calcutta, India).

RESEARCHES CARRIED OUT IN 1957

A. GENETICS, BIOCHEMISTRY AND CYTOLOGY OF INSECTS

1. *Inheritance of a New Mutant "brown-3" of the silkworm*

(By Yataro TAZIMA)

Light brown eggs are occasionally discovered in certain current commercial silkworm races of Chinese origin. It has been elucidated that the light brown egg is inherited together with transparent larval skin and black eye color and that all of those traits are not separable but are controlled by one gene.

When light brown is crossed to normal, a marked difference is observed in the color of F_1 eggs according to the direction of the cross, due to the after-effect of maternal genes. Among the F_2 eggs, *i.e.* eggs laid by F_1 females, two different cases were observed. Usually segregation does not occur within the same lot of the F_2 eggs, but in some instances segregation is clearly observed. Hence, the mode of inheritance of light brown did not seem to be simple. Under certain circumstances it behaves as if it were similar to b_1 and in other cases it behaves exactly as b_2. Both b_1 and b_2 genes are located on the sixth chromosome. Linkage test between light brown and E^{kp} of the sixth chromosome indicated that they are independent.

A particular interaction was observed between light brown and some egg color genes of the tenth chromosome. When light brown is crossed to w_1 female, characteristic white eggs, which are usually expected in this cross, become pigmented to an appreciable degree so that maternal inheritance can hardly be ascertained. On the contrary, when light brown females are crossed to w^{ol} males, the resulting F_1 eggs remain mostly white. By linkage test between light brown and *fl* (wingless, located on the tenth chromosome), it was revealed that light brown is located on the tenth chromosome. Thus a responsible gene for light brown was found to be different from b_1 and b_2 and it was named brown-3 (b_3).

Three loci, *i.e.* w_1, w_2 and w_3, have already been established on the tenth chromosome. It has also been known that crossing-over could be detectable with small frequency between w_3 and w^{ol}, although both had been reported to be allelic (KIKKAWA '47). The gene b_3 is neither allelic to w_1 nor to w_2, but seems to be pseudo-allelic to w_3. In combination

with w_3, b_3 hinders the transmission of 3-OH-kynurenine from egg plasm to serosa cells, where it would be consumed in pigment formation. This suggests that b_3 and w_3 might possess at least a part in common such as a subunit gene within their gene complex.

Though no decisive proof has been obtained yet, I am inclined to assume that w^{ol} belongs to the same group with b_3 and w_3. The hindrance hypothesis for the transmission of 3-OH-kynurenine from egg plasm to serosa cells may well explain the occurrence of the two different segregation types in F_2 as stated above by assuming that the degree of hindrance varies with the environmental conditions.

2. *Studies on a Food Preference Mutant in the Silkworm*

VIII. Selection for Higher Incidence of Non-preference Individuals and their Genetic Analysis

(By Yataro TAZIMA and Isamu MACHIDA)

Selection for higher incidence of non-preference individuals has been continued up to the tenth generation in an X-ray induced mutant strain of the silkworm by beet-feeding test (TAZIMA '54). The results show a considerable selection effect, and the percentage of incidence of non-preference individuals increased from 0.15% in F_1 to 50.5% in F_{10}. In the meantime selection for higher hatchability has also been simultaneously carried out in the same strain but it proved to be ineffective. In this strain hatching percentage has never exceeded 80% owing to a large number of dead eggs in balance. This indicates that a lethal gene which acts during embryonic stages is linked to the non-preference gene.

Crossing experiments were carried out in order to analyse the genetic behavior of the non-preference character. The non-preference individuals of the F_9 generation were crossed to normal. In F_1 offspring hatchability was recovered up to the level of the normal strain but incidence of non-preference individuals decreased to 28.9%, *i.e.* approximately to half the level of F_{10} generation (50.5%). F_2 was obtained by sib-mating of non-preference F_1 individuals; lethality appeared again resulting in 67.8% hatchability, and the incidence of the non-preference individuals was 30.3%, which was slightly higher than in the preceding generation. The non-preference individuals of F_2 were crossed again to normals. Incidence of the non-preference individuals observed was 9.6 and 9.3% in the offspring of non-preference ♀×normal ♂ and 5.0, 4.9 and 5.9% in the progeny of normal ♀×non-preference ♂.

The above results may well be explained by postulating that there ex-

ists a dominant main gene and several pairs of dominant modifiers that collaborate with the former in the manifestation of the non-preference character and that the main gene is accompanied by a close linked lethal gene. It was also noted that the cytoplasm might have some effect upon the expression of the main gene.

3. *The Resistance of Strains of D. melanogaster to DDT and Dieldrin*

(By Chozo OSHIMA)

The strains used in this experiment were started from different females captured in a single collection from each of several natural populations in Europe, Africa, Japan and United States. These strains have been cultured by mass-mating for about four years at Cold Spring Harbor and in Japan.

Forty female flies 4 or 5 days old, grown under optimal conditions, were exposed to DDT, Dieldrin or control test papers in small vials. The test papers were prepared by the WHO in Geneva. After exposure, flies were transferred into a fresh vial containing a wet paper and the number of dead flies was observed at definite intervals. Flies began to die by desiccation and starvation 25 hours after exposure to control paper.

The levels of DDT-resistance in 18 strains of 10 localities were decided on the basis of percentages of accumulated mortalities 25 hours after exposure to 4% DDT test paper for 5, 10 or 15 hours. On the other hand, the levels of Dieldrin resistance in 11 strains of 5 localities were determined by percentages of accumulated mortalities 25 hours after exposure to 0.4 or 0.8% DL test paper for 0.5, 1.0, 2.0 or 3.5 hours. The several points showing each total average mortality of these strains at several time doses are nearly linear by arranged on normal logarithmic section paper. For this reason, resistance to both insecticides appears to be normally distributed in populations.

Eighteen strains were classified into three groups, non-resistant, slightly resistant, and resistant to DDT, and the numbers of dead flies at 25 hours after exposure to 4% DDT test paper for 15 hours were analyzed statistically as to locality and strain. Highly significant differences in DDT-resistance between localities and between strains were found; the latter were larger than the former but not significantly so. Eleven strains were also classified into three groups, non-resistant, slightly resistant, and resistant to Dieldrin and the numbers of dead flies at 25 hours after exposure to 0.8% DL test paper for a half hour were analyzed statistically as to locality and strain. Highly significant differences in DL-resistance

between localities and between strains were found again but in this case the former were significantly larger than the latter.

These results indicate conclusively that any local population is extremely heterogeneous in DDT and Dieldrin resistance; locality-strain differences in two cases may be attributed to different use of the insecticides.

4. *Genetical and Biochemical Studies on Pterine Oxidation in Drosophila melanogaster*

(By Saburo NAWA, Toshifumi TAIRA and Bungo SAKAGUCHI)

The *Drosophila* homogenate is capable of oxidizing both xanthine and 2-amino-4-hydroxypteridine (AHP). It is likely that the pterine dehydrogenase in *Drosophila* is a dehydrogenase, not an oxidase, further that DPN is a more effective hydrogen acceptor than methylene blue (MB), and also that the conversion of AHP to isoxanthopterin is carried out by pterine dehydrogenase in the presence of DPN *in vivo*. The enzyme preparation was made from pupae of the wild type of *D. melanogaster*. The freshly prepared supernatant produces a considerable amount of isoxanthopterin from AHP in the absence of any external (exogenous) hydrogen acceptor. However, when the dialyzed enzyme or aged supernatant is used, no appreciable production of isoxanthopterin is observed without an external acceptor. The activity of different preparations in the presence of various acceptors is shown in Table 1.

Table 1. Effect of electron acceptor on the oxidation of AHP in preparations from *Drosophila*.

Acceptor	Preparation		
	fresh	aged	dialyzed
Water	0.55*	0.08	0.02
MB	0.9	0.58	0.7
DPN	0.9	0.6	0.75
Cytochrome c		0.1	0.02

* Activity was expressed in μ moles of isoxanthopterin produced per gram whole pupae (wet weight) per hr (pH 8.0).

The activity of pterine dehydrogenase in the presence of DPN has been found to be about equal to or rather higher than that of MB. It seems that DPN naturally present in *Drosophila* acts as an hydrogen accetor.

Under aerobic conditions, isoxanthopterin of much higher concentration is produced than that of DPN added. The relation is shown in Fig. 1.

This may be because pterine dehydrogenase is linked to the DPNH oxidase system. The pH optimum of DPNH oxidase in *Drosophila* is in the neighborhood of 6.5. The ratio of the reaction rate with DPN and with MB as an acceptor is very different at various pH levels. This may be due to the participation of DPNH oxidase in the reaction. The action of the enzyme for pterine oxidation in *Drosophila* is influenced by the concentration of DPN and the rate of DPNH re-oxidation. For example, it has been known that the mutant *rosy* lacks pterine dehydrogenase. The ratio of the reaction rate in the presence of DPN and that of MB as an acceptor varied considerably in some different mutants. This may be due to the difference of the rate of DPNH oxidation. Further experiments in this line are in progress.

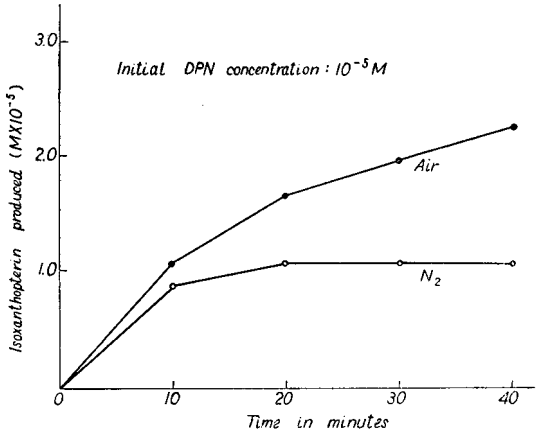


Fig. 1. The effect of the re-oxidation of DPNH on the oxidation of AHP.

5. *Purine Catabolism in D. melanogaster*

(By Toshiteru MORITA and Chozo OSHIMA)

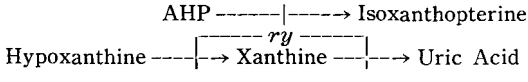
It is well-known facts that the *rosy*^{2} (*ry*^{2}) of the eye color mutants in *D. melanogaster* does not contain isoxanthopterin which occurs widely in *Drosophila*, and that the enzyme prepared from *Drosophila* can catalyze the oxidation of 2-amino-4-hydroxypteridine (AHP) to isoxanthopterin as well as hypoxanthine and xanthine to uric acid. The enzyme, therefore, has been hitherto called xanthine oxidase or pterine oxidase, but it is true dehydroganase because of requirring an electron acceptor. As an electron acceptor, diphosphopyridine nucleotide (DPN) seems to be more efficient *in vivo*, but it is rather less effective than methylene blue in case of purine-catabolism.

An eye color mutant, *rosy* (*ry*), does not contain a trace of isoxanthopterin as likely *ry*^{2}, and it accumulates a larger amount of hypoxanthine and xanthine in stead of uric acid at pupal stage as following table.

Table 1. Pteridines and purines found in *D. melanogaster* strains.

	Oregon-R			*ry*		
	Larvae	Pupae	Adults	Larvae	Pupae	Adults
AHP	±	+	+	±	++	+
Isoxanthopterin	±	++	+	−	−	−
Hypoxanthine, Xanthine	±	±	±	±	++	+
Uric Acid	±	+	+	−	−	−

Furthermore, the *ry* strain does not show any activity of xanthine dehydrogenase, and it seeems to be due to a lack of the enzyme. The same phenomenon are found in such double recessive mutants homozygous for *ry*, as *v* ; *ry*, *cn* ; *ry*, *bw* ; *ry* and *se* ; *ry*. From these results, it seems that the *ry* gene relates to both purine- and pterine-catabolisms as following scheme.



6. *Dopa Oxidase in the Eye-color Mutants of Drosophila melanogaster*

(By Toshifumi TAIRA and Saburo NAWA)

In regard to the activation of tyrosinase in *Drosophila*, some findings have been reported. A comparative analysis on the activity of Dopa oxidase of the eye-color mutants in *Drosophila* was hardly ever before carried out. Such eye-color mutants having yellow pigment as *se* or Hn^{r3} strain were used to analyze the Dopa oxidase activity in comparison with the wild type strain. As a rule, Dopa oxidase is completely activated when the homogenate of wild type prepupae was kept at 0°C in an ice-bath for one hour. Whereas, Dopa oxidase activity in the activated homogenate of *se* was much lower than that of wild type. This may be due to some inhibitation-mechanism interfering with the process of activating the enzyme. It seems that the inhibition-mechanism is probably related to the process of yellow pigment formation.

7. *Formation of Drosophila Eye Pigments*

(By Toshifumi TAIRA and Saburo NAWA)

It has been known that the wild type eye-color of *Drosophila melanogaster* is due to the presence of two pigments, the red and the brown.

It is seemed to be of particular interest to study the behavior of pteridine, since the red and yellow pigments are its derivative. The analysis of pigments and pterines was carried out by paper chromatography. The results are summarized in the following tables.

Table 1. Phenotypic effects of gene-combinations.

Genotype	Phenotype	Genotype	Phenotype	Genotype	Phenotype
w; *cn*	no color	*v*; *bw*	no color	*v*; *cl*	*cl* type
w; *bw*	no color	*cn*; *bw*	no color	*v*; *se*	*se* type
w; *se*	no color	*bw*; *se*	*bw* type	*cn*; Hn^{r3}	Hn^{r3} type
w; Hn^{r3}	no color	*bw*; Hn^{r3}	*bw* type	*v*; *ry*	light red
w; *ry*	no color	*bw*; *ca*	*bw* type	*cn*; *ca*	light red

The mutant genes *se*, *cl*, and Hn^{r3} suppress the formation of the red pigment and accumulate the yellow pigment in eyes. Therefore, the red pigment probably derived from the yellow pigment. In the body of mutant

Table 2. Relative amounts of pigments and pterines in single and compound mutants (7 day-old).

	Mutants	Or-2	*v*	*cn*	*ca*	*cn*;*ca*	*cl*	*v*;*cl*
Eye	Red pigment	##	##	#	+	+	+	−
	Yellow pigment	±	±	±	±	±	#	#
	Isoxanthopterin	±	±	±	±	±	±	±
	AHP*	+	+	+	+	+	#	#
Body	Yellow pigment	±	±	±	±	±	±	±
	Isoxanthopterin	##	##	#	#	+	##	##
	AHP*	±	±	±	±	±	±	±

	Mutants	*se*	*v*;*se*	Hn^{r3}	*cn*;Hn^{r3}	*ry*	*bw*	*w*
Eye	Red pigment	±	±	+	+	+	−	−
	Yellow pigment	##	##	+	+	±	−	−
	Isoxanthopterin	±	±	±	±	−	−	−
	AHP*	#	#	+	+	#	−	−
Body	Yellow pigment	±	±	#	#	±	−	−
	Isoxanthopterin	##	##	+	+	−	−	−
	AHP*	±	±	±	±	##	−	−

* AHP: 2-amino-4-hydroxypteridine

Hn^{r3}, accumulation of yellow pigment and decrease of isoxanthopterin are observed simultaneously. This suggests that the normal metabolism of pteridine is blocked by the gene Hn^{r3} and that the yellow pigment is a precursor of isoxanthopterin. The mutant *ry* is not capable of oxidizing AHP because of the absence of pterine dehydrogenase. However, it remains to be elucidated whether or not the low ability of the formation of red or yellow pigment in *ry* is linked to the absence dehydrogenase.

8. *Genetic Determination of Tyrosinase and Protyrosinase in Blood of Silkworm, Bombyx mori L.*

(By Bungo SAKAGUCHI)

The mutant, used in this experiment, with respect to low tyrosinase activity in the blood of larva was found recently as a spontaneous mutant in green cocoon (Gc) inbred strain at this institute. The mutant was temporarily designated as *ty*.

1. Genetics of *ty*.

Tyrosinase activity in the blood of F_1 hybrid between a wild type (N124) and *ty* showed an intermediate value of both parents. From the results of backcross and F_2 segregation, it seems to be controlled by a single ressecive gene.

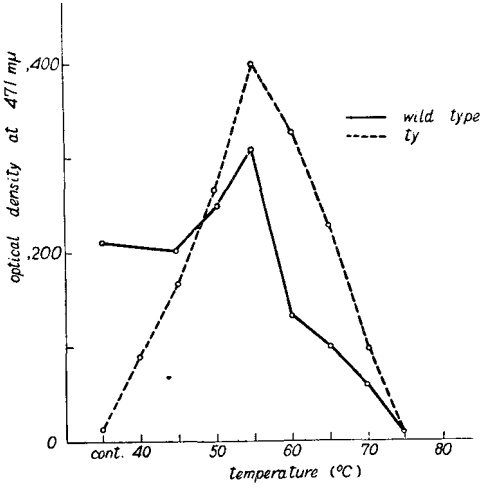


Fig. 1. Relations between enzyme activities and various heat-treatment.

2. Activation of *ty* tyrosinase.

Blood collected from the 5th inster larvae was used for the analysis as a source of the enzyme tyrosinase. Solid ammonium-sulfate was added to the blood to 30% saturation and the precipitate which contains the enzyme was centrifuged down and dissolved in a half volume of distilled water per volume of the original blood. The dissolved solution was then dialyzed over night against distilled water in the vacuum condition. The dialyzed solution consisted of partially purified enzyme preparation was used as the enzyme for the analysis of this experiment.

The tyrosinase activity was determined with photoelectric colorimator using filter of 471 mμ. The reaction mixture contained 0.4 ml of enzyme preparation, 0.5 ml of 0.01 M dopa solution and 4.1 ml of 0.1 M phosphate buffer, pH 6.8. Readings were taken at 5 minute intervals.

The heat-activation experiments were carried out for 5 minutes at the temperature ranging from 45°C to 80°C with 5°C intervals. These treatments were made prior to the determination of tyrosinase activity. The results are given in Figure 1. As shown in the table, pretreatment of 55°C for 5 minutes gave the highest activity in both enzymes obtained

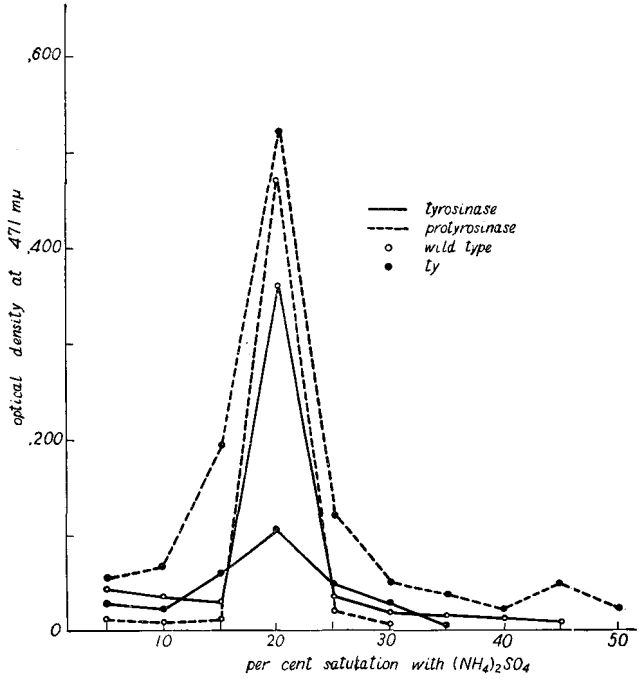


Fig. 2. Solubility of tyrosinase and protyrosinase in $(NH_4)_2SO_4$ solution.

from *ty* and wild type silkworms. The activation rate of *ty* enzyme was much higher than the *wild type* enzyme under those conditions given.

In addition to the heat-activation, activations of enzyme tyrosinase were made by such treatments with acetone, chloroform, ethyl-ether, ethyl-alcohol and temperature at 0°C for 1 to 3 hours.

The enzyme tyrosinase activated by various treatments mentioned above belongs to a catelogy of protyrosinase or latent phenolase found in eggs of Grasshopper *Melanoplus differentialis* (BODINE and ALLEN, 1938), *Drosophila*

(OHNISHI, 1954, HOROWITZ and FLING, 1955) and Broad-Bean (KENTEN, 1957). It was found from these results that protyrosinase in the blood of *ty* was much more accumulated than that of wild type. The accumulation of protyrosinase was controlled by the *ty* gene.

3. Solubility in ammonium sulfate solution of tyrosinase and protyrosinase contained in *ty* and wild type ($+^{ty}$).

Each 20 ml of blood of *ty* and $+^{ty}$ strains were separately collected, and the same volume of 0.1 M phosphate buffer, pH 6.3 was added to the each blood sample. Solid ammonium-sulfate was then added to the mixed solution to give 5 to 60% saturated concentration. The precipitate was centrifuged down and dissolved in same volume of distilled water per volume of the original blood. The dissolved solution was used as the tyrosinase preparation. This preparation was heated at 55°C for 5 minutes and used as protyrosinase in the following analysis.

Measurement of enzyme activity were carried out with photoelectric colorimeter under the conditions described earlier. Results are graphically summarised in Figure 2. The greater parts of both tyrosinase contained in blood of *ty* and $+^{ty}$ strains were collected in the fraction of 20% satulated ammonium sulfate. It was found that the solubilities of tyrosinase and protyrosinase proteins in ammonium-sulfate solution in both *ty* and $+^{ty}$ strains are of distinctly similar in nature. It seems that the gene *ty* controls the specificity of the structure of the enzyme protein or that of active site of the enzyme protein.

9. *Chromosomal Constitution of the Original Nl_2 Strain of the Silkworm*

(By Mitsuo TUJITA)

U, Nl_2, *oa* and several other genes belonging to the 14th linkage group of the silkworm form the pseudoallelic *U*-complex (or *U* region). The gene *oa* is recessive, and larvae homozygous for *oa* exhibit a transparent hypodermis. However, larvae with the genotype Nl_2/oa have a transparent hypodermis. This fact suggests that Nl_2 is due to a deletion involving the *oa* locus.

The genetic behavior found in the progeny of the cross between the original Nl_2 strain and *oa*/*oa* indicates partly trisomic inheritance and postulates the presence of a fragment of chromosome XIV. In the light of this hypothesis the following diagram illustrates the genetic constitution of the original Nl_2 strain.

The cytological and genetical study of this strain will be continued.

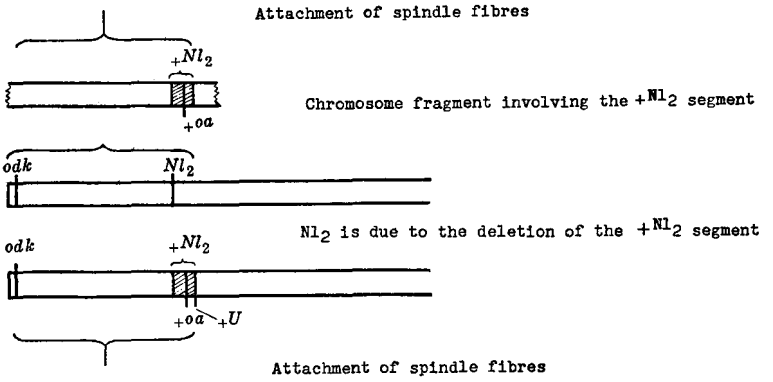


Fig. 3. Diagram of the genetic constitution of the original Nl_2 strain.

10. *Studies on the Mitochondria in the Gland Cells of Malpighian Tubules of the Silkworm Larvae by Means of Ultra-thin sections*

(By Mitsuo TSUJITA)

In my previous papers (1937, 1947), the morphology of mitochondria and and their relation to secretion in the Malpighian tubule cells of the silkworm larvae were described. In recent years I have continued to study the mitochondria in the cells of Malpighian tubules by ultrathin sections. Principal points of my observations are as follows:

The inner margin of gland cells is bordered with a brush border which consists of cytoplasmic protrusions in which mitochondria with cristae-like structure are observed.

At the beginnig of each instar mitochondria which increased their number in the cytoplasm migrate to the base of the inner margin of the gland cells and protrude into the gland lumen together with a small amount of cytoplasm. Thus, the brush border is formed. Cytoplasmic protrusions containing mitochondria, sometimes show rod-, clavate- or tadpole-shape. At the middle stage of each instar, except for the tubules of the outer layer in the rectal wall, various phases were found which indicate that filamentous mitochondria migrate from the cell body to the base of the protoplasmic processes driving toward the preformed process border. In thus manner, protoplasmic processes can be supplemented at the middle stage of each instar. It seems that in the winding as well as ascending parts, often processes change into needle-- or rod-shaped cristal. And as stated in my previous paper (1937), in the later stage of each instar granular mitochondria migrate into the lumen of the gland, where they disappear.

Similar cytological structures can be observed in the goblet cells of the mid-gut epithelium of the silkworm larvae. The goblet cell is occupied for the most part by a large vacuole or goblet, which is flask-shaped. The inner surface of the goblet is apparently bordered by a mitochondrial layer embedded in an acidophile protoplasmic matrix, in which fibrous mitochondria are arranged almost parallel to each other. It seems to be reasonable to assume that in this case the mitochondrial layer is specific form of brush border, a layer of cytoplasmic protrusions, in each of which fibrous mitochondria are contained. The so-called " Stäbchensaum " of the cylindrical cells is a typical form of brush border which consists of a number of protoplasmic protrusions.

Recently, BEAMS, TAHMISIAN and DEVINE (1956) worked with electron microscope on the cells of Malpighian tubules of the grasshopper paying special attention to the behaviour of mitochondria in the gland cells. In their paper they describe that filamentous mitochondria migrate from the cell body to the base of protoplasmic processes where they protrude into the inner margin.

B. CYTOGENETICS, GENETICS, CYTOLOGY AND BIOCHEMISTRY OF TUMORS

11. *Two Chemicals Promoting Cell Divisions in the Yoshida Sarcoma*

(By Yoshito OGAWA, Kenjiroo FUJIOKA and Yukihide ABE)

We found that two chemicals act as mitotic stimulants on animal cells. One of them is Kinetin and the other is Na-Glucuronate.

Kinetin (6-Furfurylaminopurine) was isolated by MILLER et al. (1955, 1956) from deoxyribonucleic acid from herring sperms as a stimulant of plant cell mitosis. Its effects on the division of animal cells, however, was not well known, though negative results have been reported by LETTRE et al. (1956) using animal and human cells. We have investigated the effect of Kinetin on the mitotic activity of YOSHIDA sarcoma cells transplanted to the Wister strain of rat. Injecting the solution of Kinetin into the abdomen (most effective dose is 0.15 mg. per 100 g. body weight), a significant increase in the frequency of mitotic figures was found in the tumor cells after about 120 hours (significant at the 1% level), as shown in Fig. 1. (OGAWA, Y. et al.: Nature, 180: 985. 1957)

Na-Glucuronate is well known as a growth promoting substance in animals. We have investigated the effect of Na-Glucuronate on the mitotic activity of YOSHIDA sarcoma cells using the same method as in our experi-

ment with Kinetin. When a solution of Na-Glucuronate was injected into the abdomen of rats (most effective dose is 750 mg. per 100 g. body weight), a significant increase in the frequency of mitotic figures was found in the tumor cells after 24 (significant at the 5% level) and 96 (significant at the 1% level) hours. But when injected into the muscle tissue, no significant increase was found. This suggests that effect of Na-Glucuronate on sarcoma cells may be direct.

We can find no influence upon the frequency of mitotic stages in sarcoma cells either when Kinetin or Na-Glucuronate is injected.

Further, to examine the effects of promoting activity at a combined treatment of the YOSHIDA sarcoma with both chemicals, cell division was investigated using the same method as in the above experiments. At a combined treatment, the promoting effect of Na-Glucuronate on sarcoma cell divisions was not investigated 24 hours after transplantation. But after 48 hours, we could not fined a statistically significant difference between separate and combined treatments as shown in Fig. 1.

