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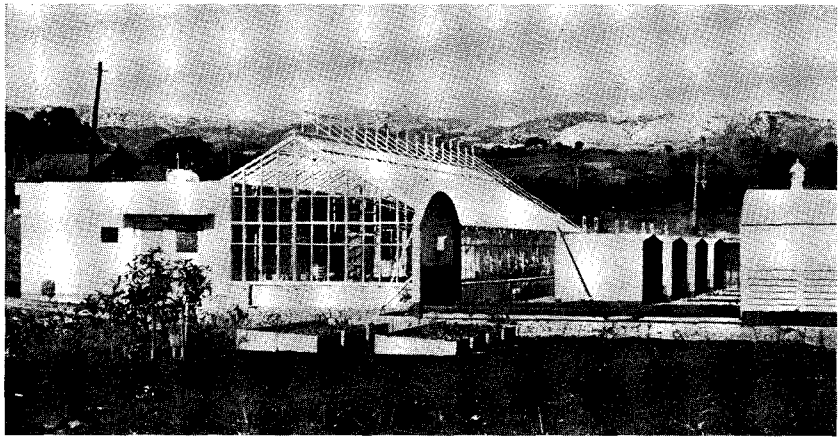

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GENERAL STATEMENT

This year has been more fruitful than last year in the establishment of research facilities, namely completion of two greenhouses, Rice Laboratory and Isolation Greenhouse, Radiation Laboratory and the Second Mousery.

The Rice Laboratory was built for studies on the origin of cultivated rice by a grant of the Rockefeller Foundation. This greenhouse has short-day installation comprising five small paddy fields (2.6×3.5 m). These fields are equipped each with a sliding darkroom chamber, which is controlled by astrodials. The Isolation Greenhouse is constructed for the isolation of plants which are crossed or self-pollinated. It is air-conditioned and has darkrooms for short-day treatments.

The Radiation Laboratory is not quite new. The old Isotope Laboratory was renamed after the new addition of 2 rooms for X-ray equipments, one room for neutron (Ra-Be 500 mg) and another for γ (^{134}Cs 6000 curie) sources.

Collecting tours were conducted by two members participating at the project for the studies on the origin of cultivated rice. Dr. Oka went to Thailand and Prof. Hirayoshi of Gifu University went to Malaya and Java. They could add many valuable wild and cultivated varieties of *Oryza* to our collection. Crossing experiments among different varieties of wild and cultivated rice are now under way.

Using silkworms, mice, *Drosophila*, grasshoppers and tumor cells, animal geneticists began studies on the genetic effects of radiation in animals.

Under the grants from the Ministry of Education, there are two projects which are the joint work of many collaborators within and without the Institute. One is the study on consanguineous marriage in Japan and the other is on the mechanism of manifestation of genes.

Several members of the Institute went to Canada and the United States to attend the Xth International Congress of Genetics (Montreal), the Ist International Wheat Genetics Symposium (Winnipeg) and International Congress of Radiation Research (Burlington).

The second summer seminar on genetics for the senior high school teachers was again very successful this year. 67 teachers from 43 prefectures attended.

ABSTRACTS OF DIARY FOR 1958

Jan. 8. Committee meeting of Animal Genetics group.
Jan. 16. Meeting of Tobacco Research workers.

Jan. 27. Meeting of Rice research group.
Feb. 14. 64th meeting of Misima Geneticists' Club.
Feb. 20. 20th Biological Symposium.
Feb. 21. 65th meeting of Misima Geneticists' Club.
Feb. 22. 17th meeting of the Board of Councillors.
March 7. 21st Biological Symposium.
March19. Committee meeting of Animal Genetics group.
March20. 66th meeting of Misima Geneticists' Club.
March24. Committee meeting of Rice Research group.
April 5. Scientific meeting of Rice Research group.
April 15. 67th meeting of Misima Geneticists' Club.
May 7. Committee meeting of Rice Research group.
May 19. 68th meeting of Misima Geneticists' Club.
May 21. Scientific meeting of Rice Research group.
June 2. 69th meeting of Misima Geneticists' Club.
June 3. Board meeting of Japan Association of Poultry Genetics.
June 4. General meeting of Japan Association of Poultry Genetics.
Committee meeting of Rice Research group.
June 10. Scientific meeting of Rice Research group.
July 11. 22nd Biological Symposium
July 12. Meeting of the Editorial Board of "The Heredity" (Iden)
July 21–24. 2nd series of Summer Seminar on Genetics.
Oct. 7. Committee meeting of Animal Genetics group.
Oct. 30. 70th meeting of Misima Geneticists' Club.
Nov. 10. Committee meeting of Rice Research group.
Nov. 11. Scientific meeting of Animal Genetics group.
Nov. 13. Committee meeting of Animal Genetics group.
Nov. 26. Scientific meeting of Rice Research group.
Dec. 2. Committee meeting of Rice Research group.
Dec. 9. Scientific meeting of Rice Research group.
Dec. 19. 71st meeting of Misima Geneticists' Club.
Dec. 23. Scientific meeting of Rice Research group.

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Misima Branch of Hatano Tobacco Experiment Station

Masao TANAKA, Head Assistants.....5

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Kan-Ichi SAKAI, Vice-president

Association for Propagation of the Knowledge of Genetics

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Kunizô FUKUDA, Emeritus Professor of Tokyo University
Kenpo TSUKAMOTO, Director of National Institute of Radiological Sciences

RESEARCH PROGRAM FOR 1958

Department of Morphological Genetics

Genetics of silkworm (TAZIMA, TSUJITA and INAGAKI)
Studies on the food preference in silkworm (TAZIMA)
Radiation mutagenesis in silkworm (TAZIMA and ONIMARU)
Genetic effects of chronic irradiation upon silkworm (TAZIMA and ONIMARU)
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 culture method (YOSIDA)
Determination and differentiation of sex in plants (TAKENAKA)
Induction of abnormal mitosis and inhibition of growth by substances extracted from certain plants (TAKENAKA)
Interspecific hybridization in *Nicotiana* (TAKENAKA and FURUSATO)
Genetics of *Pharbitis Nil* (TAKENAKA)
Cytotaxonomic studies in Poaceae (TATEOKA)

Department of Physiological Genetics

Genetical analyses on insecticide-resistance of *Drosophila* (OSHIMA)
Physiological and genetical studies on the phenotypic expression of characters in *Drosophila* (OSHIMA and TAIRA)
Studies on the origin of wheat (KIHARA)
Studies on substitution of nucleus in wheat (KIHARA)
Studies on *Agropyron* (MATSUMURA and SAKAMOTO)
Right- and lefthandedness in plants (KIHARA and LILIENFFELD)
Studies on stoneless pomegranates (KIHARA)
Physiological genetics of the reaction of Japanese morning glory to day-length (SAKAMOTO)

Department of Biochemical Genetics

Biochemical genetics of insects and microorganisms (TSUJITA, NAWA and SAKAGUCHI)
Embryological and biochemical studies of silkworm (TSUJITA and SAKAGUCHI)
Radiation biochemistry of genetic materials (NAWA and SAKAGUCHI)
Biochemistry of the mechanism underlying variations in flower colors in plants (ENDO)
Biochemical studies on the differentiation of muscle protein in animals (OGAWA)
Biochemical studies on the mechanism of cell division in animals (OGAWA)
Biochemistry of bitter substance in *Citrullus colocynthis* (OGAWA)
Genetics of virus (TSUJITA and TSUDA)
Studies on the fine structure of the cell (TSUJITA and TSUDA)

Department of Applied Genetics

Studies on breeding and genetics in poultry (YAMADA and KAWAHARA)
Polygenic mutation in quantitative characters of *Drosophila* (YAMADA)
Theoretical studies of plant breeding (SAKAI)
Studies on competition between genotypes in plants (SAKAI and IYAMA)
Population-genetic studies of "Red-Rice" growing among upland rice (SAKAI and IYAMA)
Genetic studies on "Cherry-red leaf" in tobacco plants (SAKAI and IYAMA)
Genetic studies on the migration activity in *Drosophila* (SAKAI and IYAMA)
Polyploidy and sterility in fruit plants (FURUSATO and MIYAZAWA)
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)
Radiation induced mutation in quantitative characters of rice (OKA)