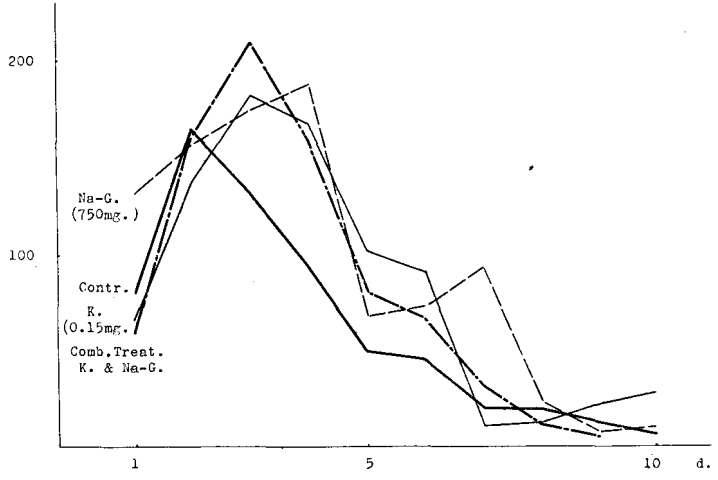


Fig. 1. Daily change in frequency of metaphasic cells (pathological figures excluded) after injecting Kinetin, Na-gluculonate and both chemicals combined into the abdomen of rat, 10,000 cells being observed per day.

This result indicates that:

1) The effect of Na-Glucuronate which appears 24 hours after transplantation of sarcoma cells is due to a different mechanism from that which acts 96 hours after transplantation.

2) The two chemicals do not help each other in promoting cell divisions in the sarcoma 48 hours and later after transplantation of sarcom cells.

12. *Biochemical Similarity of Mitotic Stimulants in Normal and Sarcoma Cells of Rat*

(By Yoshito OGAWA)

MALMGREN (1955) reported that the saline extract of mammary tumor of mice, if injected into the abdomen of rat, produced a significant increase of the mitotic index in the liver after 48 hours. In order to test if his findings are limited to his materials or can be generally true, we investigated mitotic indexes in the host liver after subcutaneous transplantation of a spontaneous fibrosarcoma which had been obtained from a Wayne-pink-eyed yellow rat.

Four days after the transplantation, a significant increase of mitotic index was observed in the host liver as shown in Table 1. It is sure that this was not due to our misjudgment of metastasis of transplanted sarcoma in view of the following points:

1) The significant increase in mitotic index appeared before the growth of transplanted sarcoma and disappeared 7 days after transplantation.

2) This fibrosarcoma was only transplantable to the Wayn-pink-eyed yellow strain of rat.

Therefore, it may be generally recognized that some mitotic stimulant produced by tumor tissue is effective in normal cells. (OGAWA, Y. et al.: Med. and Biol. 45: 110. 1957)

Is the mitotic stimulant produced in normal tissue also effective in sarcoma cells?

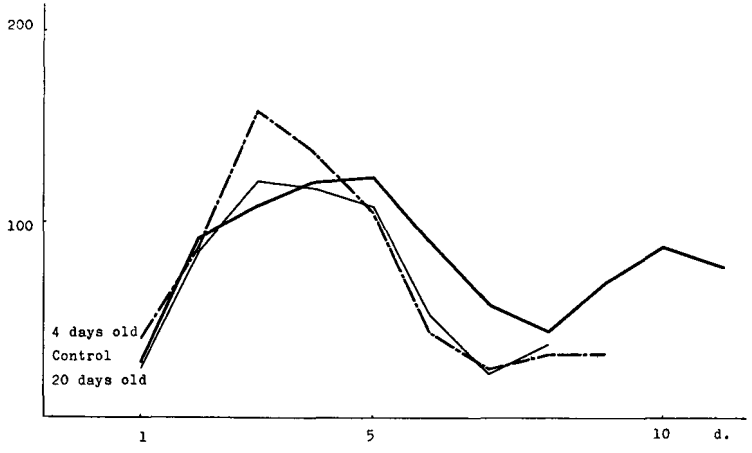


Fig. 1. Daily change of frequency of metaphasic cells (pathological figures excluded) after injecting saline extract of rat embryos into the abdomen of rat, 10,000 cells were observed per day.

Table 1. Mitotic index in transplanted sarcoma and in host liver tissue

Days after Transplantation	Mitotic Index in Transplanted Sarcoma	Mitotic index in Host Liver	Host
4	—	6.7	Wister
	—	3.3	
	—	25.0	
	—	10.0	
	—	8.3	Hybrid*
	—	13.3	
	—	0.0	
	—	3.3	
	—	6.7	Wayne
	—	45.0	
	—	6.7	
	—	10.0	
	—	0.0	
7	23.3	0.0	Wayne
	20.0	0.0	
	58.3	0.0	
14	0.0	0.0	
	80.0	13.3	
21	85.0	16.7	
	36.7	1.7	
28	40.0	8.3	
	6.7	0.0	
Control		0.0	Wister
		1.7	
		0.0	
		0.0	Hybrid*
		0.0	
		0.0	
		0.0	Wayne
		0.0	
		0.0	
		1.7	

* F_2 of Wister × Wayne

In order to answer this question, saline extract of rat embryo (Wister: 4 and 20 days old) was injected into the abdomen of the same strain of rat (Wister: 2 month old) immediately after the transplantation of YOSHIDA sarcoma, and the daily change of frequency of metaphasic cells was observed. As shown in Table 2, when saline extract from the

tissue of 4 days old embryos was injected, a significant increase in the frequency of mitotic figures was found in the tumor cells after 24 and 96 hours (both significant at the 5% level). But when the extract from the tissue of 20 days old rat was used, no significant increase was found.

It may then be concluded that the mitotic stimulant produced by sarcoma tissue is effective in normal cells and that produced by normal tissue is effective in sarcoma cells.

This suggests that there is something in common between normal and sarcoma cells in producing, and responding to, mitotsis is stimulating substance.

13. *Idiogram of a New Subline of the Yoshida Sarcoma and Some of its Properties*

(By Tosihide H. YOSIDA)

As stated in the previous report (YOSIDA 1957)*, tumor cells characterized by a new chromosomal constitution were observed in the YOSHIDA sarcoma. These cells had 40 chromosomes, including one strikingly large J- and two large V-elements, and they gradually increased in number in succeeding transplant generations. At the twentieth transplant generation the majority of tumor cells showed a new karyotype. Thus, a new subline was established in the course of serial transplantations.

Idiogramatic analysis discloses that these tumor cells have 15 rod-, 7 J- and 18 V-shaped chromosomes, among which two V's and one J are remarkably large in size. On the other hand, the original tumor cells contained 15 rod-, 8 J- and 17 V-chromosomes. On the basis of karyological analysis of these tumor cells, it appears probable that the large J-shaped element, which characterized the new subline, resulted from translocation of two long arms of the first and the sixth J-shaped elements. Furthermore, a comparison of numerical difference in the J- and V-shaped chromosomes of the two groups suggests that the short arms of two J-elements of the original cell combine to form a small V-shaped element by means of centromeric fusion.

Transplantability of this subline increased gradually, following the increase in number of tumor cells having the new karyotype. The host range for adaptability of new tumor cells was wider than that of the original tumor cells. The average life span, observed in 153 Misima-bred rats bearing the new subline, was found to be 10.3 days. The average life span of 21 Misima-bred rats bearing the stock of the Yoshida sarcoma obtained from the Sasaki Medical Institute, Tokyo, was 13 days, while that of 34 Misima-bred rats bearing another tumor strain from the Pharmaco-

* YOSIDA, T. H. 1957. Ann. Rep. Nat. Inst. Genet. Japan 7: 16–17.

logical Research Foundation, Tokyo, was 12 days.

It may be concluded from these observations that the competitive ability of the new tumor cells which developed by the chromosomal translocation, is superior to the original form.

14. *A Karyological Study on the Yoshida Sarcoma Cells Infiltrated into Organs of a Tumor-bearing Rat*

(By Tosihide H. YOSIDA)

As mentioned in the foregoing paper (in this report) new tumor cells of the YOSHIDA sarcoma are characterized by one large J- and two large V-shaped chromosomes in their chromosome complex. These cells gradually increased in number during the course of ensuing generations. At the ninth transplant generation the percentage of the new tumor cells to the original cells was 79.0 per cent in the ascites tumor, while at the tenth transfer generation this percentage increased to 88.9 per cent.

The following experiment was carried out in order to investigate the infiltrating ability of the new and the original tumor cells into organs of a tumor-bearing rat at the ninth transplant generation. From this rat, small amounts of the lung, the spleen and the liver were inserted separately into the peritoneal cavities of normal rats. In the event that tumor cells would infiltrated into organs readily, implanting the minced organs into normal hosts would serve as an inoculum, and give rise to a new ascites tumor population.

The observations have disclosed that in the case of transplantation of the liver, 90 per cent of tumor cells were of the new karyotype; while in the case of the lung and spleen transplantations, only 33.3 and 2.6 per cent of tumor cells had the new karyotype, respectively.

Based on the above investigations, it seems probable that the new tumor cells display a higher degree of infiltration in the liver as compared to that in the spleen and the lung.

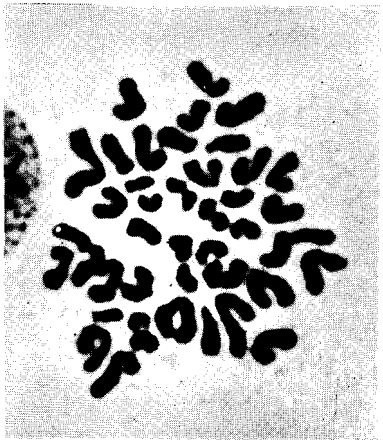
15. *Karyological Study on the Normal Somatic and Malignant Tumor Cells in the Human*

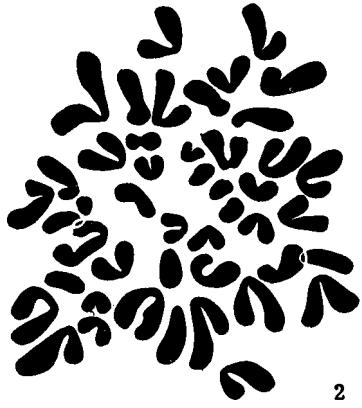
(By Tosihide H. YOSIDA and Toshihide TABATA)

The chromosomes of normal somatic cells in six human embryos and one adult were studied using squash technique—the material was pretreated in hypotonic solution*. The chromosomes were observed in twelve somatic cells which showed a clear metaphase condition (Figs. 1 and 2). Variation of the chromosome number in these cells is shown in the Table 1. As shown in the table the cells having 46 chromosomes were most frequent.

* YOSIDA, T. H. and T. ISHIHARA 1956. La Kromosomo **29**: 1005–1009.

The chromosomes of tumor cells obtained from the material of the stomach cancer, the tongue cancer, the maxillary cancer which developed by metastasis of the stomach cancer, and the HeLa cells cultured *in vitro* were studied. The number of chromosomes in the stomach cancer cells showed two peaks of variation, one of which is 60 to 70, and the other is 100 to 110. The tongue cancer cells contained from 40 to 50 chromosomes. On the other hand, the chromosome number of the maxillary tumor cells





Figs. 1 and 2. Chromosomes of a male germ cell in a human embryo ($2n=46$). Fig. 1 is a microphotograph, Fig. 2 is a drawing of the same cell as Fig. 1.

Table 1. The variation of chromosome number in normal somatic cells of the human.

No. of chroms.	43	44	45	46	47	48	Total
No. of cells observed	1	0	0	7	1	3	12

showed a variation from 30 to 40. It is readily noted that the majority of the original tumor cells in the stomach cancer has a hypotriploid and hypopentaploid chromosome constitution, whereas the tumor cells in the maxillary tumor, which developed from the metastasis of the stomach cancer, showed a hypodiploid chromosome constitution. It has been noted by YOSHIDA (this report) that the infiltrating ability of the YOSHIDA sarcoma cells into organs of a tumor-bearing rat varied according to the difference of karyotypes in these tumor cells. It is suggested from this study that the chromosome constitution of the maxillary tumor cells differ from those of the original stomach cancer on acount of selective adaptation of metastatic cells.

HeLa cells, grown in tissue culture, showed a variation from 80 to 90 chromosomes.

16. *Change in the Transplantability of the MY-mouse Sarcoma in Relation to the Change of the Chromosome Number*

(By Tosihide H. YOSIDA and Tadao HAMADA)

Studies on the transplantability of the MY-mouse sarcoma were carried out by YOSIDA (1952)*, ISHIHARA and YOSIDA (1954)**, and YOSIDA and ISHIHARA (1955)***. According to their observations this tumor was transplanted successfully to the S, S^k, S_4, D and D-103 strains of mice, which were inbred in this institute. On the other hand, this tumor failed to grow in strains of mice such as C3H, DBA/2, A, SWR and Swiss Albino, imported from the United States. Present studies on the transplantability of this sarcoma have revealed that the host range can be conspicuously extended to include such mouse strains as D, D-103, S, S_4, C57L, dba, DBA/Ma, Swiss Albino, C57BL, dd, C58, SPS, A and C3H.

YOSIDA (1954)**** studied the chromosomes of this sarcoma and noted it to contain about 40 chromosomes in number. It is interesting to note that a remarkable increase in the chromosome number was observed in this study. The majority of cells have 80 chromosomes. The stem line, which is characterized by tetraploid cells, was derived from the diploid stem line. These studies illustrate that change in the host range of transplantable tumors are related to chromosome ploidy.

17. *Effect of 8-azaguanine and its Related Compounds on the Chromosomes of the Yoshida Sarcoma Cells*

(By Tosihide H. YOSIDA and Hiroyuki HIRUMI)

8-azaguanine which is said to be antagonistic to pyrimidine metabolism causes the deformation of chromosomes in the YOSHIDA sarcoma cells. Normal chromosomes of the YOSHIDA sarcoma are generally characterized as rod, J- and V-shaped. By the intraperitoneal injection of 8-azaguanine, the shape of chromosomes of the tumor cells was observed to change to a round shape.

In order to clarify the influence of 8-azaguanine on the chromosome deformation, seven chemicals structually related to 8-azaguanine were employed in this study. They are grouped by their chemical constitutions as follows: (1) Compounds having triazolpyrimidine nucleus with a hydroxy group (—OH) at the 6-position of the pyrimidine nucleus. (2) Compound

* YOSIDA, T. H. 1952. Jour. Fac. Sci. Hokkaido Univ. Ser. VI. Zool. 11: 41-50.

** ISHIHARA, T. and T. H. YOSIDA 1954. Ann. Rep. Nat. Inst. Genet. Japan 4: 15-17.

*** YOSIDA, T. H. and T. ISHIHARA 1955. *Ibid.* **5**: 23-24.

**** YOSIDA, T. H. 1954. *Ibid.* **4**: 2.

having triazolpyrimidine nucleus with a 6-oxygen, instead of the 6-hydroxy group. (3) Chemical having triazol only. (4) Chemicals having imidazolpyrimidine with a 6-hydroxy group on the pyrimidine nucleus. (5) Chemical having imidazolpyrimidine with 6-oxygen.

The experiments were carried out as follows: 0.5 cc of ascites tumor was mixed with each compound *in vitro*, and immediately injected into peritoneal cavity of normal W-strain rats. The ascites cells were examined 6, 24, 72 and 120 hours after injection by the usual squash technique. Our observations revealed that the compounds of group 1 were most effective for chromosome deformation, showing as great degree as the 8-azaguanine. On the other hand, compounds of groups 2, 3 and 4 showed about half the effect of 8-azaguanine. No effect was found in the compound of group 5. These observations suggest that the chromosomal deformation is caused by compounds containing a certain chemical nucleus and/or a specific functional group, such as the triazol nucleus and the 6-hydroxy group.

18. *Effect of Chemical Anions on the Spreading and Splitting of Animal Chromosomes*

(By Tosihide H. YOSIDA and Tosihide TABATA)

By using hypotonic solutions of various metal chlorides YOSIDA and OGAWA (1956)*, found that the degree of spreading and splitting of metaphase chromosomes varied according to the sort of cations present in the solutions. The present report deals with the influence of chemical anions on the chromosomes in the YOSHIDA sarcoma cells.

In order to investigate the effects on anions, 16 kind of Na-, K- and Ca-salts were used: NaCl, NaH_2PO_4, Na_2HPO_4, $NaHCO_3$, Na_2SO_3, Na_2SO_4, $Na_2S_2O_3$ and NaF; KCl, KBr, $KMnO_4$, $K_2Cr_2O_7$ and $K_3[Fe(CN)_6]$; $CaCl_2$, and $Ca(CH_3CO_2)_2$. The tumor cells were treated with N/10 and N/100 solutions of the above compounds. They were then stained with acetic orcein by means of usual squash technique (YOSHIDA and ISHIHARA 1952)**. Among the solutions of Na-salts, the most satisfactory results for the splitting and spreading of chromosomes were obtained with the N/100 solution of NaCl and the N/10 and N/100 solutions of NaF. For K-salts, the N/100 solutions of KCl and KBr showed good results. In Ca-salts the N/100 solution of $CaCl_2$ was the most effective.

Of the compounds studied it can be concluded that the halogen anions cause the greatest spreading and splitting of chromosomes.

* YOSIDA, T. H. and Y. OGAWA 1956. Ann. Rep. Nat. Inst. Genet. Japan **6**: 20.

** YOSIDA, T. H. and T. ISHIHARA 1956. La Kromosomo **29**: 1005–1009.

C. DEVELOPMENTAL GENETICS OF ANIMALS

19. *Development of Muscle Protein in Early Chick Embryo*

(By Yoshito OGAWA, Takatada KAWAHARA and Jiroo MIURA)

As early as on the sixth day of development of a chick embryo, slow contractions are shown by migrating myoblasis (HERRMANN 1952). This property of contractility is present in the forming muscle well before the appearance of the usually accepted histological characteristics. In spite of the primitive morphological stage of early contractile cells, there is evidence that biochemically they have already reached a rather advanced development (DEHAAN 1956). CSAPO and HERRMANN (1951) found in chick muscle of the ninth day of development a protein with solubility and viscosimetric characteristics similar to myosin, suggesting the possibility

Table. 1. Results of precipitin reaction with actin anti-serum and saline extract of chick embryos.

Hours after Incubation	Number of Embryos	Antigen Concentration 4×	8×	16×	32×	64×	128×	Note
24	8	−	−	−				4 Embryos mixed
		−	−	−				
48	8	−	−	−	−			
		−	−	−	−			
72	4	+	−	−	−	−	−	2 Embryos mixed
		+	±	−	−	−	−	
96	2	++	+++	+++	++	+	+	*
		+	++	+++	++	++	+	
144	2	+	+	+	++	++	+++	*
		+	+	+	+	++	+++	
216	2	+	+	+	+	++	++	*
		+	+	+	+	++	+++	
Control (Non-fertilized)	8	−	−	−				4 Blastodisks mixed
		−	−	−				

* The heart tissue was carefully excised.

Actin from the heart tissue and that from the skeletal muscle showed no difference in the reaction with anti-serum.

of its existence at still earlier stages. HERRMANN (1952), however, reported later that muscle protein was not detectable until about the fourteenth day.

We obtained pure actin and myosin from the pectoral muscles of White Leghorns by the method of Szent-Györgyi (1944). This protein, at a concentration of about 15 mg. per ml., was injected intravenously into rabbits at three day intervals, until it reached a total of 60 mg. The rabbits were bled by heart puncture on the fifth day after the last injection, and the serum obtained was used for the precipitin reaction with saline extracts from chick embryos. A possible failure due to antigen-excess was prevented by using a constant volume of serum and progressively decreasing amounts of antigen.

It was found that actin becomes first detectable in the 72 hours old chick embryo (Table 1). In view of the fact that heart contractions begin about 48 hours after incubation, it may be said that the stage of the first

Table 2. Results of precipitin reaction with mysin anti-serum and saline extracts of chick embyos.

Hours after Incubation	Number of Embryos	Antigen Concentration 4×	8×	16×	32×	64×	128×	Note
48	10	−	−	−				5 Embryos mixed
		−	−	−				
72	20	−	−	−	−			
		−	−	−	−			*10 Embryos mixed
96	20	+	+	±	−	−	−	
		++	+	+	−	−	−	
120	10	++	+++	+++	++	+	+	* 5 Embryos mixed
		+	++	+++	+++	++	+	
168	3	+	++	++	++	+++	+++	*
		+	+	++	+++	+++	+++	*
		+	+	++	++	++	+++	*
240	3	+	++	++	++	++	++	*
		+	+	++	++	++	+++	*
		±	+	+	++	++	+++	*
Control (Non-fertilized)	12	−	−	−				6 Blastodicks mixed
		−	−	−				

* The heart tissue was carefully excised.

contraction of heart muscle corresponds to the stage of actin formation. (OGAWA, Y. et al.: Kagaku. 27: 253 1957)

On the contrary, in the skeletal muscle, myosin was ascertained about 96 hours after incubation (Table 2).

Myosin in the heart muscle of a chick about 16 hours after incubation was determined first by EBERT (1953). It may therefore be assumed that the appearance of heart myosin, action and myosin in the skeletal muscle occurs, respectively, about 16, 72 and 96 hours after incubation.

It seems that in the heart myosin appears before the formation of actin, while the order may be reversed in the skeletal muscle.

20. *Promoting Effect of Gonade Extracts on the Spawning of the Pearl Oyster (Pinctada Martensii)*

(By Yoshito OGAWA, Yushiroo HARADA* and Toshio AI*)

It is a serious problem in rearing pearl oysters that when the gonade is in mature condition, it becomes not only difficult to insert pears kernels into it, but operating at such a stage spoils the quality of the pearls produced. Though artificial promoting of spawning has been tried by many workers, no successful method has been reported.

The writers have injected the extract from gonades of *Pinctada Martensii* into the foot tissue of pearl oysters. The extract contained a basic com-

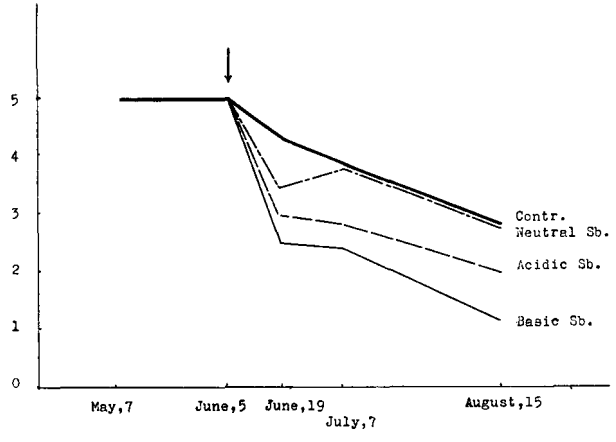


Fig. 1. Promoting effect of ether-soluble basic compounds from gonade tissues on the spawning of the pearl oyster.

* Sizuoka Fishers Experiment Station, Simizu.

pound which was ether-soluble and non-evaporative. This treatment was found invariably to bring about spawning about two weeks after injection. Completly mature and hypertrophous gonades were given grade 5 and those in faded and reduced condition grade 0. The grades between 5 and 0 represented successive intermediate stages (Fig. 1). Thus spawning could be accelerated by two months and this condition of the injected ovary was kept for a long time.

This method may help the pearl industry. Chemical purification of the effective substance is now being studied.

D. CYTOLOGY OF ANIMALS

21. *Mitotic Activity of Regenerating Liver Cells after Partial Hepatectomy*

(By Yoshito OGAWA)

Mitotic activity is often used as a sensitive and reliable criterion to measure the degree of growth and regeneration of organs. In our experiments with hepatectomy, however, we have frequently met with cases in which mitotic activity of regenerating liver cells was not proportionally related to the physiologically regenerating grade. From this point of view, the relation between tissue weight and mitotic activity in the regenerating liver after partial hepatectomy was investigated using the Wister strain of rat (No. 1–13, two month old: No. 21–25, 5–6 month old). We measured the frequency of metaphasic cells 48 hours after operation, observing 2,000 cells per liver lobe.

When more than 60% in weight of the whole liver was taken out by operation, the remaining tissue greatly increased in weight after 48 hours, but no mitotic cell was seen. On the other hand, if the part taken out of the liver was less than 60%, the increase in weight of the remaining part was relatively small but the mitotic activity was considerably high (Table 1). (OGAWA, Y. et al.: Med. and Biol. 45: 12, 1957)

Further we investigated the relation between the regenerating process and mitotic activity in each liver lobe, taking less than 60% weight of the liver. As shown in Table 2, the operated lobes decreased but the non-operated ones increased considerably in weight. However, regarding mitotic activity no remarkable difference was found between the operated and non-operated lobes.

Table 1. Regenerating process of liver tissue after partial hepatectomy.

	No.	Sex	Body Weight (g)	Weight of Removed Tissne in %	Increase in Weight of Regenerating Liver Tissue (%)**	Mitotic Index
Treated	1	♂	90.0	42.8	+ 41.6	70.0
	2	♀	127.8	46.4	+ 48.9	58.4
	3	♂	108.7	35.5	+ 54.2	46.7
	4	♀	129.5	49.6	+ 77.1	33.4
	5	♀	109.3	51.0	+ 28.0	15.0
	6	♀	106.0	50.5	+145.0	8.3
	7	♀	96.0	53.6	+250.9	1.6
	8	♂	125.6	63.2	+ 71.8	0.0
	9	♂	91.5	63.4	+288.5	1.6
	10	♀	129.7	64.6	+ 82.3	1.6
	11	♂	117.1	75.0	+162.7	0.0
	12	♀	82.9	75.0	+199.0	0.0
	13	♀	113.4	77.3	+191.6	0.0
Control	101	♀	84.9	3.9*	—	1.6
	102	♀	85.6	3.5*	—	0.0
	103	♀	82.5	3.4*	—	0.0
	104	♂	98.7	5.7*	—	1.6
	105	♂	105.7	5.0*	—	0.0

* Liver weight.

** Percent of the whole liver weight estimated.

Table 2. Variation in the weight of regenerating liver tissue after partial hepatectomy.

No.	Sex	Body Weight (g)	Weight of removed Tissue in %	Non-operated Lobes (%)*			Operated Lobes (%)*	
				I	II	VI	III+IV	V
21	♂	112.3	33.6	+48.8	+57.2	+ 41.5	−48.7	−58.2
22	♂	121.5	31.8	+89.2	+27.1	+ 27.3	−41.2	−52.0
23	♀	180.5	34.1	+46.3	+41.3	+ 3.1	−70.9	−58.4
24	♀	155.1	34.1	+49.2	−10.5	+134.0	−74.6	−46.2
25	♀	175.0	33.6	+ 1.5	+ 2.2	+ 31.8	−56.2	−71.4

* Increasing (+) and decreasing (−) percentage were estimated in percent of the whole liver weight.

Table 3. Mitotic index in each regenerating liver lobs after partial hepatectomy.

	No.	Sex	Non-operated Lobes (%)*			Operated Lobes (%)*		Mitotic Index
			I	II	VI	III+IV	V	
Treated	21	♂	0	1	0	2	2	5
	22	♂	1	3	2	3	3	12
	23	♀	0	2	4	1	0	7
	24	♀	2	1	0	0	2	5
	25	♀	3	2	1	2	0	8
	Per lob			7.1		7.5		
Control	121	♂	1	0	0	0	0	1
	122	♀	0	0	0	0	0	0
	123	♀	0	0	0	0	0	0
	124	♂	0	0	0	0	0	0
	125	♂	0	0	0	0	0	0

From these results, we may conclude that:

1) The increase in tissue weight is not always related to mitotic activity in the regenerating liver.

2) Mitotic activity may be influenced by the action of some diffusible substance which is produced in the regenerating liver tissue.

3) Young rats produce more of such mitotic stimulants than the older ones. (Table 1 and 2)

For measuring the degree of regeneration of liver tissue by mitotic activity after partial hepatectomy, the extent and place of operation should be carefully considered.

E. GENETICS AND CYTOLOGY OF CEREAL CROPS

22. *Ecogenetical Studies in Agropyron*

(By Sadao SAKAMOTO)

(I) A new ecotype of *A. tsukushiense* var. *transiens* OHWI

A. tsukushiense var. *transiens* ($2n=42$) is a very common plant in Japan, growing in fields and along road-sides. A strain which differs in several characteristics from the well known type was found as a swarm in idle lying paddy fields only, in the hilly vicinity of Misima (Table 1). In comparison with the type, plant height, first internode from top, flag leaf and

Table 1. Comparison of several characters distinguishing *A. tsukushiense* var. *transiens* and the new ecotype.

Characters	Experimental field		Natural habitat	
	transiens	Ecotype	*transiens*	Ecotype
Plant height (cm)	117.2	63.0	84.6	37.9
First internode from top (cm)	53.5	33.4	34.8	19.4
Flag leaf (cm)	17.4	14.4	18.0	7.6
Spike (cm)	26.3	14.8	25.4	10.2
No. of spikelets	23.6	13.3	23.0	9.6
No. of florets per spikelet	7.6	7.3	7.6	5.7
Empty glume (cm)	0.91	1.08	0.80	0.65
Lemma with awn (cm)	3.92	4.29	3.23	3.14
Palea (cm)	0.92	1.03	0.84	0.92
Seed length (cm)	0.54	0.67	0.49	0.60
Seed weight (mgm)	4.8	11.0	2.3	7.8
Date of first earing	21 May '57	27 April '57	—	—
Date of first flowering	21 May '57	30 April '57	—	—

spike of this strain are shorter, and the number of spikelets is much smaller but awn, empty glume, lemma, palea and seed are longer, seed weight is much heavier, and the flowering time is about 20 days earlier. Artificial hybrids between *A. tsukushiense* var. *transiens* and this strain were easily obtained and the growth of the F_1 plants was very vigorous.

This strain was recognized as a new ecotype of the above variety of *A. tsukushiense*.

(II) Natural hybrids between *A. Mayebaranum* HONDA and *A. tsukushiense* var. *transiens* OHWI

A. Mayebaranum and *A. tsukushiense* var. *transiens* are both hexaploid ($2n=42$), but their natural habitats are very different. The former is restricted to moist paddy fields or ditches, while the latter is found along road-sides and in fields.

In the sympatrical regions of both species in the suburbs of Misima, 12 clones of the natural hybrid between those two were found on the footpaths of the paddy fields. This hybrid found also in Fukuoka Prefecture, Kyusyu Island, has been referred by taxonomists to *A. Mayebaranum* var. *intermedium* HATUSIMA. Among the 12 clones 9 were non-waxy and very similar to each other, and the other 3 were waxy. One of the latter was a dwarf with short spikes. It was considered to be a hybrid with the ecotype mentioned above.

In 1956 artificial hybrids were very easily obtained, and the 17 F_1 plants were vigorous but completely sterile. The natural and the artificial hybrids were morphologically similar and resembled *A. tsukushiense* var. *transiens*,

but their spikes were not nodding when ripe due to complete sterility. Chromosome conjugation in meiosis of the natural and the artificial hybrids showed mostly 21_{II}. From this result it is assumed that both species have the same genomatic constitution. Complete sterility of the hybrid might be due to cryptic structural differences between the chromosomes of the two species. To throw light upon this point, cytogenetical studies are now in progress.

23. *Morphological and Physiological Variation among Aegilops squarrosa Strains Collected in Pakistan, Afghanistan and Iran*

(By Hitoshi KIHARA)

176 strains of *Ae. squarrosa* were collected along a route of over 6000 km in Pakistan, Afghanistan and Iran. The whole area, where *Triticum* and *Aegilops* were collected, is divided into 8 regions from geological as well as ecological considerations: Quetta, Kabul, Pulikhumri, Maimana, Teheran, Gorgan, Pahlavi and Tabriz. Morphological studies of these strains were undertaken mainly with plants cultivated in the experimental fields in Kyoto and Misima. Physiological investigations were made with a part of strains representing 8 regions.

Ae. squarrosa is a polymorphic species. Two subspecies, ssp. *squarrosa*, and ssp. *strangulata*, are clearly distinguished. Among ssp. *squarrosa*, there are known three varieties: *typica*, *anathera* and *meyeri*. There are many intermediate types between the first two, which are found almost in the whole area. *Meyeri*, which is morphologically an intermediate between the two, is found solely on the west coast of the Caspian Sea. Ssp. *strangulata* is localized along a narrow stretch of the south east coast of the Caspian Sea (Gorgan). Its occurrence along the route was estimated to cover 320 km. *Ae. squarrosa* was found very often in wheat fields. Therefore it is no wonder that we could find abundant spikelets of *Ae. squarrosa* in wheat grains sold as chiken feed. Such characters as erect culms and large grains, which can be found among cultivated wheats, are found only among *Ae. squarrosa* strains collected in wheat fields.

Many morphological variations were discovered. They are related to plant height, growth habit (procumbent or erect), waxy (non-waxy) leaves and colour of seedlings (red or green) etc. Variations in physiological characters were also found. They are related to resistance to some rust strains, earliness and habit, namely winter, intermediate and spring growth. As to these characters some local varieties seem to have adapted themselves to their habitats. For instance vars. *meyeri* and *strangulata*, which

are resistant to certain rust strains, grow in very humid places, along the coast of the Caspian Sea. All other regions are extremely arid.

Hybrids between strains from 8 regions and also hybrids between three old strains (Nos. 1–3) on one hand and 8 strains from 8 regions on the other were produced. All F_1 hybrids have shown 7 bivalents. Meiosis is normal. Fertility is normal in most cases. However some combinations show lower fertility. Hybrids of strains No. 2 give very often rise to higher sterility. Sterility of the intraspecies crosses seems to depend on the genotypes of the parents and partly on environmental conditions.

Most probably Iran is the center of diversities of *Ae. squarrosa*.

The results will be published in detail in collaboration with Mr. M. TANAKA, Laboratory of Genetics, Kyoto University.

24. *Karyotaxonomic Studies in Poaceae, V*

(By Tuguo TATEOKA)

1. The systematic placing of two grass genera was studied and their chromosomes were examined.

Brachyelytrum—This genus includes two species: *B. erectrum* Beauv. which is distributed in North America and *B. japonicum* Hackel found in Japan and Korea. Several investigators are of the opinion that *Brachyelytrum* is a member of Stipeae, while other investigators have placed the genus within Festuceae or Bromeae which are closely related to Festuceae. *B. japonicum* whose chromosomes have been examined by the author for the first time shows $2n=22$ in root tip cells, and the chromosomes are small. The chromosome features are very similar to those of Stipeae and clearly different from those of Festuceae or Bromeae. Also the characteristics of leaf structure show a relationship between Stipeae and *Brachyelytrum*. However, the spikelets of *Brachyelytrum* have distinct features by which the genus can be easily distinguished from Stipeae. Thus, it seems natural that *Brachyelytrum* should be treated as an independent tribe, Brachyelytreae, which ought to be placed near Stipeae.

Astrebla—This genus comprises four species which are endemic in Australia. According to BENTHAM (1881), GARDNER (1952), a.o., Astrebla may be referred to Chlorideae, but PILGER (1954) is of the opinion that the genus can be placed within Festuceae together with *Triodia*, *Plectrachne* and *Notochloa*. The present author has observed the somatic chromosomes of *A. lappacea* Domin and *A. pectinacea* F. Muell. Both species show forty small chromosomes in root tip cells. Since the basic chromosome number of 10 is often found in grasses, the chromosomes of *Astrebla* seem to be a multiple of 10. Not only in the basic number but also in the chromo-

some size, *Astrebla* is very similar to members of Chlorideae and clearly different from Festuceae. Further, in leaf structure, the Chloridoid subtype of Panicoid type (PRAT 1936) is shared by *Astrebla*. Thus, *Astrebla* should be linked with Chlorideae.

2. After BENTHAM's monumental treatise (1881), the system of Poaceae was submitted to studies by many investigators. The present author, also, has tried to elucidate the natural systematic grouping of grasses. Considering the features of chromosomes, leaf structure, external morphology, etc., as well as the geographical distribution, the author proposes to divide the grass family as follows. (cf. Journal of Japanese Botany 32: 275–287, 1957).

Subfam. Pharoideae
- Anomochloiformes—Trib. Anomochloeae
- Streptochaetiformes—Trib. Streptochaeteae
- Bambusiformes—Trib. Bambuseae, Trib. Olyreae, Trib. Streptogyneae
- Oryziformes—Trib. Phyllorachieae, Trib. Oryzeae
- Parianiformes—Trib. Parianeae
- Micrairiformes—Trib. Micraireae

Subfam. Arundoideae
- Coleanthiformes—Trib. Coleantheae
- Stipiformes—Trib. Brachyelytreae, Trib. Stipeae, Trib. Aristideae.
- Glyceriiformes—Trib. Meliceae, Trib. Glycerieae
- Nardiformes—Trib. Nardeae, Trib. Lygeeae
- Ehrhartiformes—Trib. Ehrharteae
- Arundiformes—Trib. Centotheceae, Trib. Phaenospermeae, Trib. Arundineae, Trib. Thysanolaeneae, Trib. Danthonieae
- Arundinelliformes—Trib. Garnotieae, Trib. Arundinelleae

Subfam. Pooideae (Festuciformes)—Trib. Festuceae, Trib. Monermeae, Trib. Triticeae, Trib. Agrosteae

Subfam. Eragrostoideae (Eragrostiformes)—Trib. Spartineae, Trib. Chlorideae, Trib. Lappagineae, Trib. Pappophoreae

Subfam. Panicoideae
- Paniciformes—Trib. Lecomtelleae, Trib. Boivinelleae, Trib. Anthephoreae, Trib. Melinideae, Trib. Trachyeae, Trib. Isachneae, Trib. Paniceae
- Andropogoniformes—Trib. Andropogoneae, Trib. Maydeae

25. *Fertility Variation due to "Gametic-Development Genes" in Hybrid Populations of Rice*

(By Hiko-Ichi OKA)

This report is an attempt to explain the change of segregation ratios and fertility variations observed in back-crosses and F_2 of two distantly related rice varieties, on the basis of the hypothesis of "Gametic-Development genes". The materials used are strains 414 (P.T.B. 10, Indica type, non-glutinous) and 563 (Kinoshita-mochi, Japonica type, glutinous), whose F_1 is semi-sterile.

First, it was found that reciprocal crosses did not differ significantly from each other in regard to the distribution of fertility in the F_2 and back-crosses (Table 1a).

Secondly, the non-glutinous gene was found to have remarkably increased against its glutinous allele both in F_2 and back-crosses. From the back-cross experiments, the gametic ratio in F_1 was found to be 80:44 or 1:0.44. In view of the fact that plants with low fertilities were mostly among heterozygotes for the glutinous gene, this change in segregation ratio seemed to be due to the G.D. genes (c.f. OKA 1953, Jap. Jour. Breed. 3: 31–39). Assuming that the parental strains 414 and 563 had genotypes $+-X_1x_2$ and $gl-x_1X_2$, respectively, the recombination fraction between + and X_1 or gl and x_1 was calculated to be 0.0645. The frequencies of various genotypes in the F_1 gametic series were computed from this value, and the frequency of semi-sterile plants (due to the G.D. genes under consideration) among hetero- and homo-zygotes for the glutinous gene was estimated to be 0.483 and 0.061, respectively. Assuming again that the fertility variation due to other causes than the G.D. genes is normal, the fertility distributions in hetero- and homozygous classes were calculated. They were found to be comparable with the observed distributions, as seen in Table 1b.

Regarding the F_2, the writer has formerly pointed out (OKA 1953, Jap. Hour. Breed. 3: 23–30) that pollen with a double dominant combination (X_1X_2) of G.D. genes tends to have a reduced fertilizing capacity (denoted by 1–s). Having computed the value of s from the F_2 ratio of ++:+gl:glgl plants taking the recombination fraction between gl and x_1 (p) to be 0.0645, it was found that s=0.836. The observed numbers of ++, +gl and glgl plants gave a good fitness to the calculated numbers thus obtained from these values of p and s. The fertility distribution in F_2 was also calculated by the same method as used in the case of back-crosses, and was found to agree with the observed distribution (Table 1c).

These experimental results may serve as an example of successful

Table 1. Distributions of good pollen percentage in hybrids between two distantly related rice strains, 414 and 563.

Cross	% of good pollen									No. of plants	Mean s(%)	Remarks
	100	90	80	70	60	50	40	30	20			
a) Comparison betweeen reciprocal crosses:												
414×563 F_2	13	12	10	6	4	3	2	3	1	54		$\chi^2=3.08$
563×414 F_2	6	11	10	4	2	4	6	2	1	46		$P>0.98$
(414×563)×563	4	9	4	12	10	9	3	4		55		$\chi^2=4.64$
(563×414)×563	4	3	4	5	11	3		1	1	32		$P>0.90$
b) Distributions in hetero- and homozygous classes fyr gl gene (in back-crosses $F_1\times563$):												
+gl Obs.	4	7	6	11	12	9	2	4		55	66.4	$\chi^2=3.60$
+gl Exp.	3.37	5.84	9.72	11.93	11.04	7.57	3.78	1.73		55	63.6	$P>0.50$
glgl Obs.	4	5	2	6	9	3	1	1	1	32	69.1	$\chi^2=9.38$
glgl Exp.	3.29	5.23	7.50	7.50	5.01	2.44	0.79	0.21	0.03	32	74.2	$P>0.05$
c) Distributions in F_2:												
++	7	5	5	2	2	3	1	2		27	76.2	
+gl	8	14	13	7	4	4	5	3	2	60	72.7	
glgl	4	4	2	1			2			13	82.3	
Total	19	23	20	10	6	7	8	5	2	100	74.9	$\chi^2=13.45$
Exp.	14.47	18.84	17.58	16.63	13.67	9.28	5.44	2.73	1.36	100	74.9	$P>0.05$

explanation of modified segregation ratios and accompanying fertility variations on the basis of the G.D. gene hypothesis.

26. *Back-Cross Experiment between Distantly Related Varieties of Rice —An Interaction between Maternal and Gametic Genotypes—*

(By Hiko-Ichi OKA)

Back-crosses were repeated between two rice varieties, 414 (P.T.B. 10, Indica type) and 504 (Taichung 65, Japonica type), using the latter as the recurrent parent. The F_1 between these two varieties showed 20% to 24% fertility in seed setting, and no significant difference was found between reciprocal crosses. In early generations of the back-cross, fertility ranged from about 10% to 80%, and plants with 30% to 50% fertility were relatively large in number. In later generations (B_5—B_6), most plants showed a 30% to 50% fertility, and the repetition of back-crosses did not bring about an improvement in fertility. In about 67% of B_5 plants, or 83.5% of B_6 plants, all chromosomes should be of the recurrent parent's origin. In fact, those plants had almost the same appearance as the recurrent

parent 504. Thus, the sterility persisting in those plants seems to suggest that cytoplasmic effect is involved, as has often been reported in this type of back-cross experiments with plant species.

Table 1. Fertility variations observed in a back-cross experiment in rice, $(414\times504)\times504\times504\cdots$.

Generation	% of seed setting									No. of plants
	90	80	70	60	50	40	30	20	10	
F_1							3	8	1	12
B_3 (8 lines)				2	14	32	35	12	6	101
B_5 (5 lines)				1	1	16	12		1	31
B_6 (6 lines)			4	3	14	6	10			37
$504\times B_5$ (6 lines)		2	3	3	12	5	1			26
B_5F_2 (6 lines)	19	18	11	4			1			53

However, having used the recurrent parent as the maternal plant in the back-crosses, the progeny, though it should have the same cytoplasm as that of the recurrent parent, was still semi-sterile. In contrast, if those semi-sterile plants were self-pollinated, the resulting plants were almost completely fertile without a remarkable segregation in fertility.

The above-given results can be simply explained by assuming that a pair of genes $A:A_1$ are concerned and if A is present in the tissue of the maternal plant, gametes with A_1 deteriorate. Thus, let the genotypes of parental varieties, 414 and 504, be AA and A_1A_1 respectively, the F_1, AA_1, will have a 50% sterility, and will produce gametes carrying only A. The gene $A:A_1$ may or may not be a group of linked genes. If it were a single locus, there might be many other multiple allelomorphs which do not bring about sterility in heterozygotes, and A_1 might have originated from those allelomorphs.

This is a hypothesis alternative to the Gametic-Development gene hypothesis (c.f. OKA 1953 Jap. Jour. Breed. 2: 217–224); the latter is essentially based on 1:1 segregation of fertile and semi-sterile plants in crossing-experiments of the design $(A\times B)\times C$, and change of segregation ratios accompanied by the predominance of semi-sterile plants among heterozygotes for the given gene pair. These phenomena may however be explained by the new hypothesis. The only phenomenon which the new hypothesis fails to explain is the restriction on recombination of independent genes in semi-sterile hybrids, while the G.D. gene hypothesis cannot account for the results of the present experiment.

In addition, the writer has suggested in the last issue of this Annual Report (for 1956) the presence of "duplicate fertility genes" by which

plants with certain recessive combinations have a low fertility. It seems that we have several different genic mechanisms which form barriers between distantly related rice varieties.

27. *A Preliminary Note on Moist Storage of Dormant Wild Rice Seeds*

(By Norindo TAKAHASHI and Hiko-Ichi OKA)

It is known in many species of plants that drying of fresh seeds is effective for overcoming their dormancy. In wild rices, however, seeds may fall on the ground at or before maturity, and may be kept wet in mud until they get a favorable condition for germination. The seeds may not have a period of drying for overcoming dormancy, but may need a surviving ability in soil. From this view point, germination of Formosan wild rice (*Oryzasativa sportanea*) seeds kept in moist conditions was studied.

1) Moist storage on filter paper in petri-dishes. Fresh seeds of Formosan wild rice lines (mentioned in this Annual Report for 1955) immediately after harvest, were laid on moist filter paper in petri-dishes, and were kept at 20°C. Few of them germinated within 30 days. The petri-dishes were then transferred to 30°C. 38% of those dormant seeds then showed germination within 24 hours. It was found further that, if the seeds were washed with tap water before transferring to 30°C, the germinating capacity was improved to be 63%. This may suggest that water washing removes some germination inhibitor or metabolite produced in seeds during the moist storage.

2) Incubation of dormant seeds in soil. Five lines of wild rice which appeared to have a strong dormancy and four cultivated varieties were used for this experiment. Fresh seeds of them were buried in loamy soil in pots at the depth of 5 cm, and the pots were or were not watered so as to provide the conditions of ordinary paddy or upland field in them. They were kept at 30°C or 20°C for 30 days. During this one month period of soil incubation, wild rices showed on the average only about 5% germination, while in cultivated varieties about 97% of seeds were germinated. Regarding the temperatures and soil conditions, it was found that under the watered paddy condition, 20°C gave higher germination percentages than 30°C, and under the upland field condition, on the contrary, 30°C gave higher germination.

The seeds which had not sprouted in soil were picked up, hulled, and were tested on moist filter paper at 30°C. They showed quick and complete germination within 24 hours. It may then be said that if dormant wild rice seeds were buried under ground, their dormancy would be removed, but they do not germinate for some reason and remain alive. This

behavior was particularly apparent when both the temperature and soil moisture were high. It seems that soil may remove some germination inhibiting substance in dormant seeds in the same manner as water washing of seees in moist storage, but another mechanism may keep them dormant so as not to exhaust them by immediate germination.

A comparison of germinating capacity of seeds kept under different conditions for 30 days is given as follows:

Incubation in soil (at 20°C)	100 %
Moist storage on filter paper (at 20°C)	38.4%
〃 , and washing with tap water	63.3%
Dry storage (at 20°C)	36.4%

F. CYTOLOGY AND GENETICS OF NICOTIANA AND SOME OTHER PLANTS

28. *Cytogenetic Studies on the Genus Nicotiana X*

(By Yô TAKENAKA)

The reduction divisions in PMC's were studied in 4 interspecific hybrids: *N. tabacum*×*N. otophora*, *N. tabacum*×*N. longiflora*, *N. gossei*×*N. tabacum* and *N. gossei*×*N. alata*.

1) F_1 of *N. tabacum* ($n=24$)×*N. otophora* ($n=12$)

At MI in PMC's of F_1 of *N. tabacum*×*N. otophora*, the number of bivalents and trivalents per cell was 12 in total. It then seems that the genome of *N. otophora* is the same as one of the genomes of *N. tabacum*. This finding is generally in agreement with GOODSPEED's data (1954) on hybrids between *N. otophora* and two varieties of *N. tabacum*.

2) F_1 of *N. tabacum* ($n=24$)×*N. longiflora* ($n=10$)

At MI in PMC's of F_1 of *N. tabacum*×*N. longiflora*, from 0 to 4 chromosome pairs were found, with the mode at 0–1. The frequency of PMC's with 2 bivalents followed that of PMC's with 1 bivalent and only univalents. PMC's with 3 bivalents were occasionally found and those with 4 bivalents were very rare. Considering such a small number of bivalents it is difficult to determine whether the few chromosomal affinities are allosyndetic between the genomes of the parents or autosyndetic between the 2 genomes of *N. tabacum*. However, KOSTOFF (1943) found usually 5 to 7 bivalents and rarely 8 or 9 bivalents in this hybrid combination. The cause of the difference between KOSTOFF's and my own results is unknown.

3) F_1 of *N. gossei* ($n=18$)×*N. tabacum* ($n=24$)

As far as I know, no report on this hybrid has been published. At MI of PMC's of this hybrid, the number of bivalents ranged from 0 to 5, with

the mode at 1. This pairing is probably due to autosyndetic affinities between the 2 genomes of *N. tabacum* which is of amphidiploid origin

4) F_1 of *N. gossei* ($n=18$) × *N. alata* ($n=9$)

The reduction divisions in PMC's of this hybrid were very irregular and appeared to degenerate. At prophase, thin chromatic fibres appeared around the nucleolus and soon gathered into a clump. Occasionally thin chromatic fibres spread over the nucleus. Then, in some cells, a large vacuole appeared in the nucleus which was partly covered by chromatic membrane. In other cells, a small chromatic sphere or ellipsoid was found in the nucelus but no vacuole. Only a very small number of cells showed chromosome-like bodies. Pollen grains in the anther were few and varying in shape and size. Moreover numerous powder-like cells and many non-reduction cells were observed. These observations indicate that *N. alata* is not related to *N. gossei*.

29. *Notes on Seedless Citrus Species*

(By Kazuo FURUSATO and Yasuo OHTA)

Investigations on seedless *Citrus* were carried out, and parthenocarpic fruits were found in species not yet reported as parthenocarpic. They are as follows:

1) *Citrus leiocarpa* Tanaka
2) *Citrus Hassaku* Hort.
3) *Citrus madurensis* Loureiro
4) *Citrus aurea* Hort., one strain, Kono No. 436, derived from a seedling of the species.
5) *Citrus grandis* Osbeck × *Poncirus trifoliata* Rafinesque
   Citrus grandis Osbeck × *Citrus Unshiu* Marcovitch
   2 fruits were set following the pollination of 4 flowers in the former cross and 1 fruit was set from 2 flowers in the latter cross. They were all seedless.
6) *Citrus nobilis* Loureiro
7) *Citrus Natsudaidai* Hayata
   Parthenocarpy occurred following phytohormone spraying of the flowers.

30. *Trifoliate Seedlings in Citrus*

(By Kazuo FURUSATO, Yasuo OHTA and Kenji ISHIBASHI)

In general, the three closely related Rutaceae genera, *Citrus*, *Fortunella* and *Poncirus*, are collectively called Citrus. Among them only one spe-

cies, *Poncirus trifoliata* Rafinesque has trifoliate leaves. Wnen pollen of this species is dusted on the stigma of other species which all have whole-blade leaves, trifoliate seedlings, presumably hybrids, are obtained, but their leaves show high variation in their shape and size among individual seedlings.

On the other hand, we obtained many trifoliate seedlings from selfing or open pollination as well as from pollinations among several species which do not have trifoliate leaves. They were: *C. Tamurana* Hort., *C. grandis* Osbeck, *C. aurea* Hort., *C. sulcata* Hort., *C. Hassaku* Hort., *C. Junos* Sieb., *C. Aurantium* L., *C. Unshiu* Marcovitch, *C. Funadoko* Hort. and *C. sp.* (Kaori-tachibana).

31. *Heteroploid Seedlings in Citrus*

(By Yasuo OHTA and Kazuo FURUSATO)

The occurrence of polyploid plants in *Citrus* has been reported by several authors. Heteroploid seedling, however, has been reported only one case. We found many heteroploid seedlings in *Citrus* which had 1 to 3 extra chromosomes besides the diploid number 18.

Among the seedlings of *C. Natsudaidai* Hayata, appeared a few (about 1%) with slender leaves in addition to some chlorophyll deficiencies as albina and others. Their growth was poor, and they often died in the earlier stages so that in many cases, no root tip could be secured for the examination of chromosomes. The somatic number of 51 plants with slender leaves were determined, and mixoploid plants were found which had cells with different chromosome numbers in the same root tip (Table 1). For the mixoploids the observed $2n$ numbers are arranged in the table in the order of their frequency. In Table 1 hetero- and mixoploids are grouped together. The table shows that about half of the seedlings were heteroploid, about 2/3 having 2x+1 and the remaining 1/3 2x+2 chromosomes. Only one plant was found with 2x+3 chromosomes.

Table 1. Heteroploid seedling in *C. Natsudaidai*

Chr. no ($2n$)	No. seedling	
18	23	25
18 19 20	2	
19	10	18
19 18	3	
19 18 20	1	
19 20	2	
19 20 21	1	
19 37	1	
20	4	7
20 18	2	
20 21 36	1	
21	1	1
Total	51	51

Similar seedlings with slender leaves have been obtained in other *Citrus* species, namely *C. sulcata*, *C. Tamurana*, *C. aurea* and *C. Kinokuni*. Heteroploid seedlings seem to be a frequent phenomenon in *Citrus*.

As to the heteroploids, it is not certain whether they were of nucellar origin. We can only say that two or more heteroploid

embryos were never found within one seed, but considering their high frequency, it seems that they may have some relation to polyembryony.

32. *Embryoculture in Citrus Natsudaidai*

(By Yasuo OHTA and Kazuo FURUSATO)

In most polyembryonic *Citrus* species hybrid embryos are usually small and morphologicall undifferentiated; they are unable to germinate if sown in the soil by ordinary methods. The present authors have attempted embryoculture with various culture media in order to induce germination of the so-called small embryos.

Experiment 1: Hydrogen-ion and sugar concentrations

To WHITE's (1943) solution 2% agar was added. The pH of the solutions varied from 5 to 7, and sucrose was added in 2, 4 or 8%. The arrangement of 12 experiment batches is shown in Fig. 1. The embryos of *C. Natsudaidai* Hayata were separated and classified according to their size into three groups, i.e. large (about as large as one half of the seed), medium and small (major axis of the embryo shorter than 1 mm). They were placed in the prepared media and kept in a thermostat at 30°C.

None of the small embryos germinated or became green. Neither did the majority of the middle size embryos germinate but a part of them became green. All of the large embryos became green and germinated, but their growth was markedly different, according to the kind of the culture medium used. The number of leaves, length of stem and main roots after 25 days are shown in Fig. 1.