Department of Induced Mutation

Studies on radiation-induced mutation rates in mice (SUGAHARA and TUTIKAWA)
Physicochemical mechanisms of radiation effects on living organisms (SUGAHARA)
Studies on radiation-protection in animals (SUGAHARA)
Genetics of mice (TSUTIKAWA)
Relation between the quality of radiations and mutations (MATSUMURA)
Radiation genetics of wheat and barley (MATSUMURA and FUJII)
Radiation genetics and its practical application (MATSUMURA)
Radiosensitivity in plants (FUJII)

Improvement of sugar beets by means of induced triploidy (MATSUMURA)
Measurement of X-, β- and γ-ray dosages (KONDO)

JOINT RESEARCHES SUPPORTED BY THE GRANT FROM THE ROCKEFELLER FOUNDATION

I. *Origin of Rice*

Section 1. Collection and preservation of *Oryza* species (H. KIHARA)
- a. Number of species being preserved....18 species
- b. Study-tour to Thailand (H. I. OKA)
- c. Study-tour to Malaya and Indonesia (I. HIRAYOSHI)

Section 2. Morphology and physiology of *Oryza* species (S. MATSUMURA)
- a. Taxonomic studies in the family *Graminae* (T. TATEOKA)
- b. Statistical taxonomy of *Oryza* species (H. MORISHIMA and H. I. OKA)
- c. Anatomical studies in rice (T. KATAYAMA)
- d. Susceptibility of wild and cultivated rice strains to blast fungus (K. KATSUYA)
- e. Radiosensitivity of wild and cultivated *Oryza* species (T. FUJII)

Section 3. Population-genetics in wild and cultivated rice (K. I. SAKAI)
- a. Estimation of genetic variability among and within populations of *O. perennis* and *O. sativa spontanea* in Ceylon (K. I. SAKAI and T. NARISE)
- b. Estimation of the percentage of out-crossing by biometrical methods (K. I. SAKAI and T. NARISE)
- c. Investigation of flowering time in wild rice (T. NARISE)

Section 4. Genic studies in wild and cultivated rice (H. I. OKA)
- a. Comparison between *O. glaberrima* and *O. sativa* (H. MORISHIMA and H. I. OKA)
- b. Crossing-experiments for the sterility of hybrids between wild and cultivated rice strains (H. I. OKA and H. MORISHIMA)
- c. Variation in seed dormancy and germinating capacity among wild and cultivated rice strains (H. MORISHIMA and H. I. OKA)
- d. Variation in photoperiodic response among populations of wild rice (T. KATAYAMA, W. T. CHANG and H. I. OKA)
- e. Investigation of rice strains collected from Jeypore Tract, Orissa, India (H. I. OKA and W. T. CHANG)

Section 5. Cytogenetics of *Oryza* species (Y. TAKENAKA)
- a. Karyo-type analysis of *Oryza* species (S. SHIMOYAMA)
- b. Determination of chromosome numbers of *Oryza* species (M. NEZU)
- c. Embryological studies in rice (Y. DOIDA)

d. Cytological studies of haploid plants in rice (C. H. Hu)
e. Studies of trisomics in rice (T. Tsuchiya)
f. Crossing-experiments of *Oryza* species (T. Katayama and H. Morishima)

II. *Studies on genetic effects of radiation on animals*

Section 1. Radiation mutagenesis in the silkworm (Y. Tazima)
a. Variation in mutation response to radiation of silkworm germ-cells. (Y. Tazima and K. Onimaru)
b. *In vitro* cultivation of silkworm spermatogenic cells (T. Sado)

Section 2. Mutation in mammals (T. Sugahara)
a. Studies on mutation rates after chronic irradiation in mice (T. Sugahara and K. Tutikawa)
b. Strain differences in mice in the response of leukocyte number to X-irradiation (K. Tutikawa)

Section 3. *Cytology and cancer problems* (T.H. Yoshida)
a. Behavior of grasshopper chromosomes in response to irradiation (in motion picture) (S. Makino and Y. Ohnuki)
b. Chromosome breaks in Yoshida sarcoma cells induced by X-irradiation (T. Tabata and H. Hirumi)

Section 4. *Radiation biochemistry of genetic materials* (M. Tsujita)
a. Influence of radiation on mitochondrial function in cellular metabolism (S. Nawa and B. Sakaguchi)

Section 5. *Population genetics* (C. Oshima)
a. Polygenic mutation, induced by X-rays, in biometrical characters of *Drosophila melanogaster* (Y. Yamada and O. Kitagawa)
b. Genetic consequences of irradiation and selection in a biometrical character of *Drosophila melanogaster* (O. Kitagawa and Y. Yamada)

FOREIGN VISITORS IN 1958

Feb. 20. Prof. Richard KUHN (Max-Planck Institute for Medical Research). Gave lecture on "Chemical Problems of Biological Resistance".

March 7. Prof. G. F. FRAENKEL (University of Illinois, U.S.A.). Gave lecture on "Insect Nutrition".

May 1. Dr. R. F. CHANDLER, Jr. and Dr. J. G. HARRAR (The Rockefeller Foundation).

June 28. Mr. L. H. CHEN and Mr. T. H. SU (Department of Agriculture and Forestry, Taiwan).

July 11. Prof. K. NARAYAN (University of Mysore, India).

Sept. 20. Mr. J. G. SERIANO (Department of Botany, University of Philippines, Philippines) and others.

Oct. 9. Dr. N. PARTHASARATHY (F.A.O. Regional Office, Bangkok, Thailand)

Oct. 20. Dr. R. F. CHANDLER Jr. (The Rockefeller Foundation)

Oct. 23. Prof. A. G. McCALLA (Dean, Faculty of Agriculture, University of Alberta, Canada) and Dr. J. HLYNHA (Grain Research Laboratory, Winnipeg, Canada)

Nov. 1. Mrs. Savitri SAHNI (Hon. Director, Birbal Sahni Institute of Palaeobotany, Lucknow, India)

Nov. 5. Messers J. HARAHOP, M. KOESRIN, P. PRAYURANONG and W. CROCKER (Colombo Plan students from Asian countries).

Dec. 3. Mr. B. G. SHAILCH (Agricultural Research Station, Dokri, West Pakistan) and Mr. A. AHMED (Agricultural Research Station, Jejgaon, East Pakistan)

Dec. 22. Mr. C. S. HUANG (Taiwan Agricultural Research Institute). Did research work supported by the Colombo Plan.

RESEARCHES CARRIED OUT IN 1958

A. GENETICS, BIOCHEMISTRY AND CYTOLOGY OF INSECTS

1. *On the E-region Composed of Several Functionally Related Genes*

(By Mitsuo TSUJITA and Bungo SAKAGUCHI)

Several cases of crossing-over between genes belonging to the *E*-region have been found. Among these, the most clear-cut example is that between E^{H} and E^{Kp}.

From the cross $+\times E^{H}+/+E^{Kp}$, individuals with the genotype $E^{H}E^{Kp}/++$ can be obtained and these are phenotypically quite different from $E^{H}+/+E^{Kp}$ individuals owing to a position effect. As $E^{H}E^{Kp}/++$ larvae phenotypically resemble $El/+$ or El/El larvae, they cannot be told apart on the basis of appearance. The homozygotes from the $E^{H}E^{Kp}/++$ larvae can be distinguished. The phenotype of E also resembles that of $E^{H}E^{Kp}/++$, but larvae with the genotype $E/+$ or E/E lack the supernumerary crescent patterns on the 4th segment. These facts suggest the possibility that the phenotypes El or E may be produced by a complex region consisting of two or more loci, such as E^{H} and E^{Kp}. To test this idea, female pupae with the genotype $E^{H}E^{Kp}/E^{H}E^{Kp}$ were irradiated with X-rays and mated with normal males. In the next generation we obtained various types of mutants, among them El or E types. These type of mutants can not be obtained by the irradiation of wild type individuals. Consequently it seems that these mutants are induced by some change in a region composed of two or more loci, such as $E^{H}E^{Kp}$.

E^{Tc} is a mutant obtained from a cross between females with the genotype $E^{H}E^{Kp}$ which were treated with X-rays, and normal males. Larvae with the genotype $E^{Tc}/+$ have supernumerary crescent patterns on segments 4 and 6, and larvae homozygous for E^{Tc} have supernumerary crescent patterns on the 4th, 6th, 7th and 8th segments and lack all abdominal legs (Fig. 1 A, B). It seems likely that the embryos with the genotype $E^{H}E^{Ca}$ develop through the blastokinesis stage, at which time embryos homozygous for E^{Ca} die but the others hatch out.

It is assumed that the E^{Tc} mutant was derived from a change at some locus or loci other than the E^{H} locus within the region $E^{H}E^{Kp}$, and that it is composed of E^{H} and E^{Ca}. The fact that recombination between E^{H}

and E^{Ca} is difficult to achieve seems to be explained by some structural defect of the region. It is well known that all of the embryos homozygous for E^{Ca} die at the blastokinesis stage but that the E^{Ca} gene is maintained in the heterozygote $E^{Ca}/+$. On the contrary, individuals homozygous for E^{Tc} are viable. Therefore, if E^{Tc} were composed of E^{H} and E^{Ca}, some intimate functional interaction might take place between E^{H} and E^{Ca}, just as with the position effect between E^{H} and E^{Kp}.

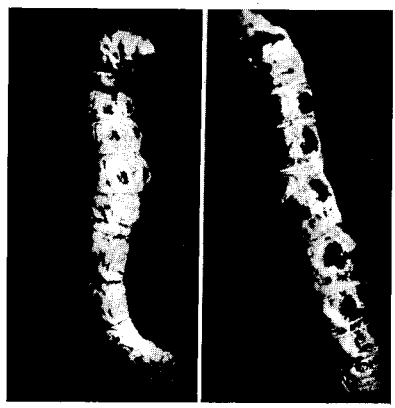

Fig. 1. Larvae homozygous for E^{Tc}.
A: abdominal view
B: dorsal view

Larvae of the heterozygote E^{H}/E^{Ca} have supernumerary crescent patterns on the 4th and 5th segments. From the cross $+\times E^{H}/E^{Ca}$, one larva with the genotype $E^{H}E^{Ca}/+$ and one normal larva were obtained in addition to a number of larvae with E^{H} and E^{Ca} genotypes. Unfortunately, the embryos in the eggs laid by the $E^{H}E^{Ca}$ type individual died, so we could not study them, but it seems that this recombinant individual is the same as that one known to have the genotype $E^{Tc}/+$.

In short, the E region of the silkworm is composed of several genes separable by crossing over, and it is characteristic of this region that it is larger than similar regions on the chromosomes of other animals. Furthermore, it may be considered that each of the genes composing the E region form a functional unit, at least from the viewpoint of size, and that there are intimate interactions in functions of the genes of which the region is composed.

2. *Structure and Function of Pleiotropic Genes of the Silkworm. II. Further Experiments to Break up the E^{El} Gene into Separate Components*

(By Yataro TAZIMA and Eiichi INAGAKI)

The attempt to break up a complex locus into several unit components with the help of X-ray irradiation was continued. The E^{El} character (additional crescent markings on the 4th segment and two pairs of extra abdominal legs on the 4th and 5th segments) was used as material for this experiment.

Heterozygous E^{El} females were *X*-rayed in the pupal stage with 4000 r and were crossed, after emergence, with untreated partners of a normal strain, which has a pair of crescents on the 5th segment. Disappearance of additional crescent markings and extra legs was carefully noted in the F_1 offspring. 185 exceptional individuals, 6.7% of the total individuals observed, were discovered as pheno-variants with respect to the characters in question. They were classsified into seven types, according to their phenotypes, as shown in Fig. 1.

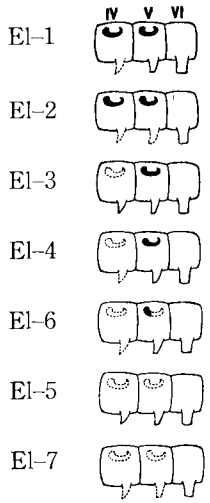

Fig. 1. Classification of discovered pheno-variants into seven types.

The results are summarized as follows.

a) Disappearance of extra abdominal legs on the 4th segment occurred most frequently, *i.e.*, 71.3% (*El*–1, *El*–2, *El*–4, *Fl*–6, *El*–7).

b) The frequency of the disappearance of two pairs of extra abdominal legs on the 4th and 5th segments was very low, *i.e.*, 2.7% (*El*–2, *El*–7).

c) Erasure of additional crescent markings on the 4th segment occurred with the frequency of 52.9% (*El*–3, *El*–4, *El*–5, *El*–6, *El*–7).

d) The frequency of the erasure of crescents on the fifth segment as well as the additional crescent markings on the 4th segment was 30.2% (*El*–5, *El*–6, *El*–7).

Among the 185 individuals four were found to be new mutants induced at the E^{El} locus, and their progenies were maintained by successive backcrossing to the normal strain. The rest of the variants were due to phenocopy, modifiers, chromosome breaks, translocations, etc.. Even though a considerable variation was observed in regard to the penetrance and expressivity of each mutant trait, the strains are characterized by different kinds of particular modification of the E^{El} character. In two strains most of *El* individuals lack extra legs on the 4th segment, while in the other two strains the majority of *El* lack both extra legs and crescent markings on the 4th segment.

Thus it seems likely that these mutants might have arisen from mutations which occurred at different unit sites of E^{El} locus. However, each site does not seem to act independently, because the modifications expressed were not confined to the character, presumably controlled by a specific site. When a mutation occurred at one unit site, it may have affected also the expression of some other unit sites. If we assume the unit sites of the E^{El} locus to be α, β, γ, δ, ε, $\cdots$ and a mutation to occur at the

γ site, the expressed character (*El*) would be modified as follows.

$$\begin{array}{l}\alpha+\beta+\gamma'+\delta+\varepsilon+\cdots \\ \downarrow\quad\downarrow\quad\downarrow\quad\downarrow\quad\downarrow \\ a+b''+c'+d''+e+\cdots=El' \text{ phenotype}\end{array}$$

3. *On Lethal Nl_1 and Nl_2 Embryos of the Silkworm*

(By Mitsuo TSUJITA)

A certain mutant of the silkworm, Nl_1 which occurred spontaneously, was discovered first by Y. TANAKA (1925). This type of mutant can also be easily induced by X-ray treatment of a certain genotype. Nl_1 and Nl_2 are mutants obtained by such artificial induction. Larvae with the genotype $Nl_1/+$ and those with the genotype $Nl/+$ have almost the same characteristics, but as larvae with the genotype $Nl_2/+$ have some different traits, they can be distinguished from the others. It has been determined that all of these mutants have a deletion of some part of the 14th chromosome in the portion extending from the *U* region to the marker *odk*.

Crossing-over experiments indicate that the order of the comparative lengths of the chromosomal deficiency in the three types is $Nl>Nl_1>Nl_2$.

It is generally known that a homozygote for a deficiency, even if it is very small, dies at a certain stage of its development. Moreover, the stage at which these mutants die varies according to the length of the deficiency. As *Nl*, Nl_1 and Nl_2 differ in the extent of their deficiencies it was expected that they might also vary in the stage at which the lethal effect was expressed. To test this point, hibernating eggs and artificially hatched eggs of the three strains were fixed in hot water or with Carnoy's fixative, and embryos homozygous for each of the *Nl* types were removed by cutting open the egg shells. The stage of development was determined by examining whole embryos or microscopic sections.

The results are as follows.