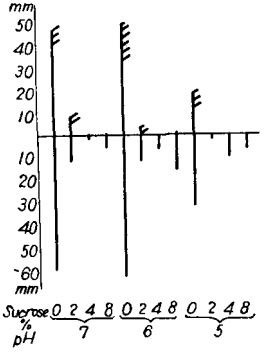


Fig. 1. Growth of Citrus seedlings in culture media of various pH and various sugar %, the diagram shows the length of stem (above) and root (below), and the flag-like appendages show the number of leaves.

These results are in accord with the situation in nature. Fruits of *C. Natsudaidai* in winter have a pH value of 5 and 11–14 % sugar, but

towards late spring and at harvest time their acidity gradually decreases. When they ripen and drop off, the content of sugars decreases and the seeds begin to germinate. Accordiogly, for the germinable large embryos a culture medium without sugar but at pH 6 is the most favorable.

Experiments 2: Minor substances

The embryos were separated and classified in two groups, large and small. They were placed in the culture media and kept in a thermostat at 30°C. The culture media contained the following minor elements in addition to the optimum medium used in exp. 1:

Naphtalen acetic acid (NAA)	100, 10, 1, 0.1, 0.01,	ppm
Vitamin B_1	10, 1, 0.1, 0.01, 0.001	〃
Vitamin C	100, 10, 1, 0.1, 0.01,	〃
2, 4-Dichlorophenoxyacetic acid (2, 4-D)	10, 1, 0.1, 0.01, 0.001	〃
2, 4, 5-Trichlorophenoxyacetic acid (2, 4, 5-T)	10, 1, 0.1, 0.01, 0.001	〃

None of the small embryos germinated or became green. It was considered, therefore, that the small and morphologically undifferentiated embryos are also phisiologically underdeveloped and unable to germinate with the addition only of the minor elements used. The large embryos became all green. Each of the elements used effected growth repression in higher concentrations and growth promotion in the optimum concentration. The results are shown in Fig. 2.

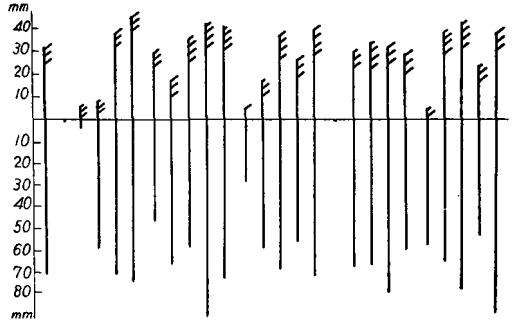


Fig. 2. Growth of Citrus seedlings in culture media added with various kinds and concentrations of minor elements. The diagram shows the length of stem (above) and root (below), and the flag-like appendages show the number of leaves.

In the case of Vitamin B_1 the maximum growth was found at a concentration of 0.01 ppm, and the number of leaves in this case exceeded that of control. This phenomenon agrees with WENT's report (WENT a.o. 1938) that Vitamin B_1 is necessary for the growth of roots in Citrus and its optimum concentration is 10^{-7} mol.

When 0.01 ppm of Vitamin C was applied, the growth was excellent. Considering the leaf number, Vitamin C may be one of the promising elements promoting the germination of embryos and the growth of

seedlings in *Citrus*. This seems to have some relation to high content of Vitamin C in *Citrus* fruits.

Thus the so-called small embryos were never germinated. This indicates that the small embryos are not only morphologically but also phisiologically underdeveloped and cannot germinate with only the help of the minor elements used in our experiments.

As to the larger germinable embryos, the growth of the seedlings was vigorous when the culture media had no sugars and contained Vitamin B_1 and C in adequate concentration pH 6.

33. *Study on Fiber of tetraploid Luffa cylindrica Roem*

(By Kazuo FURUSATO)

4x plants of *Luffa cylindrica* Roem (var. Turukubi) were obtained by colchicine treatment. The fiber of 4x was proved to be thicker and heavier in weight than that of 2x. However, the strength of the fiber was almost the same in 4x as in 2x in dry condition.

34. *A New Type of Bud Variation in Citrus*

(By Kazuo FURUSATO)

A bud variation found in Citrus Unsihu in 1949 was quite different from the many bud mutations reported up to now for Citrus.

The branch which gave rise to the observed spontaneous variation was found on a 40 years old tree.

From its apex, in spring, many long twigs sprouted forming a nest similar in appearance to a witches broom of cherry tree. In winter the whole structure some time dries up but in spring the same abnormal growth is resumed.

The leaves are lighter green and smaller than on the normal branches. Flowering was not initiated.

The abnormal branches were grafted on a Navel orange tree as stock.

Photo. 1.
Bud variation in *Citrus*.

The scion retained the abnormal growth and even flowered but the stock remained unaffected. But when again another precocious Citrus Unsihu was grafted on the normal scion as stock, some effect upon the former could be noticed.

The cause of the variation is not yet known. The abnormal growth has the character of the so-called Inebakanae disease of rice, caused by gibberellin. (Photo. 1.)

35. *Cytological Studies in the Genus Euphorbia. II. Chromosome numbers of twenty European species*

(By Shohachi SHIMOYAMA)

The large genus *Euphorbia* includes about 1,600 species which are distributed in sub-tropical and temperate regions all over the world. They represent a natural group, although various differentiations can be found among them. In 1957, the chromosomes of twenty European species were examined, and the results are given in Table 1.

Studies on interspecific hybridization are now in progress.

Table 1. Chromosom numbers of twenty European *Euphorbia* species.

Species	*n*	2*n*	Source
Euphorbia dulcis	—	12	Hungary
E. bivoniae	—	24	Austria
E. pubescens	—	14	Hungary
E. verrucosa	7	14	Germany
E. gregersenii	—	14	Sweden
E. polychroma	—	14	Hungary
E. polychroma	—	16	Germany
E. geniculata	—	28	Hungary
E. plathyphyllos	—	28	Germany
E. exigua	—	28	France
E. exigua	—	56	England
E. virgata	—	56	Germany
E. heterophylla	—	56	Germany
E. segetalis	8	16	Germany
E. lagascae	8	16	England
E. pilosa	9	18	Germany
E. amygdaloides	9	18	Austria
E. dendroides	—	18	Austria
E. Lathyris	10	20	Belgium
E. myrisinites	—	20	Belgium, Germany
E. Helioscopia	—	42	Belgium
E. esula	—	64	Germany

36. *Chromosome Studies in Japanese Callitriche*

(By Shohachi SHIMOYAMA)

The genus *Callitriche* is a single representative of *Callitrichaceae*. The genus includes about 30 species, among which only two are native to Japan. Several species of *Callitriche* have been studied cytologically by JÖRGENSEN (1923), SOKOLOVSKAJA (1932), LÖVE and LÖVE (1942) and SCHOTSMAN (1954). They have found the basic chromosome numbers of 3 and 5. The present author has investigated the Japanese *Callitriche*, and the results which have been obtained up to now are reported in this paper.

(1) *Callitriche japonica* Engelem

Callitriche japonica Engelem is endemic in Japan. The materials for cytological studies were obtained from different localities: i) Misima, Shizuoka Prefecture, ii) Odawara, Kanagawa Prefecture, ii) Tokyo and iv) Tomioka and Shimonita, Gumma Prefecture.

The chromosome number of all plants examined is 10 in the root tip cells without exception, and the somatic chromosomes are of the following kinds: two large chromosomes with median centromere, six similar medium sized chromosomes and two small chromosome characterized by a subterminal centromere.

The karyotype can be represented by the following formula.

$$K(2n)=10=2A^{m}+6B^{m}+2C^{st}$$

No meiotic irregularities have been found and all plants examined have clearly shown five bivalents at first metaphase.

(2) *Callitriche verna* L.

The materials were collected in rice-fields near Misima City (locatities—Yata, Kawaragaya, Takuchi, Aoki and Shimizu-mura). Their chromosomes were $2n=20$ in root tip cells and 10 II in the pollen mother cells without exception. JÖRGENSEN (1923) has counted $2n=20$ in Danish plants belonging to this species, and the same number has been also reported for Russian plants by SOKOLOVSKAJA (1932). Recently, SCHOTSMAN (1954) examined Dutch materials and observed $2n=20$ in root tip cells.

All of the chromosomes of this species have a subterminal centromere, and they can be divided into three groups according to size; four large (A), fourteen medium-sized (B) and two small chromosomes (C). Two of the four large chromosomes have each a satellite on the short arm. The karyotype can be expressed as follows:

$$K(2n)=20=2^{t}A_{1}^{st}+2A_{2}^{st}+14B^{st}+2C^{st}$$

Plants collected at Shimizu-mura have no satellite, and their karyotype

formula is as follows:

$$K(2n)=20=4A^{st}+14B^{st}+2C^{st}$$

In most materials meiotic divisions are entirely normal. But some chomosome irregularities, owing to chromosome stickness have been observed.

G. BIOCHEMICAL GENETICS OF FLOWERING PLANTS

37. *Leuco-anthocyanin in Variable Flowers of Camellia japonica*

(By Toru ENDO)

Leuco-anthocyanins are a group of compounds easily converted to anthocyanidin after heating with mineral acids. It is unknown, however, whether those compounds are immediate precursors of anthocyanins *in vivo*. BATE-SMITH (1953) suggested that leuco-anthocyanin was present in white flowers of *Camellia*. The present author found that the flowers of *C. japonica* contain leuco-anthocyanin which yields cyanidin irrespective of the flower color, while the red flowers contain in addition cyanidin-glycoside.

A commercial variety of *C. japonica*, Somekawa, usually has variegated flowers, but frequently produces red self-colored flowers or branches, possibly due to the reversion at different stages of a recessive gene effecting white flower color. Out of 176 flowers observed, 66 were self-colored, two were mosaic and the remaining 108 were variegated. This variety of *Camellia* seemed to be a good material for studying the action of genes controlling anthocyanin biosynthesis, since all flowers should have the same genotype except for the mutable gene.

The relative concentration of anthocyanin and leuco-anthocyanin in flowers having different color types was estimated by spectrophotometrical method. The red flowers contained about ten times as much anthocyanin as the variegated ones. Since its spectrum was similar to that of catechin, leuco-anthocyanin was measured together with other catechin-like substance. The catechin-like substance in red flowers was about 40 % more than that in variegated ones.

In addition, because of the known importance of copper enzymes in polyphenol systems, the copper enzyme activity in red and variegated flowers was estimated by manometrical methods. The results are given in the following table.

As shown in the table, no significant difference was found between red and variegated flowers in oxygen-uptake thus estimated. It appears

Table I.

Substrate	Concentration	Participating enzyme	Enzyme activity	
			variegated flowers	self-colored flowers
p-Cresol	8×10^{-3}M	Polyphenolase	15.7	7.8
Catechol	2×10^{-3}M	Polyphenolase & Laccase	20.2	20.9
p-Phenylenediamine	6×10^{-3}M	Laccase	19.0	15.7
Ascorbic acid	6×10^{-3}M	Ascorbase & Laccase	68.0	75.0

that copper enzymes, if involved in any way, participate rather indirectly than directly in anthocyanin formation.

38. *F_2 Segregation of Flower Color in Inter-varietal Crosses of Swiss Giant Pansy*

(By Toru ENDO)

Eleven crosses were made among seven varieties of Swiss Giant Pansy, Mt. Blanc (White), Rhinegold (yellow), Rasberry Rose (reddish purple), Fire Beacon (yellowish red), Alpenglow (deep red), Lake of Thun (light purplish blue) and Berna (deep purple). One of the crosses (Mt. Blanc×Rhinegold) was between two acyanic varieties, while the remaining ten were between acyanic and cyanic ones. The present report deals with their F_2 progeny, 1016 plants in total.

The color pattern of the pansy flower is classified by the relative amount of three pigment systems, i.e. carotenols, red anthocyanins (mainly composed of cyanidin-glycoside), and blue anthocyanins (delphinidin-glycoside). However, regarding each pigment system, a continuous array of intergrades in color tone was found among the F_2 plants. This seems to indicate that each pigment system is controlled by a polymeric or polygenic system.

The following results of observations may serve as evidence for the above assumption:

1) In the F_2 of Mt. Blanc×Rhinegold, the range of variation in flower color seemed to be roughly compared of 33 yellow, 84 pale yellow, and 36 faintly yellowish, though on account of the continuous nature of the variation the classification could not be a distinct one. In this case, it was found that none of the F_2 plants had the same amount of carotenol pigment as the Rhinegold parent.

2) The F_2 of Mt. Blanc × Raspberry Rose appeared to segregate into 42 reddish purple, 15 reddish purple with white stripes, 35 faintly reddish purple and 35 white with reddish purple parts at the periphery of the petals.

3) The F_2 of Mt. Blanc × Alpenglow segregated into 20 pale yellow, 56 reddish purple (which might have a smaller amount of carotenol than Alpenglow) and 16 purplish in which anthocyanins were localized at the outer parts of the petals.

4) The F_2 of Mt. Blanc × Lake of Thun segregated into 69 blue pale blue, 9 blue with white stripes and 3 white which were similar to Mt. Blanc.

5) The F_2 of Mt. Blanc × Berna segregated into 65 deep purple, 8 bluish purple and 7 reddish purple.

6) The F_2 of crosses between Rhinegold (acyanic) and five cyanic varieties showed approximately the same pattern of segregation as those between the cyanic varieties given above, though the number of segregants with acyanic flower color was larger than in the above crosses.

7) The F_2 of Rhinegold × Berna segregated into 46 deep purple, 59 deep purple with carotenol in some parts of the petals, and 20 yellowish. In this case, it seems that the carotenol pigments inhibits the formation of anthocyanin to some extent.

H. POPULATION GENETICS AND STUDIES ON QUANTITATIVE CHARACTERS

39. *Genetic Analysis of a White Leghorn Closed Flock Apparently Plateaued for Egg Production**

(By Yukio YAMADA)

I. Time-trends in the means of the population.

The population has been selected for high rate of egg production based primarily on family average since 1946. The scale of egg production was transformed to angle from percent production to January 1, as a routine in analysis of percentage data. The means of the flock were gradually improved year by year until 1952. Afterwards the means in 1954 and 1956 dropped sharply, even though the means in 1953 and 1955 returned to the same level as the highest mean.

The regression of performance on years was calculated and is given in

* This study was carried out at the Department of Poultry Husbandry, Purdue University, and details will be published in Poultry Sci., 37.

Figure 1. The linear regression was significant at 5 % level. The second degree regression was also significant. This suggests that the rate of improvement was high in the early generations reaching a maximum in 1952 or 1953, after which the means may have decreased. A severe attack of respiratory infection occurred in 1956 resulting in lowered production. On the contrary, the means of all strains kept at the same farm were extraordinarily high in 1952. These years could be considered exceptional from the standpoint of environment and may account in part for the significant curvilinearity of regression.

Considering all evidence available, it would appear that the curvilinear trends, as shown in Figure 1, may be exaggerated from the genetical point of view. The linear regression may be considered as more nearly representative of the time-trends of the means. Nevertheless, the means in later generations have not increased, indicating that a second degree regression may actually provide the best fit to the genetic means of the population. It must then be tentatively concluded that the population has reached or is approaching a genetic plateau in performance in this population.

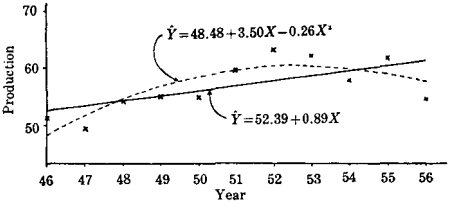


Fig. 1. Time-trends in the means of the population.

II. Time-trends in heritability estimates.

The magnitude of the heritability in a population provides a measure of additive genetic variability which determines the potential for changing population means by selection. It has been postulated that heritability would decrease during the course of selection in a closed flock, due to loss of genetic variation by fixation through selection, and also because of increasing homozygosity from inbreeding, which is inevitable in a population of small size.

The pooled heritability estimated from different components are 0.137 0.239 and 0.188 from sire, dam, and combined components, respectively. The annual estimates obtained by the variance component technique within years fluctuate greatly from year to year. So as to evaluate time-trends in heritabilities, the regression of heritability on year of hatch was calculated. This procedure requires the assumption that there is a linear relationship between heritability and year, and that the heritabilities distribute normally at random around the estimated value. Regression coefficients are listed below, with heritability estimated from the regressions for two years, 1947 and 1956.

Source	Regression coefficient	t	Probability	Heritability predicted in 1947	1956
h^2_S	$-.033\pm.017$	1.93	$.10>P>.05$	.305	.010
h^2_D	$-.024\pm.011$	2.27	$.10>P>.05$	.373	.153
h^2_{S+D}	$-.029\pm.011$	2.65	$.05>P>.02$	.340	.079

Only the regression coefficient for heritability from combined component is statistically significant. Those for dam and sire components approach significance. Although little reliance can be placed on the decline of heritability from the sire and dam components alone, the fact remains that the coefficient of regression for heritability based on the sire component was larger than that from the dam component. This would suggest a decrease in the additive portion of the genotypic variance, and could serve as contributory evidence for the hypothesis that the additive genetic variance will decrease by selection, while, the rest part of the genotypic variance resulting from non-additive gene effects remains constant.

Heritability obtained from selection experiments based on averaged annual selection differential and the annual change in the population mean was estimated to be 0.08. Compared with those obtained from variance components, the value seems quite small. Pooled estimate from sire component, however, was estimated to be 0.137 which agree fairly well from the view point of practical breeding, compared with values obtained by other components.

III. Intended and realized selection intensities.

Selection differential is defined as average superiority of selected birds over the unselected population from which they were chosen. It was calculated separately for cockerel, pullet and yearling-hen breeders at different stages of reproductive cycles, as follows:

(I_1) Intended or initial selection was measured as average superiority of the mean of birds selected as breeders over the mean for all birds in the unselected population from which the breeders were chosen. Modification of intended selection due to any association of the performance of each selected bird with (I_2) the number of eggs set from its mating during the breeding season, (I_3) the number of chicks hatched from its mating, (I_4) the number of pullet progeny housed, and (I_5) the number of pullet progeny completed their records. The last one is the realized selection. Each selection differential under (I_2)~(I_5) was the weighted means of individual or family superiority over the mean of all birds of

the unselected population.

Automatic selection due to association with family size was calculated as the mean of all progeny minus the corresponding mean of all full-sisters' average. This is the selection automatically applied because the larger the family size the more the opportunity of selecting the birds to be breeders.

Automatic selection gave a positive value which means that the larger families have a greater opportunity for birds to be selected from them than do smaller families, at least to some extent in this population.

There seems to have a tendency of intensifying family selection for cockerels and pullets until the stage of progeny housed. Selection for yearling-hens reached its maximum at the stage of chicks hatched, afterward it reduced to the level of initial selection. As the male breeders were mated to random samples of selected females, it is reasonable to find no appreciable changes in the selection differential at the stage of eggs set. After this stage, however, deliberate selection could be changed, at least to some extent, because fertility, hatchability and vaibility of progeny would be influenced by male breeder's genotypes. Selection at stages of chicks hatched and of pullets housed favored to the male breeders of superior family record. Realized selection exceeded that of intended.

Changes in family selection at the stage of eggs set were larger in yearling-hens than in pullets. Fertility or hatchability was not affected in pullet selection but did in yearlings. Viability favored a little to the birds which were descendants of superior families in pullets but not in yearlings.

Selection within family in pullets reached its maximum at the stage of chicks hatched, but selection intensity in yearlings decreased successively until the last stage of reproductive cycle. The ratio of realized to intended was 0.75. Superior yearling-hen breeders had poor hatchability and they might transmit poor genotypes of viability to their offspring.

Selection in males was stronger than that in females and that in yearling-hens was more intense than in pullets. Selection between families was much stronger than that for individual selection within families. The tendency was very much so in yearling hen breeders.

There was no systematic tendency of the changes in the ratio of realized selection and intended one during the selection experiment in which the population had reached a plateau in later half. Intended selection was realized as intense as it was deliberated.

IV. Effects of inbreeding.

During ten years of breeding the average increase in coefficients of

inbreeding among all pullets was approximately 2 percent per year, although full-sibing was avoided. It has been generally recognized that rapid or intense inbreeding leads to a decline in fitness. Therefore, it would be reasonable to expect that inbreeding in this population would be seriously opposed to the direction of selection.

The technique of regression was used for determining the effect of inbreeding on performance and size of family. Compulation was carried out on a within-year basis since this removed the between-year environmental effects on the trait, if there was any.

Of the 10 years considered, only one coefficient of regression of family average egg production on inbreeding percentage reached statistical significance. The pooled regression was slightly positive but did not approach significance. On the other hand, one regression coefficient of family size was significant and pooled regression gave negative value which did not reach any significance level. An additional analysis of the effect of inbreeding on survivor's production was conducted within-sires within-year since this would minimize the non-genetic bias. The result showed that the alternate method has changed the result only slightly and gave the negative sign for the pooled regression which still was not statistically significant. Therefore, it seems clear that inbreeding effect was not seriously opposing the selection applied in this population if at all.

V. Analysis of effectiveness of selection.

Since selection has been based on superiority of individual, family and progeny records in this flock, the expected genetic gain per year may be expressed as follows:

$$\Delta G=(P-F)h^2_{w/n}+(F-\overline{P})h^2_{fa}+(O-\overline{P})h^2_{fa'}$$

where P, F, O and $\overline{P}$ are the individual, full-sisters' average, offspring average and the mean of a population, respectively. The $h^2_{w/n}$, h^2_{fa} and $h^2_{fa'}$ are the heritabilities corresponding to each selection differential.

For the convenience of calculation, above formula could be changed as follows:

$$\Delta G=(\Delta S/2)h^2_{fa'}+(\Delta D_p/2)h^2_{w/n}+(\Delta D_F/2)h^2_{fa}+(\Delta D_o/2)h^2_{fa'}$$

where ΔS, ΔD_p, ΔD_F and ΔD_o are average realized annual selection differentials for sires and dams.

The results showed that the annual gains realized were not found to be significantly different from expected gains predicted on the basis of the annual selection differentials and heritabilities estimated from the sire component of variance ($P>.20$). On the other hand, when the expected

values were based on heritabilities from combined sire and dam component, the differences were significant ($P<.05$). Thus, the results of selection in this population cannot be considered to differ from theoretical expectations.

It is reasonable to assume that slight progress could still be made in this population, since a small amount of genetic variance still remains. However, such progress would become increasingly difficult to obtain as indicated by recent generations. Therefore, it is questionable whether further selection in this population would be worthwhile without some procedure for increasing the proportion of additive genetic variance.

40. *Observation on Natural Populations of Formosan Wild Rice Particularly on Variations in Seed Dormancy*

By Norindo TAKAHASHI and Hiko-Ichi OKA)

In this Annual Report for 1955, one of the writers (OKA) has pointed out that the Formosan wild rice (*Oryza sativa spontanea*) might be heterogeneous and heterozygous for genes controlling various characters. This report deals with variations in seed dormancy and other several characters as a continuation of the former report. Populations A and B, whose habitats seem to be similar ecologically but are apart from each other by about one kilometer, were investigated. Plants taken at random from the natural populations were grown in 1955 in Misima, and their progeny lines, 88 in total number, were grown again in this year. All of the plants came to heading in September, but only about one third of them could reach maturity on account of low temperatures in October in Misima.

Lines from both populations varied in various morphological characters. The extent of variability within population (C.V.), and the difference between the two populations as compared with the within population standard deviation (t), were as follows:

	Growing period (days)	Anther length	Awn length	Ligule length	Tiller angle	Lth/Width ratio of grain
t*	1.61	3.16	1.91	0.42	0.33	3.26
C.V.(%)**	10.4	15.1	49.8	23.0	17.4	8.1

* t value for the difference between population A and B.

** Variability within population in terms of the coefficient of variability.

For investigating the variation in seed dormancy, germination was tested immediately after harvest, and the remaining seeds were kept in

a desiccater with $CaCl_2$ at 20°C for further tests. The germination tests were made under three temperature conditions, i.e., 30°C, 20°C, and daily alternation between 30° and 20°C. Tests at 30°C generally showed higher percentages of germination than those at 20°C, and the alternation of temperature gave the same results as 30°C. However, the degree of dormancy was shown conveniently by the percentage of germination at 20°C of seeds stored for 20 days after harvest. The results are given in Table 1.

The table shows that a wide range of variation in seed dormancy exists in both populations, and population B tends to have stronger dormancy than A. It is apparent in the table that most of these wild rice lines have much stronger seed dormancy than usual cultivated rice varieties, but a few of them seem to come to the extent of cultivated varieties.

Further, correlations between the degree of seed dormancy and other characters were investigated. It was found that the degree of dormancy was significantly correlated with heading date ($r=-0.437$) and with ligule length ($r=0.323$).

Table 1. Variations in germination percentage of dorman seeds of Formosan wild rice (within 30 days).

Population	% of germination										No. of lines
	5	15	25	35	45	55	65	75	85	95	
A			1	1	1		4	5	3	2	17
B	1	2	3	1	2	1	2	3	1	4	20

41. *Genetical Studies on the Cherry-Red Leaf in Tobacco Plants*

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

In the same manner as in the last year, three lines from each of six line-groups were raised for the observation of cherry-redness in leaves. The experiment was designed according to the randomized block method with two replications, each plot containing 12 plants (First experiment). In addition, an experiment with F_1- and F_2-hybrids between lines with high and low cherry-redness (2 cross-combinations) was made with three replications, each parental, F_1- and F_2-plot containing 30, 15 and 45 plants, respectively (Second experiment). Five top leaves were sampled from each plant, and after drying, were classified into five classes, 0 to 4, by visual judgement. The index-numbers 0 to 4 were then weighted by the weighting formula which was calculated from the data of the first experiment, to maximize the ratio of between-plant-variance to within-plant-

variance. The formula obtained for this year was

$$X=0x_0+0.256x_1+0.451x_2+0.809x_3+1.000x_4\,,$$

where x_i represents the number of leaves falling on the i-th class of cherry-redness.

Analysis of variance of the score X in the first experiment showed that the variation among line-groups was significant but that within group was insignificant (Table 1). The heritability value for line means computed from variance components was 0.699. With the data for the last three years (1955–57), analysis of variance was made again (Table 2).

Table 1. Analysis of variance of cherry-redness in 18 lines of Bright Yellow tobacco (1957).

Source of variation	d.f.	Mean square
Replication	1	0.050,161
Line-group	5	0.142,177**
Line within line-group	12	0.014,630
Error	17	0.017,853

** Significant at 1 % level.

Table 2. Analysis of variance of cherry-redness with the pooled data for 1955, 1956 and 1957.

Source of variation	d.f.	Mean square
Line	17	0.200,218**
Year	2	0.101,857**
Year × line	34	0.015,867
Error	72	0.016,575

** Significant at 1 % level.

Table 2 shows that the variance among lines was significant as expected from the results obtained in each of the three years, but the interaction between year and line was insignificant. Further, the correlation coefficient between line means in 1956 and in 1957 was as high as 0.876, and was highly significant. It may then be safely concluded that the cherry-redness is primarily genetically controlled.

The results of the second experiment are given in Table 3.

Table 3 shows that the occurrence of cherry-red leaf in F_1 is at the same level as that in the parent with higher occurrence, and the F_2 mean

Table 3. Comparison between F_1 and F_2 of reciprocal crosses regarding cherry-redness. (1957).