Individuals homozygous for *Nl* die at a very early stage, during the formation of the blastoderm, as reported by ICHIMARU (1956). However, in the case of Nl_1/Nl_1, young embryos of normal appearance are formed which advance through diapause and die at the longest embryonic stage (Fig. 1).

Embryos homozygous for Nl_2 develop normally through diapause, and they die during the period between the longest embryonic stage and blastokinesis. However, their development beyond the longest embryonic stage is abnormal, and as shown in Fig. 2, strikingly malformed embryo were observed.

Although larvae with the genotype $Nl/+$ and those with the genotype

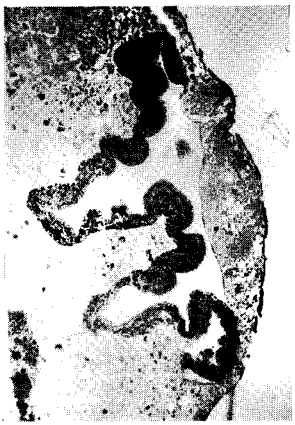

Fig. 1. Lethal embryo homozygous for Nl_1.

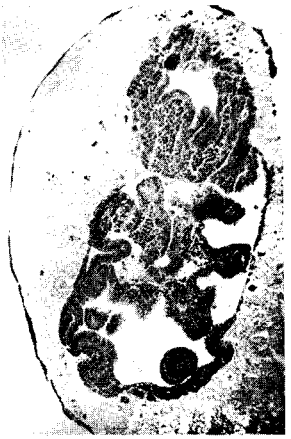

Fig. 2. Lethal embryo homozygous for Nl_2.

$Nl_1/+$ have similar characteristics, the cross-over value between *odk* and *Nl* is 2.8 and that between *odk* and Nl_1 is 5.2. This is how we determine the extent of the deficiency in each, and it is this difference which finds expression in the different stages at which their homozygous embryos die. The cross-over value between *odk* and Nl_2 is 7.2 and as the length of the deficiency in Nl_2 is smaller than in Nl_1, the development of the embryos homozygous for the former advances a little further than that of the latter.

Larvae with the genotype $Nl_2/+$ differ from those with the genotypes $Nl/+$ or $Nl_1/+$. It seems of interest to inquire why the characteristics of *Nl* and Nl_1 are almost the same and why *Nl* and Nl_2 are different. As mentioned before, *Nl*, Nl_1 and Nl_2 differ in the lengths of their deficiencies. Consequently, it is clear that the points of fusion of all three are at different places on the 14th chromosome. It seems that a position effect might be involved in the production of different phenotypes.

Thus it is shown, as suspected, that embryos homozygous for Nl_1 or Nl_2 die at a far more advanced stage than embryos homozygous for *Nl*.

Embryos having the genotypes Nl/Nl_1 or Nl_1/Nl_2 do not die at the stage of formation of the blastoderm but develop until about the longest embryonic stage or beyond. From this we learn that in heterozygotes between two deficiencies the time of death is controlled by the partner with the shorter deficiency; for example, in the heterozygotes $Nl/+$, $Nl_1/+$, and $Nl_2/+$ the normal chromosome overcomes the effect of the deficiency of its partner.

A number of larvae with the *Nl* phenotype were obtained by irradia-

tion of individuals with the genotype $E^{H}E^{K_p}/E^{H}E^{K_p}$. It might be inferred that all of these genes were involved in a deficiency in the above-mentioned portion of the 14th chromosome. However, it seems that the extent of the deficiency is not always the same.

4. *Genetic and Biochemical Studies on the Lethal Yellow Silkworm, Bombyx mori. On the Mechanism of Hardening of the Cuticle*

(By Bungo SAKAGUCHI and Mitsuo TSUJITA)

Lethal yellow silkworm larvae (*lem*^{l}/*lem*^{l}) which are produced by mothers of the genotype +/*lem*^{l} grow normally up to the youngest larval instar, but they die immediately after the first moult because they cannot feed. We have been interested in determining the exact cause of this malfunction which is connected with imperfect differentiation of the mandibular cuticle and incomplete hardening of the cuticular layer of the hypodermis. In order to determine the mechanism of cuticular hardening, various biochemical analyses were carried out.

(1) Quantitative analysis of scleroprotein and other proteins in yellow lethal and normal larvae

Larvae of the two types were taken just after the first moult for biochemical analysis. The protein found in these larvae was divided into three fractions, water soluble, alkali soluble and alkali insoluble (scleroprotein), using a modification of the method of HAKMAN (1953). The amount of proten was estimated as nitrogen. The results are shown in Table 1.

Table 1. Solubility of proteins from + and *lem*^{l} larvae.

Fractions	+		*lem*^{l}	
	mg	%	mg	%
Water soluble	89.5	76.9	104.3	89.6
Alkali soluble	16.0	13.7	8.5	7.3
Alkali insoluble	10 8	9.4	3.7	3.2
Total	116.3	100.0	116.5	100.0

(Tests were made on 1 gram of dry material divided into three parts.)

Table 1 shows that the total amount of protein found in yellow lethals is not different from that of normal larvae, but the water soluble protein of the former was found to be greater than that of normal larvae. The scleroprotein of the yellow lethal larvae, on the other hand, was shown to be less than that of normal larvae.

(2) Quantitative analysis of phenolic compounds

It is well known that the hardening of the insect cuticle depends upon the conversion of a phenol substrate into a scleroprotein. The relationship between this hardening process and phenolic compounds in yellow lethal larvae was therefore examined. A quantitative analysis of tyrosine was made by the spectrophotometric method of CERIOTT (1957) and the amount of other phenolic compounds was determined by assay with Folin's reagent. The experimental results are shown in Table 2.

Table 2. Quantitative analysis of tyrosine and other phenolic compounds in normal and lethal yellow larvae.

Fractions	Tyrosine				Phenolic compounds			
	+		*lem*^{l}		+		*lem*^{l}	
	%	*Ty/N*	%	*Ty/N*	%	*Ph/N*	%	*Ph/N*
Water soluble	85.6	21.2	86.5	17.7	88.2	32.4	88.6	26.8
Alkali soluble	9.0	20.0	11.9	26.0	7.3	25.0	8.2	30.7
Alkali insoluble	5.4	20.7	1.6	10.7	6.5	37.9	3.2	26.6
Total and Total (*Ty/N* or *Ph/N*)	100.0	21.1	100.0	18.1	100.0	32.2	100.0	27.1

Ratio of tyrosine and phenolic compounds to nitrogen are shown in percent of their substances contained in each fraction.

As is seen clearly in the table, the amounts both of tyrosine and of phenolic compounds in the water soluble and the alkali soluble fractions were greater in yellow lethal than in normal larvae. On the other hand, the amounts in the alkali insoluble fractions and the total amount of these compounds contained in yellow lethal larvae were less than in normal larvae.

From these results, then, we conclude that the phenolic compounds, one of the materials of scleroprotein are insufficient in the cuticular layer of yellow lethal larvae, resulting in imperfect hardening of the cuticle.

From our previous work (1957, 1958) and the present results, it seems evident that the *lem*^{l} gene, which allows accumulation of a yellow pigment (Xanthopterin-B), primarily affects pteridine metabolism and is accompanied by a secondary effect upon the metabolism of phenolic compounds. This, in turn, results in a decreased formation of phenol substrates, and of scleroprotein. Softness and weakness of the cuticular layer in the mandibular and other integumentary tissues are the net effects. Thus the yellow lethal larvae die because of their inability to feed.

5. *Genetic Determination of Tyrosinase and Protyrosinase in the Blood of the Silkworm, Bombyx mori. II. On the Nature of the Protyrosinase*

(By Bungo SAKAGUCHI)

A mutant of the silkworm called *ty*, which shows low tyrosinase activity, causes the accumulation of more protyrosinase in mutant blood than in that of normal strains. The nature of the protyrosinase contained in *ty* larvae was found to be similar to the tyrosinase which is produced by normal strains (SAKAGUCHI, 1958) by tests of the solubility in ammonium sulfate and immunological reactions.

To determine the nature of the protyrosinase, further biochemical and genetic studies were carried out.

(1) Quantitative analysis of copper in the blood of normal and *ty* larvae

It is well known that copper acts as coenzyme of tyrosinase. In order to determine any quantitative differences between the copper contained in protyrosinase and tyrosinase, the amount of that substance in both enzymes in the blood of 5th instar larvae of the normal and the *ty* strains was measured by the spectrophotometric method of Gubler (1953). The results of these experiments are presented in Table 1.

Table 1. Copper contents of normal and *ty* larvae.

Source \ Strain	+	*ty*
Whole blood	1.32±0.28	1.35±0.28
Whole blood (ashes)	1.43±0.35	1.51±0.41
Tyrosinase fraction	1.17	1.05
Non-tyrosinase fraction	0.50	0.47

(γ/cc blood)

As is shown in the table, the amount of copper contained in the tyrosinase and non-tyrosinase fractions of whole blood shows no significant difference between the normal and the *ty* strains.

(2) Quantitative analysis of sulfhydryl compounds

Tyrosinase activity is inhibited by reducing substances such as sulfhydryl compounds. Quantitative differences in the sulfhydryl compounds contained in the tyrosinase and protyrosinase from the blood of normal

and *ty* larvae were examined.

Quantitative analysis of the compounds was performed by measuring the reducibility of allithiamine by sulfhydryl compounds. The results are shown in Table 2.

Table 2. Quantitative analysis of sulfhydryl compound of normal and *ty* strains.

Source \ Strain	+	*ty*
Total blood	3.6±0.8	7.4±0.6
Tyrosinase fraction	1.5±0.2	2.5±0.3
Non-tyrosinase fraction	2.2±0.3	4.8±0.6

(γ/cc blood)

It is evident that in the sulfhydryl compounds contained in whole blood, the tyrosinase and non-tyrosinase fractions of the *ty* strain were significantly greater than those of the normal strain.

It seems from the results obtained that the *ty* gene does not control the amount of coenzyme (copper) in tyrosinase but controls the specificity of the apoenzyme. The mechanism of genetic control seems in addition, to be connected with the quantity of the sulfhydryl compounds.

6. *DDT-resistance of Drosophila pseudoobscura*

(By Chozo OSHIMA)

This work was carried out at the laboratory of Professor DOBZHANSKY in Columbia University. In natural populations of *D. pseudoobscura*; several kinds of inversions in the third chromosome have been observed; two of these are Arrowhead (AR) and Chiricahua (CH). Two AR/AR homozygous populations, two CH/CH homozygous populations and three AR/CH heterozygous populations have been cultured for one year in population cages. In the heterozygous populations, the frequency of both chromosomes has already reached an equilibrium; about 70% were AR and 30% CH.

About 15 pairs of flies taken from each population were allowed to lay eggs for three days in a bottle containing cream of wheat, molasses and yeast medium. Their 400 female offspring were divided into 10 groups of forty flies each. These flies, 4–5 days old, were transferred to vials containing a piece of test paper (5×6 cm^2) soaked with 0.5 or 1.0% DDT solution, and were exposed to DDT for 10 or 15 hours. After exposure,

the flies were transferred to a fresh vial containing filter paper wet with 0.4 m*l* distilled water and the number of dead flies was scored 40 hours later. The vials were kept in a room in which the humidity was about 50% and the temperature was 16°C. The results are shown in Table 1.

Table 1. Accumulated mortalities of *D. pseudoobscura* populations.

Population	Accumulated mortality 40 hours after exposure to DDT	Mean (%)
180 AR/AR	35.8	43.5
175 AR/AR	51.3	
181 AR/CH	47.0	44.9
176 AR/CH	39.5	
173 AR/CH	42.0	
182 CH/CH	30.0	27.3
177 CH/CH	16.5	

From the results of statistical analysis, no significant differences were found in DDT-resistance between different chromosomal populations, but the differences between popurations were significant. The CH homozygous populations were more resistant to DDT than the AR homozygous ones.

About 15 pairs of flies were taken from the heterozygous populations 181 and 173 and 1000 F_1 flies were exposed to 1% DDT for 5 hours. Forty hours after exposure, the surviving flies were cultured and their offspring were tested by the same method. Their resistance was found to be increased (15% in mortality). After the test, 500 pairs of their offspring were transferred into a population cage and cultured for about one month. The frequencies of both AR and CH chromosomal arrangements in populations were found by observation of salivary gland chromosomes of 150 larvae. The results are shown in Table 2.

Table 2. The frequencies of AR and CH in populations selected by DDT.

Population	Frequency of AR (%)	Frequency of CH (%)
181	80.3	19.3
173	87.0	13.0

At the same time, the frequencies of both chromosomal arrangements in the original 181 and 173 populations were examined by Prof. DOBZHANSKY

who found about 70% AR and about 30% CH in both populations. It was assumed that the number of AR was increased and the number of CH was decreased by DDT selection.

7. *DDT and Dieldrin (DL) Resistance of Drosophila melanogaster*

(By Chozo OSHIMA)

The character of insecticide resistance has been generally assumed by geneticists to be polygenic. Some factors in common to DDT- and DL-resistance have been found in Drosophila by genetic analysis. Among them a dominant gene (or genes) was found to be located on the second chromosome, but the mechanism of resistance to both insecticides was proved by the following experimental results to have some different components.

A highly resistant wild Hikone strain and a susceptible mutant strain, having *sca* (scabrous) and *ry* (rosy) genes on the second and third chromosomes respectively, were crossed. The F_1 male flies were back-crossed to *sca*;*ry* female flies and four kinds of phenotypes were obtained in the F_2 generation, as follows:

$$+\left(\frac{sca}{+}\ \frac{ry}{+}\right),\ sca\left(\frac{sca}{sca}\ \frac{ry}{+}\right),\ ry\left(\frac{sca}{+}\ \frac{ry}{ry}\right),\quad sca;ry\left(\frac{sca}{sca}\ \frac{ry}{ry}\right)$$

DDT- and DL-resistance in these flies was examined by using test papers (2% DDT, 0.4% DL), which were prepared by WHO in Geneva. Forty female flies, 4–5 days old, were exposed in a small vial to DDT for 15 hours and to DL for 1 hour; the accumulated mortality was observed in a fresh vial 25 hours after exposure. The experiments were replicated three times and the mean mortality was obtained. The results are shown in Table 1.

From the results, an important DL-resistant gene (or genes) was assumed to be located on the second chromosome and a powerful DDT-resistant

Table 1. DDT- and DL-resistance in F_2 flies of the hybrid between Hikone and *sca*;*ry* strains.

Phenotype	Accumulated mortality (%)	
	DDT	DL
+	0	0.8
sca	7.5	35.0
ry	21.5	2.6
sca;*ry*	87.0	92.0

gene (or genes) was thought to be located on the third chromosome. The latter finding is contradictory to the results previously reported. It stands to reason that the homozygous *ry* flies are susceptible to DDT. The *ry* gene inhibits the enzymatic activity of xanthine oxydase, which has an intimate relationship to the respiratory system. Since the poisonous action of DDT affects this system, the *ry* flies appear to be more susceptible to DDT. On this point the mechanism of the reaction to the two insecticides is different.

8. *Studies on the Formation of Drosophila Eye Pigments*

(By Toshifumi TAIRA and Saburo NAWA)

We have previously reported that the formation of red eye pigments has an intimate relation to pteridine metabolism, because one of products obtained by hydrolysis of the pigment was 2-amino-4-hydroxypteridine-6-carboxylic acid. Several eye-color mutants were used for a comparison of the relationship between the amount of eye pigments and of pteridines. The results are summarized in Table 1.

Three kinds of eye pigments, red, yellow, and brown, were extracted

Table 1. Relative amount of eye pigments and pteridines.