Line	Cherry-redness	Average of reciprocals	Line	Cherry-redness	Average of reciprocals
(1)	0.321		(1)	0.321	
(7)	.598		(15)	.595	
F_1 (1)×(7)	.606	.611	F_1 (1)×(15)	.622	.582
F_1 (7)×(1)	.615		F_1 (15)×(1)	.541	
F_2 (1)×(7)	.624	.639	F_2 (1)×(15)	.574	.537
F_2 (7)×(1)	.654		F_2 (15)×(1)	.500	

is near to the F_1 mean. Thus, cherry-redness appears to be dominant in these crosses.

Correlation between cherry-redness and the quality of tobacco leaf was investigated using the data of the first experiment. The quality of leaves was evaluated irrespective of cherry-redness for this purpose. The phenotypic correlation was 0.404 (significant at 1 % level), and the genetic and environmental correlation coefficients were computed to be 0.085 and 0.326, respectively. In view of the low genetic correlation coefficient, cherry-redness may be regarded as a character independent of other factors relating to quality in selection. The relatively high values in phenotypic and environmental correlations might be due to the tendency to understimating the quality of a leaf if it showed cherry-redness.

42. *Genetic Correlations of Agronomic Characters in Rice*

(By Shin-ya IYAMA)

Genetic and environmental correlations between various agronomic characters were estimated from the analysis of components of variances and covariances. The following two groups of materials were used.

Group 1. 19 varieties cultivated in Japan. The experiment was designed according to the randomized block method with six replications. Plant height, panicle length, tiller number, panicle number, plant weight, panicle weight per plant and 100 grain weight were investigated. The genetic and environmental components of variances and covariances were estimated from the data, and correlation coefficients were calculated as given in Table 1. The correlations between these characters and grain yield in three-plant-per-hill cultivation are also shown in Table 1.

As shown in Table 1, tiller or panicle number showed a positive genetic correlation with yield, but panicle length showed a negative one. This may imply that among the Japanese varieties used, the so-called " panicle-

number types" are high yielders. However, varieties with many panicle tend to have a small grain weight, judging from the genetic correlation. In addition, positive genetic correlations were found between panicle length and plant height, and between panicle number and tiller number. Genetic correlation between panicle length (or plant height) and tiller (or panicle) number was negative, but the environmental correlation was positive, resulting in non-significant phenotypic correlations.

Grain yield of the varieties in three-plant-per-hill cultivation showed a significant positive correlation with panicle weight per plant. This means that selection from single planting should result in obtaining strains which will have a high yielding capacity in the usual planting conditions.

Table 1. Genetic and evironmental correlation coefficients in rice. (Genetic correlation coefficients are above the diagonal and environmental ones below the diagonal. Correlation coefficients from Group 1 are given in the upper lines and those from Group 2 in the lower lines.)

	Plant height	Panicle length	Tiller number	Panicle number	Plant weight	Panicle or grain wt./plant	100 grain weight	Yield†	Grain+ shape
Plant height		0.594 .595	−0.334 .088	−0.363 .080	.179 .544	.034 .235	.042 —	−0.066 —	— .088
Panicle length	.159 .337		−0.420 −0.147	−0.423 −0.164	−0.206 .285	−0.309 .201	.317 —	.301 —	— .252*
Tiller number	.347 .555	−0.043 .281		1.000 .996	.657 .448	.529 .090	−0.325 —	.493* —	— .177
Panicle number	.594 .566	−0.045 .212	.948 .947		.583 .409	.513 .083	−0.318 —	.487* —	— .122
Plant weight	.458 .674	.124 .415	.707 .837	.712 .869		.961 .643	−0.057 —	.743** —	— .264**
Panicle or grain wt./plant	.493 .448	.151 .257	.561 .524	.626 .582	.803 .699		.147 —	.833** —	— .168
100 grain weight	−0.076 —	.013 —	−0.024 —	−0.020 —	.144 —	.148		.215 —	— —

\+ Phenotype correlation based on line means.
† Yield per plant of three-plant-per-hill cultivation.
* Significant at 5 % level. ** Significant at 1 % level.

Group 2. F_7 lines from Omachi × TeTep. 112 lines were taken at random from the F_6 bulk population of the cross. They were tested in an experiment arranged according to the randomized block design with three

replications. Genotypic and environmental correlations among various agronomic characters were estimated by the same method as used for Group 1. The results are also given in Table 1.

Table 1 shows that there are essentially the same tendencies as found in Group 1. But, there are some important differences in that plant height or panicle length shows a positive genetic correlation with grain yield per plant, and does not show a negative genetic correlation with panicle or tiller number. This seems to suggest that in Japan, such types of rice as showing dwarf habit and high tillering ability have been selected, resulting in the absence of correlation between panicle length and yield, and a negative correlation between tiller number and panicle length. These features may however be limited only to Japanese rice varieties. Considering the whole pattern of variation among rice varieties, both tiller number and panicle size are important as yield components.

Finally, regarding the shape of grain, Omachi has apparently shorter grain than TeTep (length/width ratio were 2.17 and 2.95 for Omachi and TeTep respectively). A wide variation in grain shape was found among the F_7 lines. The correlations found between grain shape and other characters indicate that lines with a long grain tend to have a long panicle and heavy plant weight. But grain shape showed no correlation with yield.

Heritability values: Based on the values of genetic and environmental variances obtained from the above experiments, heritability values for various characters were computed as given in Table 2. There were no marked differences in heritability values between Groups 1 and 2.

Table 2. Heritability values of the characters investigated.

Group	Heritability for	Plant height	Panicle length	Tiller number	Panicle number	Plant weight	Panicle or grain wt./plant	100 grain weight	Yield*
1	Plant	.460	.348	.393	.372	.280	.172	.500	.079
	Plot mean	.809	.748	.851	.840	.740	.646	.775	.523
2	Plant	.363	.247	.245	.158	.137	.061	—	—
	Plot mean	.899	.837	.771	.746	.713	.362	—	—

* Grain yield per plant of three-plant-per-hill cultivation.

43. *Migrating Activity in Inbred Lines derived from Two Wild Populations of Drosophila melanogaster*

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

Two wild populations of *Drosophila melanogaster*, viz. KN and TB popu-

lations (for details see the last issue of this report), which differed markedly from each other with respect to migrating activity were used in this study. Fifteen inbred lines have been derived from each of the two populations. They were investigated as to their migrating activity in every inbreeding generation. The method of investigation consisted in counting the number of flies migrated from the original tube 24 hours after the establishment of an experimental set of population-tubes. The

Table 1. Migrating activity of 12 inbred lines derived from two wild populations of *Drosophila melanogaster* in sixteen consecutive generations of inbreeding.

Inbred lines		Migrating activity in 16 inbred generations								
		1,2	3,4	5,6	7,8	9,10	11,12	13,14	15,16	Average
KN	A-2	50.63	45.63	28.05	37.92	40.63	37.71	49.38	61.88	43.98
	B-1	53.13	41.46	60.21	77.71	48.34	63.96	64.58	64.58	59.25
	C-3	42.71	33.34	25.83	58.96	44.79	56.88	57.71	65.00	48.15
	D-2	44.79	49.59	55.04	72.09	30.84	36.25	27.09	31.46	43.39
	D-3	71.46	60.21	80.80	49.38	45.21	48.75	52.50	39.59	55.99
	F-2	27.92	58.75	37.09	55.63	35.21	27.71	23.34	39.38	38.13
	H-1	22.88	45.21	18.34	43.00	23.96	25.83	25.84	16.25	27.66
TB	A-1	50.42	26.46	28.33	16.88	25.00	22.09	18.54	25.83	26.70
	B-3	18.75	20.84	50.63	50.84	36.46	42.30	46.25	41.67	38.47
	C-2	24.59	48.54	49.58	64.38	43.54	56.46	41.88	51.04	47.50
	C-3	27.50	49.58	49.79	66.63	43.96	66.25	57.29	77.50	54.81
	F-1	27.83	17.08	37.09	39.79	21.88	18.34	14.59	22.29	24.86
Average		38.55	41.39	43.40	52.77	36.65	41.88	39.92	44.71	42.41

Table 2. Analysis of variance of migrating activity of twelve inbred lines derived from two wild populations for the last seven inbred generations from 10th through 16th.

Source of variation	d.f.	Mean square
Generation	6	0.236,157**
Line	11	.534,690**
Betw. population	1	.042,287
Within population	10	.583,931**
Generation × line	66	.026,750
Error	168	.027,646

* Significant at 1% level.

number of flies tested in each plot was 80, replicated three times. The inbreeding was continued up to the 16th generation, during which the number of inbred lines were reduced to 7 in KN population and 5 in TB population. The migrating activity of 12 lines from the two populations, expressed in percent of migrated flies to the total number is presented in Table 1.

Analysis of variance was applied for the data obtained for the last seven generations (Table 2).

From the two tables given above, we find the following facts: (1) The migrating activity of flies did not show any definite decreasing tendency due to inbreeding, though there was a statistically significant variation from generation to generation. (2) The difference between two populations was not statistically significant, but intra-population variation was highly significant. (3) Interaction between generations and lines was insignificant.

These facts indicate that the same population involves genotypes responsible either for high or for low migrating activity which will be separated by inbreeding. Despite our initial expectation, the inbreeding has not brought about a marked decrease in migrating activity.

44. *Variation in Competitive Ability among F_3 Lines of the Cross between an Upland Rice Variety and "Red Rice"*

(By Shin-ya IYAMA)

To inquire into the genetic behavior of the difference in competitive ability between the upland rice and "Red rice", F_3 lines of Norin-Mochi 18 × "Red rice" were tested in a competition experiment designed as follows: One of the parents, Norin-Mochi 18, was used as the tester for competitive ability. Each of 23 F_3 lines were mix-planted with the tester variety alternately in a row. Various characters of the tester plants were measured, and the difference in row means between mix- and pure plantings was used for measuring competitive ability of the F_3 lines. The rows were arranged according to randomized block design with four replications. Plant weight, plant height, panicle length, tiller number, panicle number and panicle weight per plant were investigated. Variations in row means in mix-planted plots, as compared with the means in pure stand, are shown in Table 1.

The results of variance analysis of the data are given in Table 2.

Table 2 shows that the variations among lines in plant weight, panicle number and panicle weight per plant are significant. It is apparent from Table 1 that though "Red rice" is the strongest competitor, most F_3

Table 1. Variations in characters of the tester variety mix-planted with different F_3 lines.

Plant weight

13	15	17	19	21	23gr.	No. of lines
1	6	7	5	3	1	23
R*					N**	

Panicle number

4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	No. of line
	1	4	8	5	2	2		1	23
R							N		

Panicle weight per plant

4	5	6	7	8	9	10	11gr.	No. of lines
3	8	7	3	1	1			23
R							N	

* R: Mixed with "Red rice". ** N: Pure stand of Norin-Mochi 18.

Table 2. Analysis of variance of competitive ability (difference between mix- and singly planted plots) for F_3 lines.

Source of variation	d.f.	Mean square					
		Plant height	Panicle length	Tiller number	Panicle number	Plant weight	Panicle wt. per plant
Replication	3	32.2353	14.6658	6.7516	3.1215*	120.6994**	32.6469**
Line+	23	20.9282	1.5992	2.4536	2.4570*	28.0128*	6.5376*
Error	69	16.0051	1.1746	1.7816	1.3303	15.6635	2.2666

* Significant at 5 % level. ** Significant at 1 % level.
\+ Including "Red rice".

lines are nearly as strong as "Red rice". It may then be inferred that the progeny of crosses between upland rice and "Red rice" tends to have a strong competitive ability and to grow in numbers if mixed with a population of upland rice varieties.

45. *Genetic Variability in Wild Populations of Oryza perennis and O. rufipogon*

(By Kan-Ichi SAKAI and Takashi NARISE)

Oryza perennis and *O. rufipogon* (or *O. sativa spontanea*) are considered to be the very close relatives of cultivated rice species, *O. sativa*, and are distributed sporadically in the Ceylon island. The former species is mostly found in the western part of the island, while the latter in the eastern part. Both species grow in swamps, on the fringe of water-courses, and in paddy fields.

A number of panicles were collected from three populations of each species, and ten spikelets were taken at random from each panicle. Measurement was made on awn length and on the length and width of spikelet. The places where *O. perennis* was collected were Veyangoda, Yagoda and Bombuwela, and the places where *O. rufipogon* was collected were Illuppaiya-dichchenai, Pottuvil and a spot near the latter village. Materials were always collected from a fairly large population.

Mean values for the length and width of spikelets and awn length on an individual panicle basis are presented in Table 1.

The data thus obtained were analyzed by the method of analysis of variance in order to estimate the genetic and environmental components

Table 1. Mean values of length and width of spikelets and awn length in wild populations of two *Oryza* species.

Species	Population	No. of panicles investigated	Spikelet length (mm)	Spikelet length (mm)	Awn length (cm)
O. perennis	Veyangoda	52	8.15±0.340	2.60±0.155	6.82±0.800
	Yagoda	55	8.25±0.297	2.76±0.106	8.59±1.771
	Bombuwela	67	7.95±0.282	2.44±0.048	9.94±0.843
O. rufipogon	Pottuvil	80	7.96±0.230	2.61±0.099	9.31±1.133
	〃 (nearby)	50	7.94±0.253	2.67±0.115	7.30±0.973
	Illuppaiya.	67	8.62±0.311	2.75±0.094	10.93±1.661

of variances and covariances. Coefficients of genetic and environmental correlations were also calculated. In this analysis, it was assumed that variation within panicles (among spikelets on the same panicle) represents the environmental variation, and that among panicles is composed of the environmental variation of the same magnitude and the remaining genetic portion (each panicle represents an individual plant). This assumption is

acceptable for the length and width of spikelet empirically. In awn length, though the environmental variance among plants may not always be comparable with that within plants, the assumption may still go so as to yield a preliminary insight.

Genetic and environmental correlations of spikelet length with spikelet width, and with awn length, are given in Table 2.

It is seen in Table 2 that length and width of spikelet are not corre-

Table 2. Environmental and genetic correlation coefficients between spikelet length and other characters in wild populations of two *Oryza* species.

Species	Population	Spikelet length and Spikelet width		Spikelet length and Awn length	
		r_e	r_g	r_e	r_g
O. perennis	Veyangoda	0.1623	−0.0076	0.4953	0.4816
	Yagoda	0.1294	0.1619	0.1926	0.0325
	Bombuwela	0.3058	0.0057	0.9239	0.3866
O. rufipogon	Pottuvil	0.0209	0.3491	0.3146	0.4838
	〃 (nearby)	0.2063	0.0389	0.1381	0.6539
	Illuppaiya.	0.4669	0.1898	0.3930	0.7012

r_e: Environmental correlation. r_g: Genetic correlation.

Table 3. Genetic and environmental variance components and heritability values (h^2) in wild populations of two *Oryza* species.

		O. perennis			*O. rufipogon*		
		Yagoda	Vayan-goda	Bombu-wela	Pottuvil	near Pottuvil	Illup-paiya.
Spikelet length	σ_e^2	0.0590	0.1044	0.0568	0.0268	0.0412	0.1027
	σ_g^2	0.0827	0.0266	0.0400	0.0505	0.0272	0.0866
	h^2	58.36	20.31	41.32	65.33	39.77	45.75
Spikelet width	σ_e^2	0.0036	0.0156	0.0043	0.0040	0.0120	0.0044
	σ_g^2	0.0141	0.0199	0.0029	0.0094	0.0071	0.0504
	h^2	79.66	56.06	40.28	70.15	37.17	91.97
Awn length	σ_e^2	0.7640	0.9629	1.1353	0.1190	0.3540	0.5087
	σ_g^2	1.3829	0.5124	0.5829	1.0304	0.9110	1.8027
	h^2	64.41	34.73	33.92	89.65	72.02	77.99
% of genetic variance in average		67.48	37.03	38.51	75.04	49.65	71.90

lated genetically, or, if correlated, only slightly. In contrast, spikelet length and awn length are correlated significantly. Of special interest in this connection may be the very low genetic correlation between spikelet length and awn length in the Yagoda population, unlike the other five populations.

The values of genetic and environmental components of variance, and the heritability value (genetic variance in per cent of the total variance) were estimated as given in Table 3.

Looking at Table 3, we find that among three populations of *O. perennis*, the Yagoda population is genetically the most heterogeneous, and in *O. rufipogon*, the Pottuvil and Illuppaiyadichchenai populations appear to be heterogeneous to the same extent.

From the results of investigations described above, the following conclusions will be drawn: (1) The two species cannot be distinguished from each other in so far as spikelet measurements or awn length are concerned. (2) There is a large inter-population variation within the same species. (3) Though length and width of spikelets are not correlated genetically, spikelet length appears to be genetically correlated with awn length. (4) The magnitude of genetic variability seems to differ from population to population.

46. *Heritability of Grain Shedding and Other Characters in Rice*

(By Kan-Ichi SAKAI and J. J. NILES*)

This report deals with the estimation of the heritability values of a few of economically important characters of rice in Ceylon. The method adopted is the estimation of genetic and non-genetic components of variation by the method of analysis of variance in F_3 lines. The materials used were hybrid populations between Pachchai perumal or Panduruwi and Vellai Illankalayan.

The method of analysis is as follows: Let the number of F_3 lines be x, the number of individuals within each line be y, the number of individuals within a parental (pureline) plot be z, and the number of those pureline varieties be r. On the basis of measurements taken on each individual F_3 plant, the analysis of variance table can be constructed as follows:

* Department of Agriculture, Peradeniya, Ceylon.

Source of variation	Degree of freedom	Mean square	components of mean square expected
Between lines	$x-1$	M_1	$\sigma^2+y\sigma_s^2$
Within lines	$x(y-1)$	M_2	σ^2
Within pureline varieties	$r(z-1)$	M_3	σ_e^2

In this table, σ_e^2 stands for environmental variance, σ^2 includes besides environmental variance, genetic variance due to segregation of genes within F_3 lines, and σ_s^2 is the genetic variance due to genetic differences between lines. The genetic variances involved in σ^2 and σ_s^2 are expected, for F_3 hybrid lines of autogamous plants, to be.

$$\sigma^2=\sigma_e^2+\sigma_w^2 ,$$

$$\sigma_w^2=\frac{1}{3}\sigma_g^2+\frac{2}{3}\sigma_h^2 ,$$

and

$$\sigma_s^2=\frac{2}{3}\sigma_g^2+\frac{1}{3}\sigma_h^2$$

where σ_g^2 and σ_h^2 are the variances due to fixable and non-fixable effects of genes, respectively.

The heritability value of a given character in the F_3 generation can be estimated by

$$h^2_{(F_3)}=\frac{\sigma_g^2}{\sigma_g^2+\sigma_h^2+\sigma_e^2}$$

The heritability value of the same character in F_2 can be estimated by

$$h^2_{(F_2)}=\frac{\frac{2}{3}\sigma_g^2}{\frac{2}{3}\sigma_g^2+\frac{4}{3}\sigma_h^2+\sigma_e^2}$$

Heritability values were computed by using these formulas. The regression of F_3 progeny lines on the F_2 parents, and the number of effective factors (by Panse's method), were computed further. The results thus obtained for degree of grain shedding,* panicle length and plant height are given in Table 1.

Table 1. Heritability values, F_2-F_3 regression, and number of effective factors for grain shedding and other characters.

Character	Cross	Heritability value F_3	Heritability value F_2	Regression of F_3 lines on F_2 parents	No. of effective factors
Grain shedding	$P_1 \times V$	0.093	0.053	0.082	6
〃	$P_2 \times V$	0.392	0.300	0.239	2
Panicle length	$P_1 \times V$	0.069	0.041	0.155	11
Plant height	$P_1 \times V$	0.612	0.481	0.465	3

P_1, P_2, and V: See text.

* The degree of grain shedding was assessed by running a glass roller over each of the panicles to be tested on an inclined board. The data given in percentage were transformed into an index-number before analysis.

I. RADIATION GENETICS IN ANIMALS

47. *Radiation Mutagenesis in the Silkworm*
I. Changes in Sensitivity of Silkworm Germcells to X-rays at Different Stages of Gametogenesis

(By Yataro TAZIMA and Kimiharu ONIMARU)

Marked differences between different developmental stages have been revealed in the sensitivity and mutability of the spermatogenic cells of *Drosophila* and mouse treated by X-rays. In both animals germcells in different phases exist together in the same testis and the response pattern was determined by testing successive broods at a definite interval after irradiation. It may, however, be difficult to tell exactly which type of spermatogenic cells were tested when the adult males had been irradiated. The investigation can be more exactly carried out in silkworm which offers the advantage of almost simultaneous development of the germcells in the gonads in accordance with the development of the individuals, both male and female. Therefore, the present work was undertaken with the silkworm.

Wild type females and/or males were irradiated with 250, 500, 1000, 2000, 3000 and 4000 *r* at several stages of larva and pupa and were mated to non-irradiated double recessive *pe re* partners. Five days after being laid, the eggs were covered with pigments according to their genotypes. They were counted seven weeks later, so as to make it easy to distinguish

pe mutants from unfertilized eggs and early embryonic lethals. The results were as follows.

1) Changes in the number of eggs laid per mother

By the irradiation of the female with 1000 *r* X-rays, no noticeable change was observed among groups irradiated at various stages, but when irradiation dose was higher than 2000 *r* a noticeable decrease was found in the egg number among groups irradiated early. Irradiation at late pupal stage showed no appreciable decrease in the egg number, even at 3000 *r*.

Male germcells, however, responded quite differently to X-rays from those of the females. A marked sensitive period was revealed in the early fifth stadium when the germcells were in early spermatocyte stage.

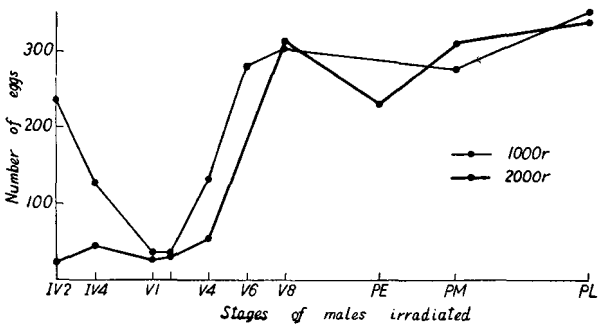


Fig. 1. Average number of eggs laid per female mated with males irradiated at different developmental stages.

In this stage decrease in the number of eggs was clearly observed even at 500 *r*.

2) Frequency of unfertilized eggs and early lethals

No affected stages were found when females were irradiated with 1000 *r*, while in the male a remarkable difference was observed with the same dose of X-rays according to the irradiated stage. The incidence of unfertilized eggs showed its peak at early fifth stadium, which coincides with the most sensitive stage in respect to the egg number. The spermatogonium in the third larval stadium is fairly resistant to X-rays as well as the mature sperm in the pupal stage.

Thus the sensitivity of germcells to X-rays was revealed clearly in both sexes of the silkworm. It is noteworthy that there is an extraordinary sensitive stage in early fifth stadium of the male when the germcells are very badly affected.

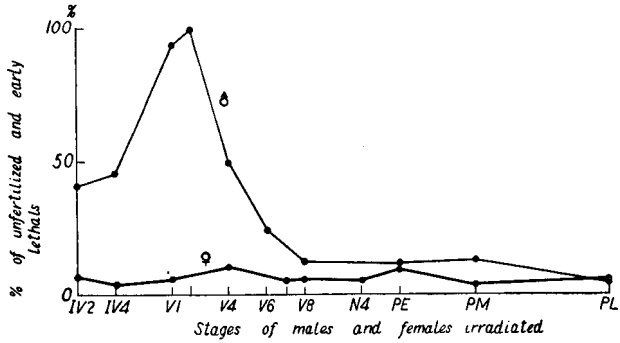


Fig. 2. Irradiated stages of males and females and rates of unfertilized eggs.

48. *Radiation Mutagenesis in the Silkworm*
II. Mutation Response Pattern of Silkworm Germcells to X-rays According to Stage and Sex

(By Yataro TAZIMA and Kimiharu ONIMARU)

Since conspicuous differences were found in the sensitivity to X-rays of silkworm germcells at different stages of the gametogenesis, a similar detailed analysis of mutation response was made in respect to the rate of recessive visible mutations at specific gene loci and that of dominant lethal mutations at various developmental stages of the gametogenesis.

1) Recessive mutation rate at specific gene loci

Experiments were carried out by the same method as mentioned in the foregoing article. According to this method most of the F_1 eggs are expected to be of wild type, but some eggs may show recessive mutant characters of the non-irradiated parents as a result of recessive point mutation or deficiency at marked loci. From the number of exceptional eggs mutation rates were calculated at each irradiated stage and plotted with the developmental stages of the parents.

The mutation rates in the male germcells showed remarkable differences according to the developmental stages of the irradiated individual, while the female germcells showed mostly the same results at different stages prior to mid-pupal stage but after this stage sensitivity suddenly increased.

2) Dominant lethals

The incidence of dominant lethals was analysed by the percentage of

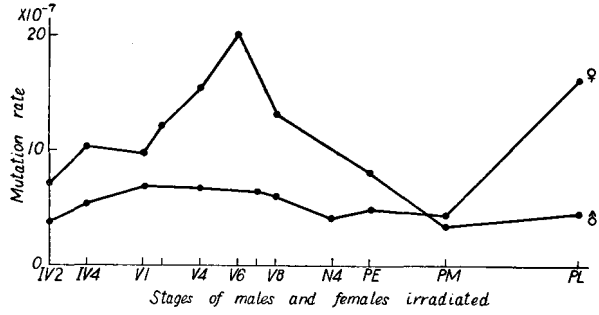


Fig. 1. Change in mutability with the development of Silkworm germ-cells.

dying embryos per total number of fertilized eggs at four different developmental stages, *i. e.* stadium 4 day 2, stadium 5 day 2, stadium 5 day 6 and late pupal stage. The response pattern of dominant lethals was mostly the same as that of recessive visible mutations at specific loci, except that higher doses than 2000 *r* were required to reveal the differences in sensitivity. Irradiation group at stadium 5 day 6 showed higher mutation rate than at stadium 5 day 2, which was consistent with the most sensitive stage with regard to the visible recessive mutation but inconsistent with the stage of lowest fertility. To discuss this point a more thorough investigation is required.

If these results are referred to the stages of gametogenesis, it may be concluded that the germcells of the silkworm are most sensitive to X-rays in some stages of spermiogenesis and oöcyte, while oögonium, spermatogonium and mature sperm are less sensitive.

49. *Radiation Mutagenesis in the Silkworm III. Comparison of X-ray Induced Mutation Rates in the Silkworm with Those of Drosophila and Mouse*

(By Yataro TAZIMA)

X-ray induced recessive mutation rates were calculated from the data obtained from mutations at loci $+^{pe}$ and $+^{re}$, and compared with those of *Drosophila* and mouse as follows.

It is evident that visible mutation rates in the silkworm are remarkably higher than those found in *Drosophila*. The former are about 7 times as high in mature sperm and 28 times as high in spermatogonium as the latter. It is, however, necessary to bear in mind that in the silkworm

	Mutation rates per r per locus	
	Spermatogonium	Mature sperm
Bombyx	4.2×10^{-7}	4.5×10^{-7}
Drosophila melanogaster	1.5×10^{-8} (Alexander '54)	6.0×10^{-8} (Alexander '54)
Mouse	2.5×10^{-7} (Russell '51)	

mutation rates were calculated with respect to mutant characters expressed in the egg stage in contrast to those observed in *Drosophila* which were obtained with respect to adult characters. Actual incidence of mutation in *Drosophila* might be much higher than the above data if earlier characters were tested.

50. *Tissue Culture Analysis of Delayed Lethal Irradiation Effect in D. melanogaster*

(By Tsutomu SUGAHARA and Masakatsu HORIKAWA)

It is well known that the effect of radiation on *Drosophila* larvae is not their immediate death but a delay of pupation and a decrease of pupation- and imagination rate in a wide range of radiation doses. Furthermore, it was observed by the authors that the body weight of irradiated larvae, whose pupation was delayed, did not only not decrease but was considerably larger than in the control larvae. In the present investigation, the mechanism of this phenomenon has been studied in some detail using the method of tissue cultured irradiated organs and discs.