Eye pigment			Strain	Pteridine						
				Head			Body			
Red	Yellow	Brown		YP	BFS	T	YP	BFS	IXP	T
100	7	100	Ore.-R	14	48	62	7	14	59	80
75	5	0	*v*	11	57	68	12	29	63	104
39	24	129	Hn^{r3}	75	135	210	25	33	14	72
26	17	15	$v;Hn^{r3}$	69	142	211	23	42	12	77
3	100	122	*se*	304	286	590	12	23	84	119
3	80	13	*v;se*	288	305	593	14	23	96	133
33	4	73	*ry*	6	74	80	8	94	0	102
16	3	0	*v;ry*	6	63	69	9	78	0	87
0	0	88	*bw*	0	0	0	0	0	0	0
0	0	82	*bw;se*	0	0	0	0	0	0	0
0	0	0	*v;bw*	0	0	0	0	0	0	0

Standard; Red and Brown: Oregon-R, Yellow: *se*

YP: Yellow pteridine, BFS: Blue fluorescent substance, T: Total amount of pteridine, IXP: Isoxanthopterin.

individually by a double extraction method, and their amounts were measured photometrically. Several kinds of pteridines were eluted from paper chromatograms, and the relative intensities of their fluorescence were measured by fluorophotometer. The amount of eye pigment and pteridine were affected by the following conditions: (a) food, temperature, and crowding during the larval stage (b) age after emergence. The flies were cultured under optimum conditions.

From the results summarized in Table 1, the following hypotheses can be suggested. (1) Red and yellow eye pigments have an immediate relation to pteridines, because such strains as *bw*, *v;bw*, and *bw;se*, lacking any red eye pigment, have no pteridines either in head or in body, but show a trace of pteridine at the period of emergence. The *bw* gene inhibits the formation of red and yellow pigments. (2) It is a well-known fact that the *v* gene blocks the formation of brown pigment derived from tryptophane metabolism. The amount of red eye pigment of *v* is about 80 percent of that of the wild strain, although there is no abnormality in its formation. In double mutants containing the *v* gene, such as *v; se*, *v;Hn*^{r3}, and *v;ry*, the amount of red and yellow pigments are markedly decreased. It seems that some interaction between the metabolisms of pteridine and tryptophane might exist. In the case of *v;ry*, the amount of red pigment is about half of that of *ry*. It appears that strong effective interaction takes place in red pigment-formation under such restricted condition as *ry*. (3) The *v;se* and *v;Hn*^{r3} strains have a small amount of brown pigment in spite of the *v* gene. This is assumed to be due to an overproduction of brown pigment in both *se* and *Hn*^{r3} in comparison with the wild strain. (4) In the heads of *se*, *Hn*^{r3}, *v;se*, and *v;Hn*^{r3} flies a large amount of BFS as well as of yellow pteridine (the main constituent of yellow pigment) is recognized. BFS consists of two main components 2-amino-4-hydroxypteridine (AHP) and biopterin. On the other hand, one component of BFS in *Hn*^{r3} and *v;Hn*^{r3} flies can not be oxidized by xanthine oxidase and thus is not AHP, but it is thought to be an analogue of AHP. These mutants have also a yellowish-green fluorescent substance which is catalyzed by xanthine oxidase into a purplish-blue one in the alkali range. The analyses of these substances are now in progress.

9. *Purine Metabolism in Drosophila melanogaster II*

(By Toshiteru MORITA)

It is a well-known fact that the *rosy*^{2} (*ry*^{2}) eye-color mutant of *D. melanogaster* does not contain isoxanthopterin, which generally occurs in

this fly. Neither does another *rosy* mutant, ry^1, contain even a trace of isoxanthopterin at any developmental stage. On the other hand, at pupal and imaginal stages, large amounts of hypoxanthine and xanthine, precursors of uric acid, are produced, but no uric acid, which is the end product of nitrogen-metabolism in the insect, is formed.

The purine content, detected by methods of chromatography and optical analysis at several developmental stages of Oregon-R and ry^1 strains, are shown in Table 1. It is very interesting to note that hypoxanthine is accumulated in the heads of adult ry^1 flies.

Table 1. Purine content at various developmental stages in *D. melanogaster*.

	Oregon-R		*ry*		
	Hypoxanthine μg/mg	Uric Acid μg/mg	Hypoxanthine μg/mg	Xanthine μg/mg	Uric Acid μg/mg
3rd instar larvae	0.0	0.06	0.1	0.10	0.0
Early pupae	0.11	0.70	0.85	0.28	0.0
Mid pupae	0.06	0.80	1.55	0.48	0.0
Late pupae	0.05	1.10	1.85	0.52	0.0
Adult, male head	0.33	0.0	2.63	0.0	0.0
Adult, male body	0.03	0.65	0.37	0.19	0.0
Adult, female head	0.09	0.0	1.38	0.0	0.0
Adult, female body	0.0	0.30	0.20	0.20	0.0

The enzyme, prepared from pupae, can catalyze the oxidation from 2-amino-4-hydroxypteridine (AHP) to isoxanthopterin, as well as from hypoxanthine and xanthine to uric acid. This enzyme, therefore, has been called xanthine oxidase or pterin oxidase, but it is assumed to be a true dehydrogenase because it requires electron acceptors such as DPN or methylene blue for oxidation. As the homogenate can also catalyze the oxidation of DPNH to DPN, xanthine dehydrogenase seems to link a DPNH-oxidase system by the medium of DPN *in vivo*.

The activity of xanthine dehydrogenase was not actually recognized in ry^1 flies, but the activity of DPNH-oxidation could be distinguished. A similar phenomenon was found in the double mutants $v;ry^1$, $cn;ry^1$, $se;ry^1$, and $stw^3;ry^1$. Both the deficiency of uric acid and the accumulation of hypoxanthine and xanthine in *ry* flies are assumed to be due to the lack of xanthine dehydrogenase activity.

The activity of guanase, which catalyzes the conversion of guanine to

xanthine, was observed in pupal stages of both Oregon-R and ry^1 strains.

From these results, the process of uric acid formation in *Drosophila* is assumed to proceed according to the scheme illustrated in Fig. 1.

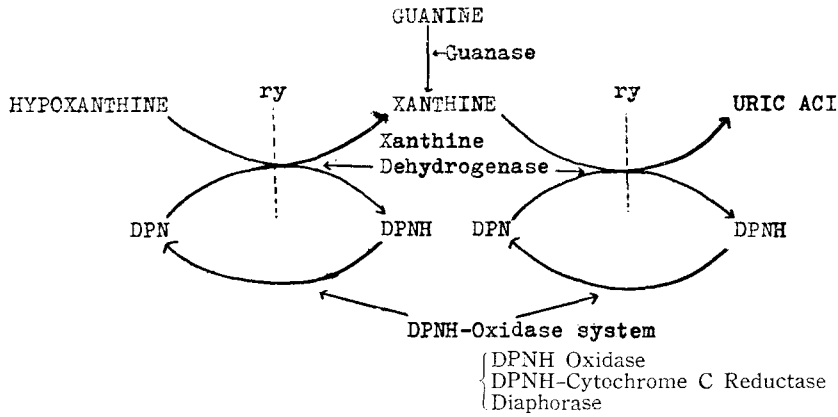

Fig. 1. The pathway of uric acid formation and relative metabolic systems in *Drosophila*.

10. *The Chromosome Number of an Indian Wild Silkworm, Theophila religiosae* Helf.

(By Yataro TAZIMA and Eiichi INAGAKI)

The Indian wild silkworm is usually known as *Theophila huttoni* West. Adalbert SEITZ's opinion (1933) is that the proper name of the insect is *Theophila religiosae* Helf., according to the rules of nomenclature.

The larva of the Indian wild silkworm is quite similar to that of the Japanese wild silkworm, *Theophila mandarina* Moore except for the following characteristics: 1) the Indian wild silkworm has a larger body than the Japanese form and 2) in the former species each segment other than the 1st to 5th segments has a caudal horn-like nodule on the subdorsal line and a small nodule on the basal line, while the latter species lacks the nodules completely. The larva of the Indian variety is trimoltic, as is that of the Japanese wild silkworm. The egg of the Indian silkworm is about 1.3 times longer and 1.4 time wider than that of the domesticated silkworm, *Bombyx mori* L.. The cocoon of the Indian wild form is yellow or pale green, and elliptic. The insect is found wild on mulberry trees in south Himalaya and Assam.

We examined the chromosomes of the Indian wild silkworm. The

Table 1. Observed data on chromosome number at metaphase in first spermatocytes.

No. of chromosomes per metaphase plate	No. of small chromosomes							Total metaphase plates observed
	1	2	3	4	5	6	7	
31	3	9	9	13	1	1	0	36
30	0	0	2	2	0	0	1	5
29	1	1	1	1	0	0	0	4

material was collected at Kalimpong, India, by the senior author in 1957. Thanks to the kind assistance of Dr. D. C. Sarker, Deputy Director of Industries, West Bengal, the testes of the insect were fixed directly at the habitat by Bouin's solution. Heidenhain's iron-hematoxylin method was used for staining. n=31 chromosomes were found in the nuclear plates of both first and second spermatocytes. The chromosomes differed in size, and three or four of them were very small.

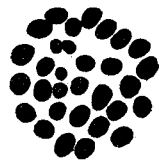

Fig. 1. Chromosomes at metaphase in first spermatocyte.

The domesticated silkworm which is now commercially reared in India and Japan has 28 chromosomes (n), while the chromosome number of the Japanese wild silkworm is 27 (n). It therefore does not seem probable that the Indian wild silkworm might have been the ancestor of the domesticated silkworm in India, considering the difference in chromosome number, although studies on hybridization between the Indian wild silkworm and *B. mori* L. or *T. mandarina* have not been carried out yet.

11. *Studies on a Silkworm Poison Emanating from Tobacco Plants*

(By Mitsuo TSUJITA, Saburo NAWA and Bungo SAKAGUCHI)

It has long been known that when silkworm larvae are fed on leaves taken from mulberry trees near a field of tobacco plants, they are often poisoned. It is clear from this fact that some poisonous substance evaporates from the tobacco plants and adheres to the mulberry leaves. There are two different ideas resulting from studies on the nature of this poisonous substance. YABUTA (1931, '32) concluded that it was nicotine and HASEGAWA (1931) insisted that it was trimethylamine. As it is necessary to know the nature of the toxic substance if we want to protect silkworm larvae from the damage caused by tobacco plants, the present studies

were undertaken.

(1) The season of the emanation of the posionous substance from tobacco plants.

Since 1956 special mulberry fields have been planted in which the trees are placed in rows 3 meters apart from ridge to ridge (this distance is twice as wide as usual) and 0.76 meter between one stump and the next. In the spring of every year young tobacco plants were planted mid way between every two rows.

For the purpose of studying the season when the poisonous substance emanates from tobacco plants and the silkworm larvae show symptoms of poisoning, experiments were carried out using leaves taken from the special mulberry field, and the following results were obtained.

i) The poisonous substance emanated from tobacco plants for a short period during their maturity. According to records kept during the past three years, the date of the beginning of emanation varies from year to year. In 1956 it began about July 14, and in 1957 about July 9. As we had a relatively dry rainy season in 1958 it was expected that poisonous substance would be transferred earlier than usual. However it was delayed until July 29, and after few days the poison reached its peak.

ii) Judging from the symptoms of the silkworm larvae it appears that the poisonous substance is a kind of neurotoxin.

(2) Nature of the poisonous substance

To determine the nature of the poisonous substance and to observe the toxic effects of nicotine and trimethylamine on silkworm larvae several experiments were carried out. The conclusion is that nicotine is far more poisonous than trimethylamine to the silkworm larvae, and that the symptoms of the two are different. Symptoms of poisoning displayed by silkworm larvae which are mulberry leaves contaminated by the natural emanations from tobacco plants markedly resemble those of larvae which ate leaves painted with dilute nicotine sulfate solution or fumigated with nicotine gas.

From these observations it seems clear that the poisonous substance is nicotine, not trimethylamine. Thus, our next step was to try to detect nicotine and determine its amount in the poisoned mulberry leaves. Therefore, the extraction of nicotine from the following samples was carried out. A) Normal leaves (control), B) Mulberry leaves fumigated with nicotine gas (each mulberry tree was put in a wooden framework covered with a polyethylene sheet and fumigated with nicotine gas), and C) Mulberry leaves contaminated under natural circumstances from adjacent tobacco plants.

The procedure for the extraction of nicotine from the leaves is as

follows:

Mulberry leaves 1 kg
↓ homogenized in 0.1N HCl (4 *l*)
filtrate
↓ concentrate to 500 m*l* in vacuo, and filtered after addition of 10 N NaOH (20 m*l*)
filtrate
↓ extracted continuously with petroleum ether for 24 hours
pet. ether layer
↓ extracted with 0.1 N HCl (10 m*l*)
aqueous layer
↓ steam distilled, after addition of 10 N NaOH (1.5 m*l*)
distillate

Nicotine was collected on Amberite IRC-50 resin column (hydrogen form, 60-100 mesh) and then eluted with 0.1 N HCl.

Several fractions of the extract were used for chemical analysis.

From chemical analysis it was proved that the poisonous substance in sample (C) is nothing more than nicotine.

(3) Nicotine content of the mulberry leaves

The nicotine content of several samples of mulberry leaves naturally contaminated with nicotine to various degrees was calculated by the method of Willits, Swain, Connelly and Brice (1950). The yield of each of the samples was doubled because it was estimated that half of the nicotine content of the mulberry leaves was lost in the course of extraction. The results showed that nicotine content varies in different samples from 1 – 10 mg per kg mulberry leaves.

Next, when mulberry leaves painted with various diluted nicotine sulfate solutions (the ratio of the weight of the free nicotine to that of mulberry leaves was 1 – 10 parts per million) were given to silkworm larvae, they showed various degrees of poisoning, ranging from very weak to very strong. These symptoms, which vary according to the dose of nicotine, are almost identical with those of larvae which fed on leaves contaminated naturally to various degrees.

On the contrary, trimethylamine is so much less toxic to silkworm larvae than nicotine and if want a violent response it is necessary to give far greater dose than with nicotine. We have not yet found trimethylamine in mulberry leaves which were naturally contaminated by tobacco plants. If such leaves contain any trimethylamine, it must be a

very small amount. Consequently, its effect upon silkworm larvae may be considered to be negligible.

(4) Relative amounts of nicotine given off by several races of tobacco plants.

It is quite clear that the chemical methods described above are indispensable to clarify the nature of the toxic substance. But the nicotine content of the contaminated leaves is so small that it is necessary to take great care in its extraction as this procedure is troublesome. Therefore, chemical analysis alone does not offer a simple method of determining the degree of contamination of mulberry leaves, but our biological method, in which the strength of response is observed in larvae fed on leaves contaminated with nicotine, is much simpler and more useful than the chemical method.

The relative amounts of nicotine given off by several races of tobacco, including Bright yellow, Enshū, Daruma, Matsukawa and White Burley were determined by the above-mentioned biological method. The results showed that the first four races emanate relatively large amounts of nicotine, but the last one an amount considerably smaller than the other.

(5) Experiments on the resistance of silkworms to nicotine

Various races of silkworm, including the Japanese races N 111, N 112, N 115, N 122, N 124, N 501, the Chinese races S 108, S 110, S 115, S 124, S 501, their hybrids, N 112×S 110, N 124×S 124, N 501×S 501, N 115×S 108, and another race, Daizo, were examined for possible nicotine resistance. However, as far as the present experiment went, no conspicuous differences were found among the races.

(6) Nicotine contamination of other plants.