Wild strains (*Oregon*, *Canton-S*, *Kochi*, and *Samarkand*) and several eye-color mutants (*bw*, *w*, *v*, *cn*, *v bw*, *cn-cw Bar*, *bar-3* and *Dp*/*In*(*3L*)*P*, *In*(*3R*)*C*, *Sbel*(*3*)*e* of *D. melanogaster* were used as material. Third-instar larvae (about 80 hours old after hatching at 25°C) grown under sterile conditions were irradiated with various doses of X-rays (160 Kvp, 25 mA, Imm Al filter, 370 r/min, at the distance of 30 cm). They were dissected, and the various organs and tissues were removed under a binocular microscope in a sterilized glass chamber. They were cultured in a synthetic medium, and the sensitivity for irradiation was determined by observing their growth and differentiation. Some of the results obtained from a wild strain (*Oregon*) are shown in Table 1.

When the irradiated (0–20 Kr) eye discs were cultured together with the normal cephalic complexes (ten bodies), they showed pronounced growth and differentiation as in normal eye discs. But after high doses

Table 1. Growth and differentiation rate of the irradiated eye discs in tissue culture.

Organs and discs cultured	G.* and D.* rate	Doses (Kr) 0	0.5	1	3	5	10	15	20	25
N.* eye disc+I.*C.C.*	G	+	+	+	+	+	+	+	±	−
	D	+	+	+	−	−	−	−	−	−
I. eye disc+N.C.C.	G	+	+	+	+	+	+	+	±	−
	D	+	+	+	+	+	+	+	±	−
I. eye disc+I.C.C.	G	+	+	+	+	+	+	+	−	−
	D	+	+	+	−	−	−	−	−	−
Culture medium	G	+		+			+		+	+
	D	+		+			+		+	+
Extracted metamorphic hormone	G	+		+			+		+	+
	D	+		+			+		+	+

* G.=Growth, D.=Differentiation, C.C.=Cephalic complex (ten bodies), N.=Normal, I.=Irradiate.

(25 Kr), neither growth nor differentiation were observed.

Otherwise, when normal eye discs were cultured together with the irradiated (3 Kr) cephalic complexes (ten bodies), the eye discs showed pronounced growth, but no differentiation. Since the cephalic complex is known to exert hormonal control on growth and metamorphosis, it seems that the normal differentiation mechanism of organs and discs of the cephalic complex was virtually destroyed after low doses (3 Kr). Hence, one might suspect that *bar-3* mutant which showed high radiosensitivity in whole body irradiation test of thirteen strains used would be due to higher sensitivity to radiation damage of the cephalic complex than other strains.

Furthermore, the metamorphic hormones already secreted from the cephalic complexes were not influenced even after high doses (25 Kr). The result obtained in this experiment shows that the delay in pupation and the decrease in imagination rate are due to radiation damage of the cephalic complex.

51. *Structure and Function of Pleiotropic Genes in the Silkworm I. An Experiment to Break up E^{pl}, E^{Kp} and E^{Ne} genes into Separate Components by X-rays (a Preliminary Note)*

(By Yataro TAZIMA and Eiichi INAGAKI)

In genetically well analysed organisms some genes have been known to

control so diverse characters that no relation between them would seem to exist. For instance E^{Kp} controls the appearance of extra legs and extra markings in different segments of the silkworm. The question arises if it is possible to divide the gene into some unit components which control the seemingly different characters. Is there any change in the manifestation of each component when they are separated or when they are brought together under a new sequence? In order to get an answer to these problems, the present study was initiated.

E^{El} (extra crescent markings on the fourth segment and extra legs on the fourth and fifth segments), E^{Kp} (extra crescents on the sixth segment and extra legs on the fifth segment) and E^{Nc} (no crescents on the fifth segment and extra legs on the same segment) were used as materials. X-ray irradiation was administered to the males of those mutants at the pupal stage. Irradiated individuals were crossed to untreated partners of normal strains (with crescents on the fifth segment) after emergence. F_1 offspring was examined carefully for dislocation of crescent markings and extra legs. Several exceptional individuals were discovered with respect to both characters and classified into E, E^{Ca}, E^{D}, E^{Kp}, $\cdots$ etc. according to their phenotypes, the corresponding mutant types being already known (Table 1). Genetic analysis has not yet been carried out.

Table 1. Aberration induced by X-rays in E^{El}, E^{Kp} and E^{Nc}

Cross	Aberrations induced
$+/+$♀$\times E^{El}/+$(X-rayed) ♂	E, E^{Ca}, E^{D}, E^{Kp}, E^{N}, E^{Nc} and new types
$+/+$♀$\times E^{Kp}/E^{Kp}$ (X-rayed) ♂	E^{Ca}, E^{Nc}, homo E^{Kp} and a new aberrant with regard to abdominal leg
$+/+$♀$\times E^{Nc}/+$(X-rayed) ♂	E^{Kp}

Most of the mutations occurred in the direction from complicated types to simpler ones, but in very few cases the reverse was true. The frequency of appearance of the aberrants was far higher than would have been expected from a single gene locus which indicates that the E-allelic gene is not a single unit locus but an aggregate of small unit loci which control different characters.

52. *Silkworm Larvae with Extra-abdominal Legs Caused by a Chromosome Fragment*

(By Mitsuo TSUJITA)

Female pupae of the genotype $E^{H}E^{Kp}/E^{H}E^{Kp}$ were irradiated with X-

rays of 8000 r, and the imagines were mated to normal males. The larvae with supernumeral abdominal legs caused by a chromosome fragment were found in the offspring of this cross. Small extra-abdominal legs were produced on the 4th and 5th abdominal segments of the mutant larvae. Those on the 4th segment were often missing.

As the phenotypic manifestation resembles that of E, the gene symbol E' was given to this mutant.

From the cross $+\times E'$ or $E'\times E'$ the E' larvae segregate as shown in the following tables:

Table 1. $+\times E'$

Hatching rate	+	E'	Total
92.6	1158	363	1521

Table 2. $E'\times E'$

Hatching rate	+	E'	Total
87.8	823	892	1715

The number of the E' larvae in Tables 1 and 2 is less than that which is theoretically expected to segregate. The E' homozygous individuals could not be ascertained because they are hardly different from the heterozygotes. To examine the linkage relation between E' in question and E^{Ca} belonging to the E region of chromosome VI, cross experiments were carried out.

From the cross $E'/E^{Ca}\times +$ and from its reciprocal four types $+$, E^{Ca}, E' and $E'E^{Ca}$ segregated as shown in the following table.

Table $E'E^{Ca}\times +$

	+	E^{Ca}	E'	$E'E^{Ca}$	Total
Total of two batches	94	63	64	16	237

Table $+\times E'E^{Ca}$

	+	E^{Ca}	E'	$E'E^{Ca}$	Total
Total of two batches	850	377	568	134	1929

From the experimental results shown above, it is evident that E' does not link to chromosome VI.

According to my cytological observation, a very small chromosome which is probably derived from breakage of chromosome VI could be observed at the maturation division of the first spermatocyte of the E' individuals. It seems that the characteristic trait of E', supernumerary abdominal legs, is produced by the genetic constitution of E' on the chromosome fragment.

Furthermore, it may be inferred that by the treatment with X-rays this fragment was formed as a result of breakage of chromosome VI, at the end of which $E^{a}E^{Kp}$ loci are located. As stated above, mutant larvae have two supernumerary abdominal legs but they have not the extra crescent patterns on the dorsal side of the 4th segment. Why these patterns do not appear?

It is possible that in spite of the presence of E^{a} locus the extra crescent patterns are not produced owing to a position effect. On the contrary, it may be due to a defective structure of the E^{a} locus. On this point further studies are required.

The attachment of spindle fibres on the chromosome fragment should be taken into consideration.

53. *Effect of Chronic Gamma Irradiation on the Breeding Behavior of Mice at Different Developmental Stages*

(By Tsutomu SUGAHARA, Kiyosi TUTIKAWA, Yoshihiko SUGIURA, and Tomizo TANAKA)

As previously reported (Annual Report No. 7), the effect of chronic irradiation on the breeding behavior was different according to the developmental stages and sex of the irradiated mice. A similar experiment was undertaken on a larger scale with respect to the number of animals and the number of developmental stages in order to obtain more detailed information about the difference between the effects.

Mice of the CBA strain kept in aluminum cages were subjected to chronic gamma irradiation. Upon completion of irradiations, all animals were mated with mice of NH strain by placing one male with one or two females. Irradiations were made from the γ-ray source of ^{60}Co in a specially designed irradiation room, for 22 hours per day and 5 days per week. A total dose of 450 r was reached in about 80 days in the first series, and that of 366 r in about 70 days in the second series.

The treated mice were divided into four groups, A, B, C, and D according to their developmental stages during irradiation. Group A consisted of animals receiving radiation from fertilization, through birth, until the age of 50 to 60 days. Group B was composed entirely of adult animals. In group C and D, the irradiation started with birth and weaning

respectively.

The results of matings examined during three months after irradiation are summarized in Table 1.

Table 1. Breeding behavior during three months after irradiation.

Dose	group	Sex	No. of irradiated animal	Sterility	Mean litter size	% of control	Sex ratio
450r	A	♂	31	0	5.55	81.7	61.0
		♀	34	100	—	—	—
	B	♂	20	40	4.42	65.2	71.7
		♀	12	8.3	4.83	71.2	56.3
	C	♂	13	15.4	5.2	77.1	62.5
		♀	18	88.9	—	—	—
	D	♂	9	88.9	—	—	—
		♀	13	61.5	3.7	54.6	43.3
366r	A	♂	10	10	5.32	73.8	48.0
		♀	7	100	—	—	—
	B	♂	9	0	4.25	62.6	65.6
		♀	6	16	4.91	72.3	50.0
Control			23	—	6.78	100	54.5

Sterility and litter size showed similar tendencies among different groups in each sex. In the female, sterility percentage and litter size reduction became less pronounced in the order of the age of irradiated animals. In the male, opposite results were obtained on these points with the exception on group D. As for sex ratio, which has recently been regarded as one of the most useful means to estimate the genetic effects of radiation on man, the results were rather controversial, though in the case of irradiated males of group A a statistically significant increase was observed in the proportion of males. With regard to the changes of breeding behavior following irradiation, the males seemed to be able to recover from the damage, and the recovery was earlier for younger males. On the other hand, the females, seemed to become permanently sterile sooner or later, especially the younger females which were sterile from the beginning.

Recessive mutations at three loci of the double recessive NH mice were not observed in F_1 consisting of 819 animals.

54. *Biochemical Studies on the Radioprotective Effect of Cysteamine*

(By Goro YAMAMOTO, Saburo NAWA and Tsutomu SUGAHARA)

It has recently become known that in mammals cysteamine is one of the most effective protective agents against lethal effect of radiation. In order to clarify the mechanism of its protective effect, disturbance of nucleic acid metabolism induced by X-irradiation was studied in the spleen and thymus of the mouse, some partly with and partly without cysteamine administration before irradiation.

Female mice of the NCF_1 strain (NH×CBA) were used for the experiment. Groups of three or four animals were sacrificed at one time and dissected to obtain the spleen and thymus. Each organ was weighed immediately and nucleic acids were extracted by the Schneider method. DNA content determined photometrically by diphenylamine color reaction, and RNA content by orthin color reaction.

Total DNA content in the spleen and thymus as well as their weight were markedly decreased when the whole body was irradiated, even with relatively low doses of X-ray as Table 1 shows.

Table 1. Total DNA content and weight of spleen and thymus, 24 hours after X-irradiation.

	Spleen		Thymus	
	DNA content in %	Weight in %	DNA content in %	Weight in %
0r	100	100	100	100
68r	55.4	56.2	61.8	59.4
136r	38.2	43.5	35.2	43.4
300r	22.9	36.5	17.1	28.2

Concerning the effect of cysteamine, DNA and RNA content in the organs were studied one, two and four days after irradiation with 300 r. Animals examined were divided into four groups; 1) those injected intraperitioneally with a dose of 0.15 mg/body weight of cysteamine thirty minutes before X-irradiation, 2) those injected at the same time intraperitoneally with 0.5 cc of physiological saline, 3) those injected intraperitoneally with the same dose of cysteamine as the first group but without X-irradiation, 4) untreated controls. The mice of group 1) could survive even when exposed to a lethal dose of 800 r. The weights, total DNA and RNA contents of spleen and thymus of the treated animals as ex-

pressed in per cent in relation to controls are shown in Figs. 1 and 2.

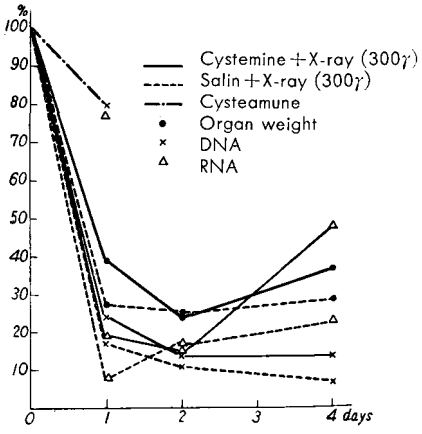


Fig. 1. Changes in weight, total DNA and RNA contents of the thymus after treatments.

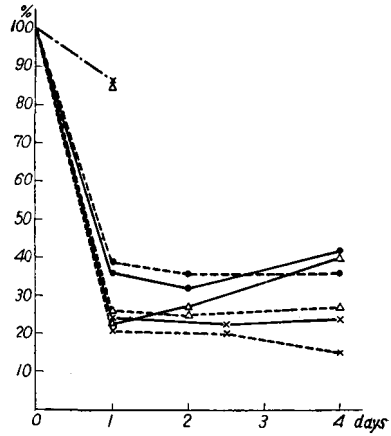


Fig. 2. Changes in weight, total DNA and RNA contents of the spleen after treatments.

In both organs reduction of the three value at one and two days after irradiation was quite similar in both cysteamine-treated and cysteamine-untreated group. However, at four days after irradiation the results were different depending on the presence or absence of cysteamine treatment. Recovery, especially in RNA content, was seen in the treated, but not in the untreated group. In addition, it should be emphasized that both DNA and RNA content were reduced by the administration of cysteamine alone. Cysteamine itself seems to be a toxic substance to nucleic acid metabolism and yet able to protect the metabolism from the radiation damage by enhancing its recovery. Such a contradictory phenomenon was also observed in body weight. The reduction of body weight just after X-irradiation was more marked in the cysteamine-treated group than in the untreated one. After four days, on the contrary, the recovery of body weight was striking in the cysteamine-treated group even when irradiated with a dose lethal to the untreated.

55. *Melanotic Tumors Induced in Drosophila melanogaster by Ionizing Irradiation*

(By Toshifumi TAIRA and Toshiteru MORITA)

Melanotic tumors are induced easily by ionizing irradiation in larval

tissues of *D. melanogaster* if early larvae are exposed to radiation with 800 and more roentogens. The frequency of induced melanotic tumor differs according to mutants or dosages. In the former case, it seems to depend on differences in gene constitution in respect to metabolism. In the latter case, it may be due to the ionizing intensity of the agent. The results are shown in Table 1.

The isogenic line of Hn^{r3}, one of the eye color mutants, shows the highest frequency of induced melanotic tumors. Therefore, it was used to analyze developmentally and biochemically the formation-mechanism of melanotic tumors induced by ionizing irradiation. The effects of trypotophan and cysteamine added to a normal medium on the frequency of induced melanotic tumors were examined. The results are given in Table 2.

By adding tryptophan, the frequency of induced melanotic tumors is distinctly increased, but it is conversely decreased by adding cysteamine to the culture medium.

Although constituents between irradiated and non-irradiated groups of prepupae were made a comparative study chromatographically, there are not any distinct differences among the free amino acids besides excessive phenylalanine in the irradiated group without melanotic tumors.

Table 1. Frequencies of the induced melanotic tumors by ionizing irradiation in *D. melanogaster* strains.

Strain	Dosage (Kr)	No. of treated 1st larvae	No. of pupation	No. of pupae with melanotic tumors	% of induced melanotic tumors
Oregon-2	0	300	288	0	0
	3	680	453	10	2.2
	5	550	383	16	4.2
	8	603	360	11	3.1
e^{11}	0	300	283	0	0
	3	150	84	10	11.9
	5	578	224	18	8.1
	8	315	71	7	9.9
Hn^{r3}	0	102	82	0	0
	3	78	55	16	29.1
	5	50	41	7	17.1
	8	123	6	1	16.6*

*) Decrease of frequency with higher dosage seems to be due to increase of larval lethality.

Table 2. Frequencies of melanotic tumors induced by ionizing irradiation after adding tryptophan and cysteamine to the culture medium (Hn^{r3}, 3 Kr).

		No. of treated 1st larvae	No. of pupation	No. of pupae with melanotic tumors	% of induced melanotic tumors
Non-irradiated	Control	300	253	0	0
	Tryptophan (5%)**	300	233	0	0
	Cysteamine (20 μg/ml)	300	155	0	0
Irradiated	Control	500	488	78	16.0
	Tryptophan (5%)**	550	500	144	28.8
	Cysteamine (20μg/ml)	330	141	9	6.4

**) Tryptophan is added at 5 per cent of a given yeast powder to the medium.

56. *Differential Sensitivity to X-ray Irradiation of Male and Female Day-old Chicks*

(By Takatada KAWAHARA)

A study of mortality in response to whole body exposure to X-rays has been carried out in day-old chicks of two breeds, namely White Leghorns (WL) and Barred Plymouth Rocks (BPR) and their reciprocal F_1 hybrids. The irradiation was made under the following conditions: single dose of 800 r, at 180 KV, 25 mA tube current, added filter 0.3 mm copper and 0.5 mm aluminum, in air at the target distance of 50 cm, intensity 82.5 r/min. All experimental birds were reared in electric brooders and were fed a commercial chick starting mash. Observations were made up to 20 days after irradiation. Lethality directly due to irradiation by X-rays apparently occurred within 20 days after treatment, afterwards the increase in mortality was negligible compared with that of non-treated controls. The percent mortality and its statistical analysis are given in Table 1.

In general, the males were more radiosensitive than the females. Differences between sexes of pure-breds were significant ($\chi^2=11.64$, $P<.01$), however, those in hybrids approached the significance level ($\chi^2=3.75$, $.10>P>.05$).

Table 1. Mortality of various mating types exposed to irradiation by a single dose of 800r.

	Breed or cross	Sex	No. tested	Dead birds		χ^2	
				No.	%		
Treated (800r)	WL	♂	53	46	86.8	11.22**	11.64**
		♀	47	29	61.7		
	BPR	♂	15	15	100.0	4.53*	
		♀	16	12	75.0		
	WL♀ × BPR♂	♂	47	40	85.1	4.41*	3.75
		♀	65	44	67.7		
	BPR♀ × WL♂	♂	55	41	74.6	0.59	
		♀	43	29	67.4		
Non-treated	WL	♂	20	0	0		
		♀	16	0	0		
	BPR	♂	17	0	0		
		♀	12	0	0		
	WL♀ × BPR♂	♂	10	1	10.0		
		♀	9	0	0		
	BPR♀ × WL♂	♂	11	0	0		
		♀	12	0	0		

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

57. *A New Spacing in the Wide Angle X-ray Diffraction Pattern of Dried Striated Muscle*

(By Tsutomu SUGAHARA, Masayoshi OZAWA*, and Nayao UESUMI*)

Striated muscle dried at its normal length has been reported to show typical wide angle X-ray fiber diagram with a main meridian and a equatorial spacing around 5.1A and 9.6A analogous to that of α-keratin. Dried actomyosin film, a contractile protein of the muscle, was reported to show a wide angle diagram similar to that of the dried muscle. Based on those diagrams ASTBURY presented his famous molecular model of α-keratin type. Very rarely, however, a number of spots, as are usually found in LOTMAR-PICKEN diagrams, have been obtained, which PAULING and COREY

* Mie Prefectural University School of Medicine.

believed to support their concept of a helical configuration of fibrous protein. While examining the X-ray diffraction pattern of dried striated muscle by an automatic recording X-ray diffraction apparatus, we found the diffraction peak to be around 5.9A which had never been noticed in the above mentioned diagrams. The same spacing was observed in dried and powdered muscles as well as in dried actomyosin gels.

Muscle specimens were taken from psoas muscle of the rabbit. Actomyosin was prepared from rabbit muscle by the method of SZENT-GYÖRGY with repeated purifications. Actomyosin gel formed in distilled water was washed several times to remove salts completely, for even a small quantity of residual salts could disturb the X-ray pattern with their diffraction peaks. All diffractions were done by a NORELCO automatic recording X-ray diffraction apparatus operating at 40 kVp with Ni-filtered copper Kα-radiation.

Some tracings of the patterns obtained from $2\theta=5°$ to 20° are reproduced in Figure 1. Curve a) was obtained from a resting muscle dried at normal length; the spacings perpendicular to the fiber axis are shown. Curve b) was obtained from a muscle in *rigor mortis* dried at its original length in the body; curve c) represents a resting muscle dried and powdered; curve d) correspond to dried and powdered muscle in *rigor mortis*; curve e) was obtained from actomyosin gel. Sharp peaks around 5.9A spacing were observed in all curves. They were of different height according to the various specimens. In the case

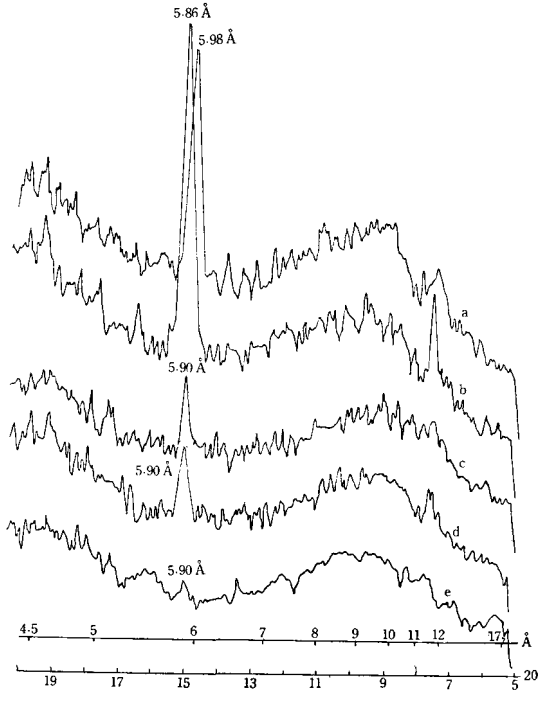


Fig. 1. Tracings of the X-ray diffraction pattern of dried muscle from $2\theta=5°$ to 20°. Explanation seen in the text

of the dried muscle fixed at its normal length the peak was quite prominent and sharp, and more so in *rigor mortis*. Slight variation in spacings and fairly large differences in their height and half value width appeared to occur according to the physiological condition of the muscle; their significance is not yet clear. A comparison of the curves obtained in two directions, parallel and perpendicular to the fiber axis, showed that the spacing was oriented mainly perpendicularly to the axis.

Further, a peak around 5.1A, usually recorded by conventional film method, has not been found. In some cases, there appeared to be one or two other sharp peaks especially in the region of 3.7A. However, as their incidence was not consistent, the experiments are now being repeated. Since the above data were markedly different from those of previous workers, the origin of the curves should be taken into consideration. Anorganic substances, if cristallized in the muscle by loss of water, can give sharp diffraction peaks characteristic of their crystal lattice, which are classified and tabulated in Hanawalt's Table.

Substances showing about 5.9A diffraction peak can hardly exist in the muscle according to the Table. It may be possible that the diagram was originated mainly from the non-contractile component of the muscle, for example the muscle membrane. The fact that the actomyosin gel showed a peak around 5.9A, though its height was very low, seems us to be against this possibility.

In conclusion, while these data are not sufficient to warrant a new proposal of the molecular configration of muscle protein, they point out that 5.1A spacing is not suitable for its elucidation.

J. RADIATION GENETICS IN PLANTS

58. *Effect of X- and γ-irradiation on Chromosome Aberrations in Vicia faba*

(By M. NEZU and S. MATSUMURA)

The beans were soaked in tap water for 48 hours (16°C) and immediately after the removal of the seed coats irradiated by X- and γ-rays in water. The dose of both rays was measured by ferrous sulphate dosimeter (FRICKE's method) in order to secure irradiation at the same dose (600 r) for the same time. After irradiation the beans were kept in a dark room at 20°C and their root-tips were fixed 24 and 30 hours later, and then treated by FEULGEN squash method. The observations of chromosomal aberrations were arbitrarily limited to cells in early telophase

Table 1. Comparison of chromosome aberrations of X- and γ-rays induced in *Vicia faba* roots.

Section	Total no. of cells observed	Percentage of abnormal cells (%)	Average no. per cell		Average of breaks per cell
			Bridges	Fragments	
Control 24 hr	400	2.3	0.0	0.023	0.023
X-24 〃	300	70.3	0.669	0.716	1.170
γ-24 〃	300	57.0	0.306	0.582	0.760
X-30 〃	650	62.6	0.300	0.775	0.938
γ-30 〃	650	39.4	0.184	0.425	0.538

just before the formation of nuclear membranes. In this stage, the chromosomes are grouped at both poles, and are stained as strongly as at anaphase or metaphase. We could easily observe bridges and fragments and ascertain their relationships. The percentage of cells with abnormal chromosomes was about 70% and 57% 24 hours after X- and γ-irradiation respectively, while after 30 hours, it was reduced to 63% and 39% respectively. This indicates that X-irradiation is about 1.2–1.6 times more effective than γ-irradiation. At the former, the average number of bridges per cell is 2.2 times more after 24 hours than at the latter, and 1.6 times more after 30 hours, while in respect to fragments it is 1.2 and 1.8 times more respectively.

It is assumed that the breakage occurred when the chromosomes were not yet split, so we estimated the number of breaks as follows: a fragment or a bridge depends on one chromosome break, and when there are more bridges than fragments the number of breakages is estimated from the number of bridges and *vice versa*. The average number of breaks per cell, after 24 hours, was 1.17 in X- and 0.76 in γ-irradiation, while after 30 hours it was 0.938 and 0.538, respectively. As to chromosome breakages, X-irradiation produced them about 1.7 times as many as γ-irradiation.

59. *Effects of Temperature and Irradiation Time upon Mutations Induced by Radiations*

(By Seiji MATSUMURA)

Dormant seeds of *Triticum monococcum flavescens* were exposed to X- and γ-rays at the dosage 10 and 20 kr. The growth of seedlings, the single-spike fertility and chromosome aberrations of treated plants (X_1)

and the chlorophyll mutations in X_2 were compared for acute and chronic irradiation. At acute irradiation with X- and γ-rays treatment was given either immediately before sowing or the irradiated seeds were kept for 30 days in storage at room temperature (about 20°C) or at 5°C. At chronic γ-irradiation with ^{60}Co the treatment lasted 54 days. Also, the effect of β-radiation by ^{32}P was examined for comparison.

The data are shown in Table 1. The relation between the inhibition of seedling growth and dosage, temperature in storage and irradiation time coincides roughly with the relation between the percentage of induced sterility and all those conditions. X- and γ-irradiations were far more effective at 20 kr than at 10 kr. In the case of 30 day storage, γ-rays inhibited the growth of seedlings and reduced the fertility more than X-rays, while irradiation applied just before sowing showed the reverse relation. It was found further, especially with γ-rays, that low temperature had the strongest inhibitory effect. At 10 kr acute γ-irradiation was

Table 1. Genetic effects of ionizing radiation in *Triticum monococcum*.

	Dosage (kr)	Length of seedlings* (cm)	Fertility of spikes in X_1 (%)	Chromosome aberrations in X_1 (%)	Gene mutations in X_2 (%)
Control		17.69 (11.08)	74.62	0.00	0.0
30 day storage at room temperature	X-10	14.99	81.82	0.00	0.0
	X-20	13.53	11.59	19.05	16.7
	γ-10	8.96	60.32	14.29	5.7
	γ-20	6.79	32.78	25.00	11.1
30 day storage at 5°C	X-10	13.81	61.95	0.00	2.3
	X-20	11.63	33.53	54.17	14.3
	γ-10	9.73	60.58	4.08	9.1
	γ-20	3.45	8.34	40.00	33.3
Acute irradiation just before sowing	X-10	10.71	62.32	7.50	6.1
	X-20	4.29	34.65	20.00	13.3
	γ-10	12.00	64.38	10.00	5.3
	γ-20	6.75	40.10	38.46	5.6
Chronic irradiation	γ-10	14.69	66.45	4.08	5.3
	γ-20	5.37	15.47	28.57	50.0
^{32}P	β-10	(12.66)	68.60	0.00	1.9
	β-20	(14.14)	79.28	2.50	4.1

* X- and γ-irradiated seeds were sown October 25th and the seedlings were measured 27 days after sowing. ( ) Sown October 27th and measured 25 days after sowing.

more effective than the chronic one, while at 20 kr the reverse relation was observed.

The frequency of ears with chromosome aberrations in X_1-plants was strikingly higher at 20 kr than at 10 kr. In most of the cases of induced chromosome aberrations ④+5_{II}, often $6_{II}+2_I$, ⑥+4_{II}, ④+④+3_{II} or ④ +$4_{II}+2_I$ and seldom $1_{III}+5_{II}+1_I$ or asynaptic 14_I have been observed. The effect of γ-ray was generally stronger than that of X-rays. Also, irradiation just before sowing and 30 day storage at low temperature produced more chromosome aberrations than storage at room temperature after irradiation. On the other hand, the effect of chronic γ-irradiation was less. This must be due to the longer time of chronic irradiation, because two-hit aberrations, such as translocation, are limited in time.

The frequency of head progenies with chlorophyll mutations in the X_2-generation increased with the increase of radiation dosage. Because of the small number of observed head progenies, due to a lower survival rate, the results with 20 kr irradiation were unsufficient. But they were roughly in accord with the observations of chromosome aberrations.

The effects of β-irradiation were unexpectedly slight. It was found from another experiment with seed absorption of ^{32}P-solution that the actual dosage of β-rays was very low.

60. *Crosses between Various X-ray Induced Recessive Mutants in Wheat*

(By Taro FUJII)

Several mutant strains of *Triticum monococcum flavescens*, namely chlorina, basi-viridis II, virido-albina, slender and irregular-ear were crossed with each other. All of these mutations were in crosses with normals simple recessives. In F_2 dihybrid segregation was observed and double recessive segregants were obtained.

Chlorophyll content of double recessive plants from the cross virido-albina×chlorina was slightly decreased in the seedling stage, compared with virido-albina itself. When they were placed in the phytotron (20°C, 80% relative humidity), their leaves gradually turned to light green and increased the chlorophyll content, until its amount was restored to the chlorina level, but a further increase was never observed. Seedlings obtained from the double recessive plants from the cross virido-albina× basi-viridis II had no chlorophyll just like albina and died out a half month after germination, even in the phytotron.

Double recessive plants in a cross virido-albina×slender or irregular-

ear were in the seedling stage similar as to chlorophyll to virido-albina itself. When both were placed in the phytotron, their chlorophyll content gradually became restored and about a month later was as that of the normals.

From these experiments it follows that the chlorophyll content of virido-albina could be recovered in the cross combinations with slender and irregular-ear which have a normal chlorophyll content. But the virido-albina gene was hypostatic to the chlorina gene and the same behavior was shown by the double recessive plants between basi-viridis II and chlorina (Ann. Rep. No. 7). Albina-like plants obtained from the cross between virido-albina and basi-viridis II must have been genetically different from albina mutants but they behaved like the latter.

61. *Radiosensitivity in Plants*

(By Taro FUJII)

Determination of radiosensitivity in plants, such as LD 50, is important in radiobiological studies and in plant breeding by radiation. Radiosensitivity is quite different in different plants. A comparison of radiosensitivity in many plants, especially in related plants of various polyploid series, were carried out in 1957.

A. Experiments in crop plants

Dry dormant seeds of several crop plants were irradiated by γ-rays from ^{60}Co source. Dose ranges were 10–100 kr, and radiosensitivity was examined by the decrease of germination rate. Some of the results are shown in Table 1.

Germination rate in most of these plants gradually decreased with the increase of doses, but that of *Cryptotaenia*, *Lilium* or *Oryza* abruptly decreased to 0 at a certain dosage. Germination ability up to the appearance of cotyledons developed in several species, for instance, *Glycine* or *Raphanus*, not much even at 100 kr, but most of the seedlings died immediately after the germination, and only a few plants could survive. Radio sensitivity must therefore be determined by the percentage of seedlings which had developed 2 or 3 leaves.

From this experiment, the genera *Cosmos* and *Brassica* were most resistant to radiation, while *Cryptotaenia* and *Lilium* were most sensitive.

B. Experiments in *Triticum* and *Aegilops*

Differences of sensitivity between di-, tetra- and hexaploid plants were compared in several species and varieties of *Triticum* and *Aegilops*. Dry

Table 1. Germination rate in several crop plants.

Material	Cont.	20 kr	40 kr	70 kr	100 kr
Cosmos bipinnatus	70%	82%	64%	56%	43%
Capsicum annuum	50	19	0	0	0
Vinca major	42	20	0	0	0
Cryptotaenia canadensis	82	0	0	0	0
Impatiens balsamina	81	66	43	55	0
Glycine max	90	84	78	1	0
Arachis hypogaea	88	81	88	42	2
Vicia faba	99	73	15	4	1
Raphanus sativus	60	47	48	34	26
Brassica campestris	97	100	100	98	98
Fagopyrum esculentum	97	91	75	16	0
Lilium philippinense	42	0	0	0	0
Zea mays	89	91	89	61	26
Oryza sativa	81	81	67	0	0

dormant seeds were used for this examination which were irradiated from 20 to 70 kr by γ-ray. Radiosensitivity was determined by the decrease of germination rate and the average length of the seedlings. Genus *Aegilops* showed more tolerance to radiation than genus *Triticum*, in tetraploid species of *Aegilops* the germination rate remained even at 40 kr, compared with that of the control, while that of the tetraploid *Triticum dicoccum* showed a marked decrease at 30 kr. A part of the results is shown in Table 2. Diploid species of *Triticum* and *Aegilops* were the

Table 2. Germination rate in cereals.

Material	2*n*	Genome	Cont.	20 kr	30 kr	40 kr	70 kr
T. aegilopoides	14	AA	30.0	6.3	0.0	0.0	0.0
T. monococcum	〃	〃	61.3	47.5	38.0	12.5	21.3
T. dicoccum	28	AABB	62.5	73.4	33.8	21.3	0.0
T. durum	〃	〃	50.0	66.3	55.0	42.5	15.0
T. Spelta	42	AABBDD	71.3	72.2	76.3	50.0	10.0
T. vulgare	〃	〃	75.0	62.5	50.0	42.5	15.0
Ae. cylindrica	28	CCDD	86.3	82.5	78.8	56.3	0.0
Ae. uniaristata	14	M^uM^u	58.2	71.3	81.3	12.5	0.0
Ae. squarrosa	14	DD	72.5	66.3	65.0	32.5	0.0
Ae. ventricosa	28	DDM^vM^v	61.3	77.2	63.8	70.0	0.0

most sensitive to radiation, and the hexaploid *Triticum* species were not more resistant than the tetraploid ones.

Differences of sensitivity were also observed in different varieties (or species) of a species (or groups). These facts show that radiosensitivity depends not only on the kind and number of genomes but also on the kind of alleles present.

62. *Radiosensitivity of Several Commercial Varieties and Useful Mutants Induced by X-rays in Tobacco*

(By S. MATSUMURA and T. FUJII)

Dry seeds of 5 commercial varieties, Bright Yellow, Dixie Bright 101, Golden Wilt, White Burley and Matsukawa, were exposed to the same dosage (30 kr) of X- and γ-rays. There was a difference in radiosensitivity among those varieties. The germination rate of treated seeds markedly decreased in Bright Yellow, Dixie Bright 101 and Matsukawa, especially at γ-irradiation. In these commercial varieties the germination and the growth of seedlings were more delayed.

The mutant "early" of the variety Bright Yellow was irradiated in 1956 with X-rays at 30 kr. In the X_2-generation many recessive mutants were observed, such as abnormal flowers, narrow leaves, etc.

"Early" (No. 6), "pubescent" (No. 12) and "yellowish green" (No.

Table 1. Yield in kg and value in Yen of leaves of X-ray mutants of flue-cured tobacco (1957).

Strains	% of dry matter	Yield in kg per Tan*	Value in Yen per kg	Average grade	Return per Tan* in Yen
Bright Yellow	16.5	136	222	5.25	30,206
6-2-2A	16.2	115	222	5.25	25,526
6-2-2B	15.7	123	229	5.15	28,526
6-3	19.0	143	226	5.20	32,172
12-2A	15.8	135	312	3.76	42,084
12-2B	15.6	155	287	4.21	44,453
12-2C	14.5	149	311	3.76	46,330
13-2A	15.1	133	281	4.31	37,440
13-2B	15.3	129	269	4.51	34,706
13-2C	15.5	120	323	3.54	38,755

* Tan=*ca.* 0.1 ha.

13), mutants of Bright Yellow, were of good quality and valuable for the improvement of tobacco. Table 1 shows the results of comparative experiments with Bright Yellow as standard in the field of our institute in 1957. From this table No. 12–2 is the best and No. 13–2 is next to it. Both strains show clearly better results than Bright Yellow. Simultaneously similar comparative experiments with these strains were carried out on a large scale in several Tobacco Experiment Stations of Japan Monopoly Corporation (Mito, Utsunomiya, Hatano, Misima, Iwata and Hyôgo). There was a difference among the experimental fields. Generally No. 12–2 and No. 13–2 again showed better results, especially in the southern districts. The latter strain was slightly better than the former. No. 12–2 is not earlier than Bright Yellow and its flue-cured leaves, especially lower ones, are thick and rough, and have hard texture and low water-absorptiveness. No. 13–2 is a few days later than Bright Yellow and has slightly more leaves, of which the upper ones are thin and shiny and have a fine texture and high absorptiveness. Both strains are of good quality and very promising in the southern districts.

63. *Induction of Bud-sports by X- and γ-rays*

(By S. MATSUMURA and T. FUJII)

In *Cinnamomum camphora* seedlings one year old and cuttings were exposed to X-rays (180 KVP, 3 mA, 240 r/min) and γ-rays (^{60}Co, 0.6–1 kr/hr) at the dosage 1, 2, 4 and 6 kr. The number of successful planting and sprouting per seedling or cutting shoot decreased in proportion to the increase of radiation dosage. X-rays were more effective in this respect than γ-rays (Tab. 1). Also, the frequency of plants with abnormal or deformed leaves increased roughly with the increase of dosage.

In the variety Nôrin No. 1 of sweet potatoes (*Ipomea batatas*) each root-tuber was longitudinally cut in 2 pieces. Each half was subjected to X-ray (180 KVP, 25 mA, 154 r/min) and γ-ray treatments (20 days by ^{60}Co) at 2.5, 5, 10 and 15 kr. The other half was untreated and used as control. X-rays effectively inhibited sprouting, while the effect of γ-rays was not so much. When X-irradiation was applied the frequency of sprouted tuber-halves and the number of sprouts per tuber-half decreased with the increase of dosage. When treated with X- and γ-rays, sprouting was always markedly delayed, especially at higher dosage. After planting of these sprouts, white, white-stripe, deep color and oblong shape were often found as chimera in new root-tubers.

Table 1. Effect of radiations on sprouting in *Cinnamomum camphora*.

Treatments (Dose)			Cont.	1 kr	2 kr	4 kr	6 kr
Seedling	X-ray	No. of seedling sprouted (%)	97	83	70	9	7
		No. of sprout per seedling	3.2	3.0	3.1	2.4	2.0
		No. of abnormal seedling (%)	3 (3.1)	8 (9.6)	12 (17.1)	3 (33.3)	1 (14.3)
	γ-ray	No. of seedling sprouted (%)	96	98	94	91	50
		No. of sprout per seedling	2.8	2.9	3.4	2.9	2.6
		No. of abnormal seedling (%)	1 (1.0)	7 (7.1)	7 (7.4)	10 (10.9)	13 (26.0)
Cutting	X-ray	No. of seedling sprouted (%)	86.96	77.55	77.51	38.00	17.65
		No. of sprout per seedling	1.4	1.5	1.2	1.2	1.3
	γ-ray	No. of seedling sprouted (%)	62.50	59.18		33.33	
		No. of sprout per seedling	1.5	1.2		1.1	

64. *Induced Polygenic Mutations in Rice*

(By Hiko-Ichi OKA and Jiro HAYASHI*)

From seeds of a pure line of rice, a control and two X-rayed (6,000 r and 12,000 r) plots were prepared. They were grown in bulk for four generations. Plants with any recognizable change (sterility, dwarfness, etc.) were removed from the plots during the X_2 to X_4 generations. 75 plants from each plot were taken at random from the X_4, and the resulting X_5 lines were tested in an experiment of split plot design with three replications. Heading date and plant height were observed.

The results of this experiment showed that (1) the distribution was completely normal in all plots, (2) the control and X-rayed plots had the same population mean, and (3) the standard deviation became larger due to irradiation. Further, selection in both directions from the X_5 populations showed that X-rayed plots had larger heritability values than the control plot. These facts may indicate that in polygenes for quantitative

* Ohara Institute for Agricultural Biology.

characters, mutations with plus and minus phenotypic effects occur equally frequently. The rate of increase of genetic variance due to 1,000 r irradiation was 0.015 (day) in heading date, and 0.084 (cm) in plant height. (c.f. Jour. Heredity, 49: 11–14)

65. *Effects of γ-radiations upon Germination and Growth of Rice Plants*

(By T. OHTA, S. KAWASHIMA and S. MATSUMURA)

Dormant seeds of paddy rice were exposed to γ-rays by ^{60}Co at 5–70 kr. Just after irradiation they were sown in an air-conditioned greenhouse at 28°C and 80% humidity. There was no marked difference in the germination rate between untreated and treated seeds at less than 40 kr, but the germination rate was reduced to 60% at 50 kr and further to 22% at 70 kr. The number of surviving seedlings a month after sowing decreased with the increase of the dosage of more than 30 kr. It is assumed that LD–50 and LD–100 are about 45 kr and 70 kr, respectively. The growth of seedlings from irradiated seeds was delayed in proportion to the dosage of more than 20 kr.

In some seeds stored about 2 months at room temperature (about 15°C) after irradiation, the effect of γ-rays upon the germination and growth of seedlings seemed to be more pronounced at higher dosage (50 kr or more). Also, when 30 day storage at 0°C was used after irradiation, the germination and growth of seedlings were delayed about 10 days, compared with 25°C storage. When the seeds irradiated at 30, 50 and 70 kr were sown at 20°C and 28°C, the growth of seedlings at low temperature was more delayed and the effect of γ-rays at 50 and 70 kr was smaller than at high temperature. In seeds one year old LD–50 is between 50 and 70 kr and radiosensitivity may decrease with the aging of seeds.

Without the soaking (water content, 13.7%) and after the soaking for 4 hrs (21.9%), 12 hrs (27.4%), 24 hrs (31.1%) and 48 hrs (35.0%), the seeds were exposed to γ-rays at 5–50 kr. Radiosensitivity in respect to the growth of seedlings increased with the increase of water content. It was sudden in the seeds irradiated after 4 hrs soaking, increasing later gradually with the increase of soaking time. When the seeds were soaked in 0.75% solution of NaCl for 72 hrs and then irradiated, they could not germinate at all even at 40 kr.

Young seed roots about 7 cm long were irradiated. At 4 kr the elongation of roots was inhibited and at 12 kr the lateral roots were nearly all inhibited.

66. *Radiosensitivity and Mutants in Barley Induced by Irradiation*

(By S. MATSUMURA and T. FUJII)

Dormant seeds of several commercial malt varieties of *Hordeum distichum* were subjected to X- and γ-ray treatments. X-rays were applied without filter, at 160 KVP, 3 mA and 10 kr, and γ-rays were applied with ^{60}Co, at 10, 20 and 60 kr. Radio-sensitivity of different varieties was compared as to germination and survival rates and the growth of seedlings. *Hordeum* had generally a lower sensitivity than *Triticum*. There was no difference in the germination rate between untreated and treated seeds (X—10, γ—10 and γ—20 kr). In two varieties with more sensitive germination, Early Golden Melon and Asahi No. 5, the germination rate was reduced to about a half of that of untreated seeds at γ—60 kr. On the other hand, the growth of seedlings was slightly delayed at X—10 and γ—10 kr, and was markedly inhibited at γ—60 kr, and most of the seedlings died before heading in Asahi No. 5. There was a marked difference concerning survival rates between different varieties.

Various kinds of chlorophyll mutants, such as albina, chlorina, viridoalbina, etc., were found among the seedlings of the X_2-generation. The frequency of head progenies with mutations generally increased in proportion to the radiation dosage. At the same dosage (10 kr), X-irradiation was more effective in this respect than γ-irradiation. Three varieties, Early Golden Melon, Asahi No. 5 and Spratt Archer, had the highest rate of chlorophyll mutants, while it was lowest in Extra Plumage and Plumage Archer with lower survival rates. It is assumed that the effect of radiation on physiological and genetic conditions is different with different kinds of radiations and varieties used.

67. *Effect of AET upon Radiosensitivity of Wheat Seeds*

(By S. MATSUMURA and S. KAWASHIMA)

To examine the protection effect of AET (Amino-ethyl-isothiuronium) upon radiosensitivity, seeds of *Triticum monococcum flavescens* were treated with 0.0001 and 0.001% AET-solution for 24 hrs just before X- and γ-irradiations at 5 and 9 kr.

Seeds treated with 0.001% solution showed no higher germination rates and no better growth of seedlings than the water-soaked ones, while when treated with 0.0001% solution rather better germination and growth of seedlings were observed. X-rays were more effective than γ-rays. At X-irradiation, especially when 0.001% treatment was used, AET unexpec-

tedly increased the inhibiting effect of radiation. In γ-rays the protection effect of AET was generally not found at 9 kr, while only the treatment with 0.0001% AET at 5 kr slightly promoted germination and growth. This promotion might be due to the effect of 0.0001% AET, mentioned above. The protection effect of AET upon radiation could not, therefore, be easily determined.

68. *Diffusion of Radioisotopes in Soaked Wheat Seeds*

(By S. KONDO, N. YOKOTA and S. KAWASHIMA)

A simple method used to induce mutational changes in plants by radiations is to soak their seeds in water solutions containing radioactive isotopes. These solutions shall be called by their abbreviation; RI solutions. This method is, however, accompanied by difficulties in the measurement of given doses.

The soaking experiments were performed in a room of constant temperature of 21±0.5°C with dry seeds of wheat, *Triticum monococcum flavescens*, soaked in RI solutions of pH 6 to 7 which contained 10 μc/cc of ^{32}P, ^{131}I, ^{90}Sr, ^{137}Cs, ^{65}Zn, each. Each set of experiments comprised 10 seeds soaked in an RI solution of 2 cc. The changes in the radioactivity of the seeds were detected by a G-M counter and X-ray film. The number of counts of RI solutions was measured by a Philips G-M counter, Model 4035, using a mica window G-M counter at 13.5 cm distance, with a working voltage of 725 volts. After soaking the seeds in an RI solution for a given time, each seed was rotated one revolution on a filter paper and then counted as an intact seed. To eliminate the self-absorption error in counting, each seed was ground into a fine powder and then counted. The count per minute (cpm) for these methods are denoted by A and B, respectively. The cpm's of A and B were compared with the cpm due to the RI contained in the

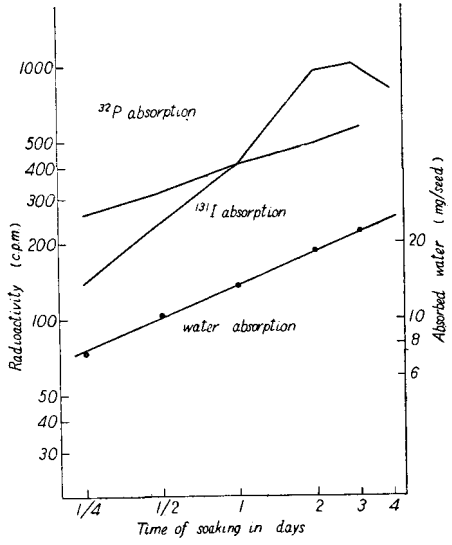


Fig. 1. Absorption curves for wheat seeds soaked in radioactive solutions.

outside solution, C. The amount of the outside solution was exactly equal to the weight increase of the seed at that time. Then, B/A or C/A can be used as a measure of the average concentration of the RI in the water absorbed by the seed. The amounts of RI solutions diffused in the wheat seeds per days soaked are shown in Fig. 1 and Table 1. The water absorption shown in Fig. 1 is indicated by the average weight

Table 1. The concentration of ^{32}P on, and inside the soaked wheat seeds, *T. monococcum flavescens*, relative to the outside solution.

Days soaked	Outside radio-activity, A	Surface and inside radioactivity		Relative activity	
		Before grinding, B	After grinding, C	B/A	C/A
1/4	500 cpm/seed	99 cpm/seed	145 cpm/seed	0.20	0.29
1/2	797 〃	120 〃	164 〃	0.15	0.21
1	1030 〃	147 〃	171 〃	0.14	0.17
2	1420 〃	384 〃	452 〃	0.27	0.32
3	1750 〃	483 〃	601 〃	0.28	0.34
4	1820 〃	387 〃	505 〃	0.21	0.28

The symbol A denotes the cpm/seed of the outside solution of an amount equal to the increase in weight of a soaked seed; B and C those of wheat seeds after soaking as an intact seed and then being ground, respectively.

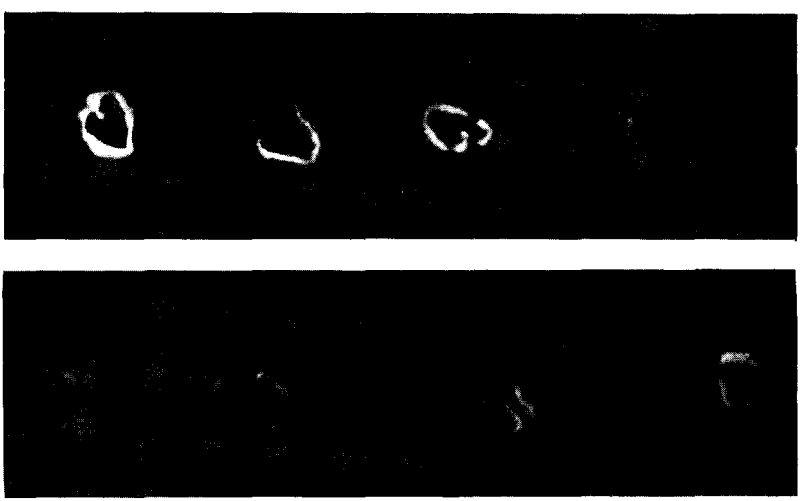


Fig. 2. Macro-autoradiographs of wheat seeds soaked 3 days in ^{32}P and ^{131}I solutions.

increase per seed for 50 seeds. From Table 1, we find that the RI concentration inside the seeds in lower than in the outside original solution and that the quick measurement of cpm of intact seeds results in a relatively good approximation to the true cpm of ground seeds. In fact, the macro-autoradiographs in Fig. 2 show that RI solutions are mostly

Table 2. A comparison of adsorbance ratios of radioisotopes on the soaked wheat seeds, *T. monococcum flavescens*.

Days soaked	^{65}Zn	^{90}Sr	^{137}Cs	^{32}P
1/4	8.06	—	1.33	0.20
1/2	—	—	—	0.15
1	4.47	4.33	0.83	0.14
2	3.14	—	0.83	0.27
3	2.97	—	0.78	0.28
4	2.57	—	0.70	0.21
4*	3.24*	—	1.83*	0.28*

All the values are expressed in B/A except for those with asterisks which are in C/A. The B/A and C/A are defined in Table 1.

adsorbed on the seed coats and that the RI concentrations inside the seeds are very low for ^{131}I and ^{32}P. The abrupt change in the absorbed amount of ^{32}P shown in Fig. 1 may indicate that the biological function of the seed coats changes between one and two days after being soaked. This change could not be detected by ^{131}I. An interesting fact is shown in Table 2 that ^{65}Zn, ^{137}Cs and ^{90}Sr are very strongly adsorbed on wheat seeds compared with ^{32}P.

We may thus conclude that the seed coats of wheat are serving as filter for radioisotopes. It should be noted that the uptake of RI solution by plants may always be performed only through such semipermeable membranes. If such membranes could be synthesized, they might be used for decontamination of RI contaminated solutions. Due to the semipermeability of the seed coats, the dosimetry of soaked seeds in ^{32}P or ^{131}I solutions can be made with about 10% accuracy only if the radiations from outside the seeds are taken into account. In the case of ^{65}Zn, ^{90}Sr, and ^{137}Cs, the adsorbed or absorbed amounts also considerably contribute to the dosage given to seeds.

K. GENETICS OF MICROORGANISMS

69. *Transductional Analysis of Monophasic Types of Salmonella*

(By Tetuo IINO and Joshua LEDERBERG*)

The specificity of flagellar (H) antigen in *Salmonella* is controlled by two distinct loci, phase-1 by H_1 and phase-2 by H_2. Which one is manifested in a given clone depends on the phase determinant at the H_2 locus. That is, the alternation of H_2 state leads to the alternative expression, which has been known as phase variation of *H* antigen (the Annual Report, 1956).

Some *Salmonella* strains do not express two phases but only one. Those strains are called monophasic-1 or -2 strains depending on their fixed phase, either phase-1 or phase-2 respectively. Three additional groups of the genes which are involved in the production of *H* antigen were discovered by transductional analysis of the monophasic strains.

S. abortus-equi CDC-26 is stable in phase-2, enx-type. A rare alternative phase can occasionally be obtained by antiserum selection, resulting in an equally stable phase-1, a-type. Transductions were carried out from enx-phase of CDC-26 to i-phase of a diphasic strain of *S. typhimurium*, TM2 i : 1.2 (such transduction is designated CDC-26 (a) : enx—x TM2 i : 1.2). Among 65 transductional clones which had been selected on semisoli nutrient gelatine-agar media (NGA), 4 expressed diphasic a : 1.2 type, 42 diphasic i : enx and 19 monophasic-2 enx which carried a hidden H_1^i. Thus, when *a* is transduced the resultin transductions remain diphasic, whereas when enx is transduced a fraction of the transductional clones becomes monophasic. By anti-enx selection, i-phase cultures were obtained from the monorphasic enx-transductional clones. The i-phase cultures obtained were also monophasic. The stabilization of the H_2 state in *S. abortus-equi* is therefore caused by a factor which is linked to H_2. The factor will be given a symbol Vh_2^-.

S. typhimurium SW1061 is a monophasic-2 mutant of a diphasic strain TM2 i : 1.2. The culture reacts to anti-1.2 serum but not to anti-i serum. However, the strain frequently produces nonmotile *H*-negative (non-flagellar) subclones, which in turn revert to motile cells with 1.2 antigen in successive cultures. From the transduction, diphasic *S. abony* CDC-103 *b* : enx—x SW1061, monophasic-2 enx, diphasic *b* : 1.2 and a a small number of i : 1.2 types were obtained. The change from the monophasic type to diphasic types was always coupled with the loss of the ability to oscillate

* Department of Genetics, University of Wisconsin, Madison, Wis., U. S. A.

between motile and non-motile types. These results are consistent with the following explanation. In SW1061, H_1^1 is inactive; on the other hand $H_2^{1.2}$ changes its state as in usual diphasic strains. Consequently, when H_2 is active, phase-2 antigen, 1.2, is produced, and when H_2 changes to inactive, that is both H_1 and H_2 are inactive, the cell produces H antigen and becomes non-motile. The production of diphasic i : 1.2 type suggests that the inactivation of H_1 is not caused by an intrinsic change of H_1 itself but by an inhibition of its function by a gene linked to H_1. The linkage and the recombination between H_1 and the controller of H_1-activity were confirmed on Fla_1^+ (linked to H_1) transduction SW1061—*x S. heidelberg* Fla_1^- r : 1.2, from which monophasic-2, -(i) : 1.2, and diphasic i : 1.2 as well as diphasic r : 1.2 were obtained. The controller of H_1-activity is designated Ah_1^-. Ah_1^- was discovered in two other monophasic-2 strains of *S. typhimurium*, SW629 an SW547. All of three Ah_1^- are non-allelic and linked to H_1 and Fla_1. The parallel type of gene, Ah_2^-, which inhibits the function of H_2 was discovered on four monophasic-1 strains of *S. typhimurium*. All of them are linked to H_2. Both Ah_1 and Ah_2 are phase specific but not concerned with the specificity of antigen types, which are detemined exclusively by H_1 and H_2.

L. MATHEMATICAL GENETICS

70. *Chance of Fixation of Mutant Genes*

(By Motoo KIMURA)

Because of its importance in evolutionary genetics, the probability of fixation of mutant genes has been studied by Fisher, Haldane and Wright. However, due to mathematical difficulties involved, so far only a few cases have been successfully worked out.

In the present report I will present a quite general solution in which an arbitrary degree of dominance is taken into account.

Let N be the effective size of a population and let s and sh be the selective advantages of the mutant homozygote AA and heterozygote AA' respectively over the wild type homozygote $A'A'$, then the probability of fixation of a mutant gene A which has appeared in the population may be given by

$$u=\int_0^{1/2N} e^{-2cDx(1-x)-2cx}dx \Big/ \int_0^1 e^{-2cDx(1-x)-2cx}dx,$$

where $c=Ns$ and $D=2h-1$. For an advantageous but nearly recessive mutant gene ($s>0$, $0<h\ll 1$), the above formula may be substituted by

$$u=e^{-2Nsh^2/(1-2h)}\sqrt{\frac{2s(1-2h)}{\pi N}}\Big/\{1-2\Phi(\sqrt{4Nsh^2/(1-2h)})\},$$

unless $2Ns$ is small. Here $\Phi(x)$ stands for the error function

$$\Phi(x)=(1/\sqrt{2\pi})\int_0^x e^{-x^2/2}dx.$$

As an example, consider a case with $N=10^3$ and $s=10^{-1}$. If the mutant gene is completely recessive ($h=0$), $u\approx 0.8\times 10^{-2}$. With slight phenotypic effect of $h=0.01$ in the heterozygote $u\approx 0.9\times 10^{-2}$, while with $h=0.1$, $u\approx 2.3\times 10^{-2}$.

(cf. KIMURA, M. 1957. Ann. Math. Stat. 28:882-901)

71. *Effect of Natural Selection in a Finite Population*

(By Motoo KIMURA)

In the last report of our institute, I have presented the results of investigation on the interaction between natural selection and random genetic drift, assuming complete dominance between a pair of alleles. The present report concerns with a more general case with an arbitrary degree of dominance.

Consider a pair of alleles A and A' with respective frequencies of x and $1-x$ in a population of effective size N. If we designate by s, sh and 0 the fitnesses of three genotypes AA, AA' and $A'A'$ respectively measured in Malthusian parameters, then the probability density, $\phi(x, t)$, that the frequency of A lies between x and $x+dx$ satisfies the following partial differential equation:

$$\frac{\partial\phi}{\partial t}=\frac{\partial^2}{\partial x^2}\left\{\frac{x(1-x)}{4N}\phi\right\}-\frac{\partial}{\partial x}\{sx(1-x)[h+(1-2h)x]\phi\}.$$

The smallest eigenvalue λ_0 giving the rate of steady decay may be expanded into a power series in Ns as follows:

$$2N\lambda_0=1+K_1(Ns)+K_2(Ns)^2+K_3(Ns)^3+K_4(Ns)^4+\ldots,$$

where

$$K_1=-\frac{1}{5}D,\quad K_2=\frac{1}{2\cdot 5}+\frac{2^2\cdot 3}{5^3\cdot 7}D^2,$$

$$K_3=\frac{1}{2\cdot 5^3\cdot 7}D-\frac{2^2}{5^5\cdot 7}D^3,\quad K_4=-\frac{1}{2^3\cdot 5^3\cdot 7}-\frac{7^3}{2\cdot 3^3\cdot 5^5}D^2-\frac{2^2\cdot 3^5}{5^6\cdot 7^3\cdot 11}D^4,$$

etc, in which D represents degree of dominance such that $D=2h-1$. Thus for the case of no dominance $D=0$ and for the case of complete dominance $D=1$ or -1 according as A is either dominant or recessive.

72. *Zygotic frequencies in a Partially Self-fertilizing Population*

(By Motoo KIMURA)

In higher plants, practice of self-fertilization is quite wide-spread, with the result more homozygotes may be found in a population than would be expected under exclusive random mating.

We shall consider an infinitely large population and designate by R the probability that an ovule is fertilized by a pollen taken as a random sample from the entire population and by $S(=1-R)$ the probability that it is fertilized by a pollen from the same plant. Thus R and S may be termed the proportion of cross- and self-fertilization respectively.

(i) *One locus segregating*: Let A_1, A_2,..... be an arbitray number of alleles with respective frequencies of x_1, x_2,..... Assuming that mutation, selection and migration are absent, the equilibrium frequency of the homozygote A_iA_i may be given by

$$F_r\{A_iA_i\}=\frac{2Rx_i^2+Sx_i}{2R+S},$$

while the frequency of the heterozygote A_iA_j $(i\neq j)$, may be given by

$$F_r\{A_iA_j\}=\frac{4Rx_ix_j}{2R+S}.$$

If the population is not in equilibrium, deviations of zygotic frequencies from their respective equilibrium values may be decreased at the rate of

$$(S+2R)/2$$

per generation.

(ii) *Two loci segregating*: Let x_i be the frequency of the i-th allele A_i in the first locus and let y_k be that of the k-th allele B_k in the second locus. If r is the recombination fraction between these two loci, the equilibrium frequencies of double homozygote $A_iA_iB_kB_k$, single heterozygotes $A_iA_iB_kB_l$, $A_iA_jB_kB_k$ ane double heterozygote $A_iA_jB_kB_l$ are given respectively by

$$F_r\{A_iA_iB_kB_k\}=\frac{2Rx_i^2y_k^2+Sx_iy_k}{2R+S}-\frac{2RSLx_iy_k(1-x_i)(1-y_k)}{(2R+S)(2R+S+LS)}$$

$$F_r\{A_iA_iB_kB_l\}=\frac{4Rx_iy_ky_l[(2R+S)x_i+LS]}{(2R+S)(2R+S+LS)} \quad (k\neq l)$$

$$F_r\{A_iA_jB_kB_k\}=\frac{4Rx_ix_jy_k[(2R+S)y_k+LS]}{(2R+S)(2R+S+LS)} \quad (i\neq j)$$

and

$$F_r\{A_iA_jB_kB_l\}=\frac{8Rx_ix_jy_ky_l}{2R+S+LS} \quad (i\neq j,\ k\neq l),$$

where $L=2r(1-r)$. These results show that distributions of zygotic frequencies between two loci are not independent under partial self-fertilization even when the two loci are on different chromosomes. For example, with two unlinked loci each containing a pair of alleles in equal frequencies the coefficient of departure from random combination of homozygous phases, i.e.

$$\theta_{(ii,kk)}=\frac{F_r\{A_iA_iB_kB_k\}}{F_r\{A_iA_i\}F_r\{B_kB_k\}}=1+\frac{R(1-R)}{3+R}$$

is always larger than unity, giving the maximum value of 1.07 when $R=2\sqrt{3}-3\approx 0.464$.

73. *Overdominant Genes in an Partially Self-fertilizing Population*

(By Motoo KIMURA)

Behavior of heterotic genes in a random mating population has been rather well understood, but the one in a non-random mating population has not yet been fully analysed. In plants, however, self-fertilizing mechanism is quite wide-spread and investigation into the behavior of heterotic or overdominant genes in a partially self-fertilizing population should be required.

Let R be the probability that an ovule is fertilized by a pollen taken as a random sample from the entire population (assumed to be very large) and $S\,(=1-R)$ be the probability that it is fertilized by a pollen produced from the same plant. Consider a pair of alleles A_1 and A_2 and designate the selective values of three genotypes A_1A_1, A_1A_2 and A_2A_2 by $1-s_1$, 1 and $1-s_2$ respectively, then one of the following conditions must be satisfied in order that the alleles A_1 and A_2 are maintained in stable equilibrium:

(i) If $s_1, s_2>1/2$, a stable equilibrium is possible for any degree of self-fertilization.

(ii) If $s_1=s_2=1/2$, the proportion of self-fertilization must be smaller than 1, i.e. $S<1$.

(iii) If at least one of s_1 and s_2 is less than 1/2, the proportion of self-fertilization must be restricted such that

$$S<\frac{2s_2(1-s_2)}{s_1+s_2-2s_1s_2},$$

assuming here that $s_1\geqq s_2$.

If one of the above conditions is satisfied, the equilibrium genotypic frequencies may be given by

$$\begin{cases} P_{11}=(1-\check{f})\hat{x}_1^2+\check{f}\hat{x}_1 \\ 2P_{12}=(1-\check{f})2\hat{x}_1\hat{x}_2 \\ P_{22}=(1-\check{f})\hat{x}_2^2+\check{f}\hat{x}_2 \end{cases},$$

where P_{11}, $2P_{12}$ and P_{22} are the frequencies of A_1A_1, A_1A_2 and A_2A_2 respectively and $\hat{x}_1$ and $\hat{x}_2$ are the equilibrium frequencies of A_1 and A_2 such that

$$\hat{x}_1=\frac{i_s}{1-\check{f}}\left(\frac{1}{s_1}+\frac{\check{f}}{s_2}\right), \qquad \hat{x}_2=1-\hat{x}_1 ,$$

in which $i_s=s_1s_2/(s_1+s_2)$ and

$$\check{f}=\frac{1}{4i_s}[2(1-i_s)-(1-2i_s)S-\sqrt{D}] ,$$

where

$$D=4(1-i_s)^2+(1-2i_s)^2S^2-4(1+i_s)(1-2i_s)S .$$

It should be noted here that $\check{f}$ is different from the ordinary inbreeding coefficient defined for neutral genes which is

$$f=\frac{S}{2R+S}$$

under partial self-fertilization. For example, with $S=R=0.5$, $s_1=s_2=0.5$, the condition (ii) is satisfied and $\check{f}=(5-\sqrt{17})/4\approx 0.22$ which is different from $f=1/3\approx 0.33$.

74. *On the Decrease of Fitness due to Deviation from Optimum in Quantitative Characters.*

(By Motoo KIMURA)

It has been confermed for several simple quantitative or meteric characters such as height and weight that phenotypically extreme individuals (deviants) are less fit than those near the population average.

The present report is an attempt to treat this problem based on the assumption that for each locus concerned to a metric character, fitness of each gene combination is determined independently of its contribution to the metric character.

Consider a locus with an arbitrary number of alleles, say, $A_1, A_2, \cdots A_n$ and designate the frequency of homozygote A_iA_i by P_{ii} and that of heterozygote A_iA_j $(i\neq j)$ by $2P_{ij}$. We assume that a large number of

loci are concerned for the determination of the metric character. If Y is the measure of the metric character which distributes normally with mean $\overline{Y}$ and standard deviation σ then the average fitness of individuals with measurement Y may be given by

$$(1) \qquad \bar{a}(Y)=\bar{a}+\left(\frac{Y-\overline{Y}}{\sigma^2}\right)\sum \sigma_{ay}+\frac{(Y-\overline{Y})^2-\sigma^2}{2\sigma^4}\sum \mu_{12}(a, y)\ ,$$

where $\bar{a}$ is the average fitness of the population and the summation is over all relevant loci. In the above formula σ_{ay} and $\mu_{12}(a, y)$ are given by

$$\sigma_{ay}=\sum_{ij} a_{ij}(y_{ij}-\bar{y})P_{ij}$$

and

$$\mu_{12}(a, y)=\sum_{ij} (a_{ij}-\bar{a})(y_{ij}-\bar{y})^2P_{ij}$$

in which a_{ij} and y_{ij} stand for the contributions of A_iA_j to the fitness and the metric character respectively.

If $\sum \mu_{12}(a, y)<0$, there exists an optimum value of Y for which $\bar{a}(Y)$ takes its maximum value. Thus we obtain

$$\frac{\bar{a}(Y)-\bar{a}(Y_{opt})}{\left(\frac{Y-Y_{opt}}{\sigma}\right)^2}=-\frac{1}{2}\frac{\sigma_g^2}{\sigma^2}\theta\ ,$$

where Y_{opt} is the optimum value of Y, σ_g^2/σ^2 is the heritability of metric character and θ is given by

$$\theta=-\sum \mu_{12}(a, y)/\sigma_g^2\ .$$

As an important application of the above result, we assume that in each locus concerned a mutant gene is maintained in equilibrium due to counter-balancing effect of mutation and selection. This leads to

$$\theta=1/\overline{(1/h)}\ ,$$

where h is the selective disadvantage of the mutant heterozygote and $\overline{(1/h)}$ means the average value of $1/h$ weighted according to the additive genetic variance of the metric character in each locus.

M. TECHNICAL NOTE

75. *Dose-rate Distribution in the ^{60}Co Gamma Ray Room*

(By S. KONDO and S. MATSUMURA)

The facility for ^{60}Co gamma ray irradiation was built in May, 1956[1].

The plan of the gamma-ray room and the sectional view of the ^{60}Co container are presented in Figs. 1 and 2.

The lead filters shown in Fig. 2 were designed to produce three regions of roughly constant but different dose rates. For simplicity's sake in the design only the primary radiation was taken into account. The dose-rate distribution on a horizontal line passing through the point 20 cm below the source was measured with a solid CdS: Ag crystal detector having 5 mm in diameter and 23 mm in length. Fig. 3 shows that the first and second filters give fairly good plateau regions, constant to about ±5% for annuli at least 5 cm wide at the distance 20 cm below the source, whereas the third filter provides no real plateau. Apparently when the attenuation factor is greater than 10, secondary radiations and boundary effects of the filter must be taken into account in the design of such a facility.

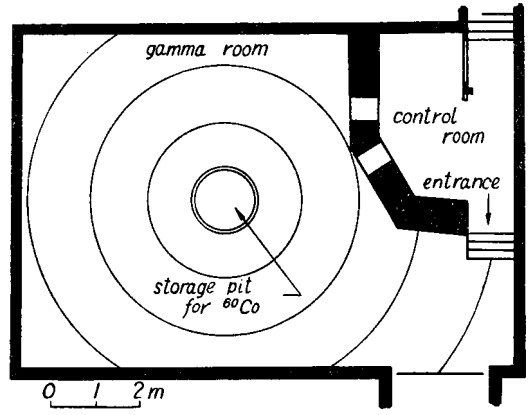


Fig. 1. Gamma ray irradiation room of the National Institute of Genetics.

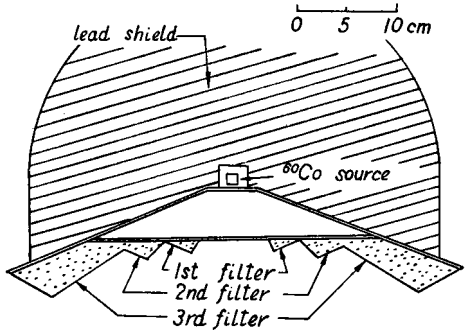


Fig. 2. The sectional view of the container of ^{60}Co.

The total emission of the source was determined in two ways. With the source 200 cm above the floor an inverse square curve was taken with the FRICKE ferrous sulphate chemical dosimeter[2]. The dose rate was also measured at a single distance with a Victoreen ion chamber. The effective source strength S in curies was calculated as:

$$S=\frac{d}{(1.35x^2)10^4}$$

where d is the measured dose rate in r/hr at x cm from the source and one curie of ^{60}Co is taken as producing $1.35\cdot10^4$ r/hr at 1 cm. The values obtained are given in Table 1; they indicate good agreement between the

two methods. Our best estimate of the effective source strength was 31.3 curies as of Nov. 1, 1957. For these measurements the ampoules containing the ferrous solution were covered by a 3 mm thick lead petri dish to eliminate secondary and back scattered radiations from the ^{60}Co container. Table 1 also shows that the inverse square law holds well from 9 to 60 cm from the source. If, however, the ferrous dosimeter is unshielded, the apparent source strength varies appreciably when the detector is closer than 20 cm to the source or farther than 160 cm from it.

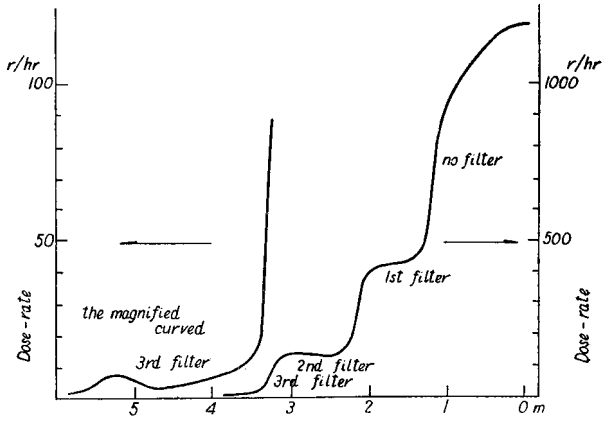


Fig. 3. The distribution curve of dose rate along a horizontal line 20 cm below the ^{60}Co source.
The measurements were made with the use of a small CdS gamma meter on December 24, 1956.

Table 1. Measurements of the effective source strength by FRICKE ferrous dosimeter and Victoreen ion chamber.

Distance between center of detector and center of source	Effective source strength in curies		
	FRICKE ferrous sulphate		Victoreen ion chamber
	Direct result	Corrected for lead shielding	
9.2 cm	26.9	31.0	—
11.6 〃	26.5	30.6	—
18.2 〃	27.4	31.4	—
31.2 〃	27.6	31.8	—
60.0 〃	—	—	31.2

The degree of hazard to operating personnel was checked by the following measurements. When the source was at the bottom of its 100 cm deep storage pit, the radiation at the floor level above the pit was initially 170 mr/hr which was reduced to 13 mr/hr by the addition of a 3 mm thick

shield plate. The average dose rate from scattered radiations at the entrance, when there were no doors, was 4 mr/hr.

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76. *Temperature Dependence of the Window Glass Dosimeter*[1]

(By Sohei KONDO)

Measurement of gamma ray dose by means of changes in the absorption coefficient of ordinary window glass has been reported previously[2]. To improve the accuracy of this glass dosimetry method, the temperature dependence of the glass dosimeters has been empirically investigated. In general irradiated glass loses its induced color at a rate strongly dependent on the storage temperature. The following equation of fading obtains for window glass irradiated with ^{60}Co gamma rays at 22°C

$$D(t,\tau)=D(0,\tau)f(t/\tau)\,, \qquad (1)$$

where $D(t,\tau)$ denotes the value of $D(0,\tau)$ at t hours after the end of irradiation, $D(0,\tau)$ being the increase in absorption density immediately after the τ-hour irradiation*. The fading factor $f(t/\tau)$ can be expressed by:

$$f(t/\tau)=(1+t/\tau)^{1-\lambda}-(t/\tau)^{1-\lambda}\,, \qquad (2)$$

where the fading index λ follows the relation:

$$\lambda=\exp(-11+0.03\,T)\,, \qquad (3)$$

and T is the temperature in degrees Kelvin.

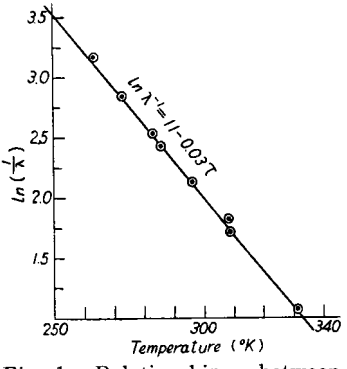

Fig. 1. Relationship between fading index λ given in (2) and temperature T.

The experimental data (Table 1 and Fig. 1) show satisfactory agreement with equations (2) and (3). The relation between the total dose R given to the glass at 22°C and the increase in absorption density $D(0,\tau)$ of the glass is given by:

$$R=(1222\pm 2)D(0,\tau)\tau^{0.154}\,, \qquad (4)$$

* Absorption density D is defined by $I/I_0=10^{-Dx}$ where I and I_0 are the intensities of transmitted and incident radiations, for the present case with wave length 4200 Å; and x denotes the thickness of the glass dosimeter.

where R, $D(0, \tau)$ and τ are expressed in kr, cm^{-1} and hours, respectively. The system of glass dosimetry employed using equations (1) to (4) yields an accuracy of $\pm 2\%$.

Fig. 2 shows the comparison of the response of the glass dosimeter irradiated at 22°C with that at 89°C. From this experiment we conclude that the fading index λ in equation (2) differs from the power index on τ in equation (4). The latter causes the non-linearity in the relationship between R and the induced absorption increase D even well below the saturation of D.

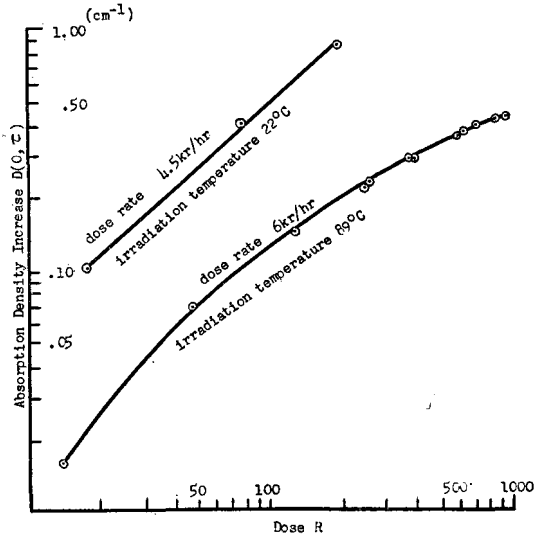

Fig. 2. Dependence of absorption density increase of glass on irradiation temperature.

Table 1. Fading of relative absorption coefficient, $D(t, \tau)/D(0, \tau)$, with time t for $\tau=16$ hr at 22°C with dose rate about 3 kr/hr.

°C \ t (hr) / Fading		1	4 (+5)	8.5 (+10.5)	23 (+25)	46 (+48)	63 (+71)
16°C $\lambda=0.1$	Calculated	0.973	0.926+	0.891+	0.838+	0.795+	0.766+
	Observed	(**)	0.930+	0.909+	0.848+	0.785+	0.746+
36°C $\lambda=0.18$	Calculated	0.947	0.880	0.826	0.729	0.666	0.608
	Observed	(**)	0.854	0.798	0.706	0.659	0.582
58°C $\lambda=0.35$	Calculated	0.875	0.750	0.657	0.519	0.428	0.376
	Observed	(**)	0.732	0.625	0.532	0.442	0.391

** Adjusted to equal the observed values.

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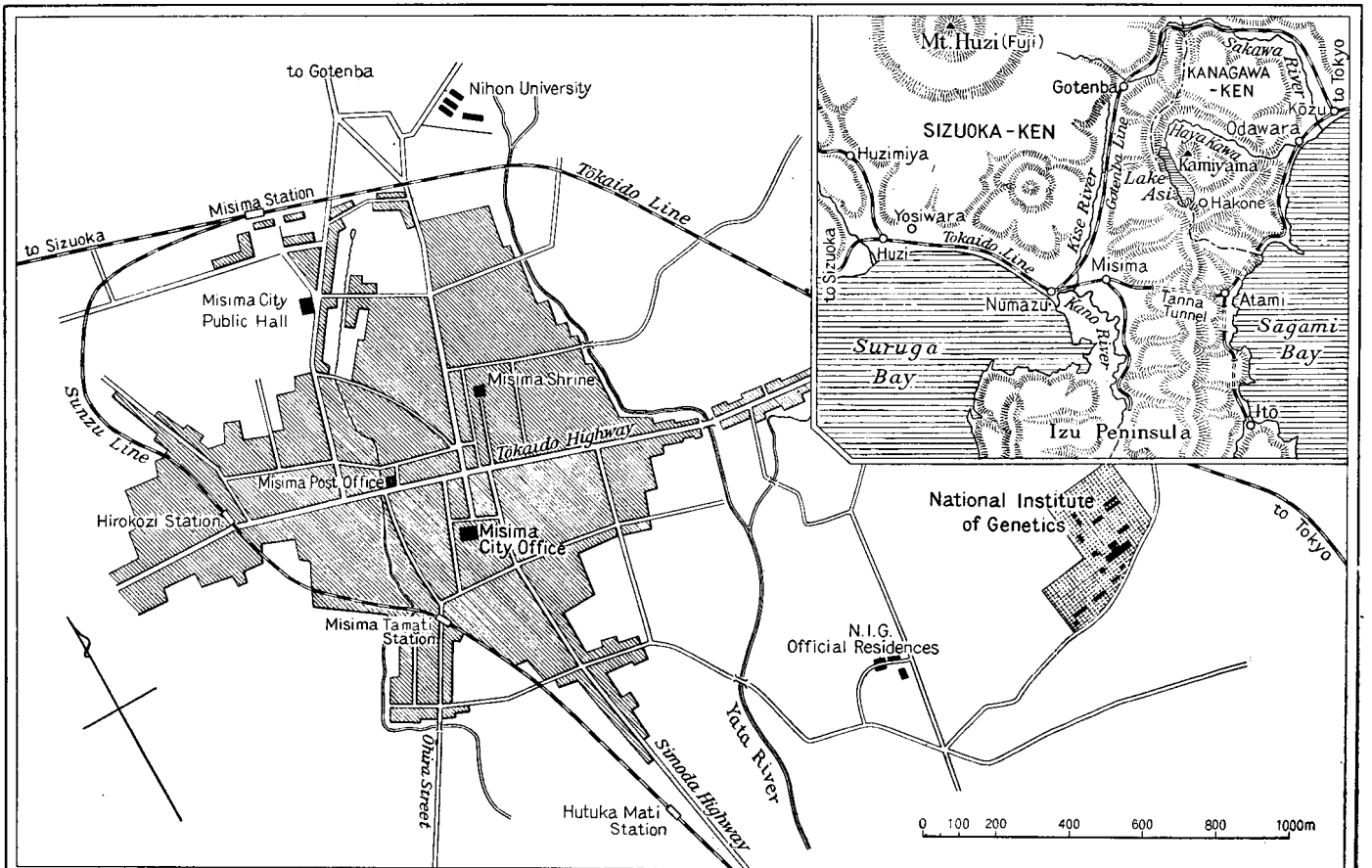
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