Plants of the dandelion or fudansō (*Beta vulgaris* L. *var. cicla* L), planted in flower pots were put near a field of tobacco plants during the period of emanation of nicotine, in July and August, and after few days their leaves were given to silkworm larvae. It was found that these plants were also contaminated with nicotine. This fact proves that other plants including crops close to a field of tobacco would be contaminated with nicotine, although the amount is so small that it probably has no important effect upon human beings or domestic animals.

(7) Survey of the actual situation of the silkworm poisoning at a farm.

Recently we learned that silkworm larvae reared on a farm in Sizuoka-ken were poisoned by mulberry leaves grown near a field of tobacco plants, and one of the authors, Tsujita, went to the farm to determine the actual situation; about 4 kg of the damaged mulberry leaves were brought back to our Institute for chemical analysis. The results of this analysis confirmed our experiments with mulberry leaves taken from the

special field. Our observations and experiments are completely consistent with the actual example of the poisoning of silkworm larvae which occurred at the nearby farm.

(8) Conclusion and consideration

It may be safely concluded from these experimental results that the toxic substance, or at least its principal constituent, which emanates from the tobacco plants, mainly during their mature stage, is nicotine and not trimethylamine.

It may be said that nicotine is given off principally from the leaves of tobacco plants. As the volatilization temperature of nicotine is 60–100°C and in the fields the temperature never rises so high even on summer days, volatilization of nicotine from the surface of tobacco leaves is impossible. However, it is assumed that free nicotine molecules are contained in vapour evaporating from the secretions or from the disintegrating cells of the hairs which cover the surface of the tobacco leaves, and it is this vapour which adheres to the leaves of other plants. Studies of the exact mechanism of the emanation of nicotine from tobacco plants are now under way.

B. GENETICS, CYTOLOGY AND TAXONOMY OF CEREAL CROPS AND RELATED PLANT GROUPS

12. *Studies on the Nucleus-substitution and -restoration in the Reciprocal Hybrids, Triticum vulgare×Aegilops caudata*

(By Hitoshi KIHARA)

As already reported, *T. vulgare* (2n=42) and *Ae. caudata* (2n=14) were used in this experiment. Substitution and restoration of the nucleus were carried out by successive back-crosses of *T. vulgare* as the male parent to the reciprocal hybrids between the two species. Chromosome numbers of the first backcross generation (SB_1 and RB_1) varied from 36 to 52. 49-chromosome B_1-plants were most frequent. Both 49-chromosome SB_1- and RB_1-plants had usually $21_{II}+7_I$. To obtain B_2-plants only 49-chromosome B_1-plants were used.

In 1958 SB_9 (9th back-cross generation of substitution line) and RB_9 (9th back-cross generation of restoration line) strains were obtained.

Chromosome conjugation in meiosis of both strains showed invariably 21 bivalents. Seed-fertility of both strains was normal; pollen-fertility was zero in SB_9, resulting in complete male-sterility, and was perfect in RB_9. Most of the RB_9-plants showed all characteristics of *T. vulgare*. SB_9-

plants, which are assumed to have the *vulgare* genomes in *caudata* plasma, were in certain respects different from *vulgare*, namely as to male sterility and right- and left-handedness of the spikelets.

Extensive studies of the karyotypes of both parents and plants of the backcross generations (B-plants) were carried out.

a) *T. vulgare* is characterized by 2 sets of Sat-chromosomes, namely Sat-1 and Sat-2. The remaining ones are V- or L-shaped.
b) *Ae. caudata* has in the haploid complement 2 Sat-chromosomes (C-Sat-1 and C-Sat-2), 2J (J_1 and J_2) and 3 i-shaped chromosomes.
c) Karyomorphologically, the 4 Sat-chromosomes (Sat-1, Sat-2, C-Sat-1 and C-Sat-2) are quite distinct from each other.
d) The 3 i-shaped chromosomes can be identified by their size. However, identification is difficult if one or two are found in later generations.
e) The 3 i chromosomes of *Ae. caudata* can be easily distinguished from *vulgare* chromosomes, when they are mixed with *vulgare* chromosomes. However it is difficult to recognize the two J's, especially J_1.

Using these morphological characteristics of *vulgare* Sats and *caudata*-chromosomes, the writer could follow the progress of substitution and restoration of the nucleus in the course of backcrosses. All B_3-plants have attained the 6x-level in this experiment. However this does not guarantee complete genome substitution or restoration.

The effects of single *caudata*-chromosomes on the morphology of plants could be in some cases clearly ascertained. For instance, C-Sat-2 is supposed to have a gene (or genes) for restoring male fertility in the substitution strains. This gene manifests itself when C-Sat-2 is substituted for one *vulgare*-chromosome or added to the *vulgare* genomes. This gene (or genes) is completely linked with the gene for black awns.

In order to investigate whether or not fertility restoring genes can be found in other hexaploid wheats, *T. spelta*, *T. compactum* and *T. macha* were crossed as the male parents with SB_8-plants. Pollen-fertility of these hybrids was as follows:

a) $SB_8 \times$ *T. spelta* was male sterile, as was SB_9.
b) $SB_8 \times$ *T. compactum* was partially male fertile (seed-fertility by selfing was 2.5%).
c) $SB_8 \times$ *T. macha* died before shooting. The same was observed when *T. vulgare* and *T. macha* were crossed (KIHARA 1949).

Contrary to C-Sat-2, C-Sat-1 has no effect on the morphology of B-plants either by addition or by substitution. J_1 is supposed to have a dominant gene for the waxy character of stems and leaves. Other *caudata*-chromosomes seem to have no effect when they are added or substituted singly to *vulgare* genomes.

First metaphase plates of PMC of several 42-chromosome B_3-plants having one C-Sat-1 (or C-Sat-2) and 41 *vulgare*-chromosomes show almost always 2_{II}. From these observations it was revealed that C-Sat-1 conjugates either with Sat-1 or one of the non-Sat chromosomes of *vulgare* and C-Sat-2 conjugates either with Sat-2 or with one of non-Sat chromosomes of *vulgare*. This relationship might be something similar to the homeologous relationship between the 3 wheat genomes, A, B and D.

As the pairing of chromosomes at MI of PMC of F_1 (*T. vulgare*×*Ae. caudata*) varies from 0–6, complete pairing of C-Sat-1 or C-Sat-2 with its respective *vulgare* partner in substitution lines seems to indicate that the pairing is governed by genotypic control. The writer proposes the term facultative conjugation for the pairing between two apparently semi-homologous chromosomes.

After careful observation of the stage following immediately the last contraction in F_1, maximum association of 28 chromosomes into 14 pairs was found. Their number varied to a certain extent. However it is always much larger than the number of bivalents at MI. This association is dissolved mostly before early metaphase (Fig. 1 a–c).

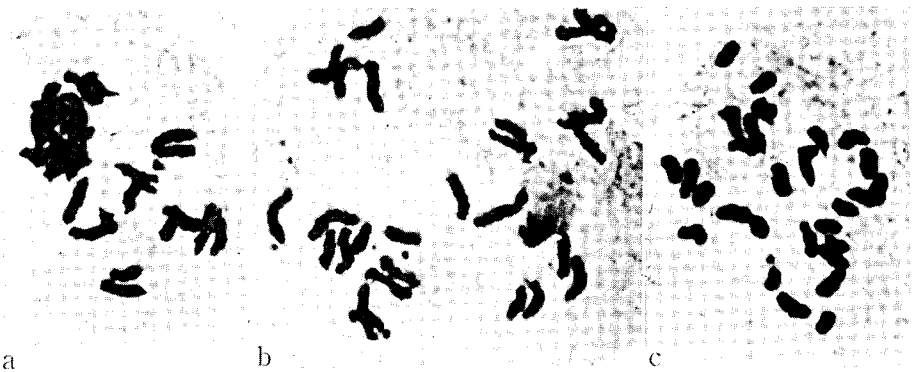

Fig. 1. Chromosome association in late prophase (a–b) and early metaphase (c) of *T. vulgare* var. *erythrospermum*×*Ae. caudata* (F_1).

Among 18 49-chromosome B_1-plants, which were supposed to have arisen from the unreduced female gametes of F_1, 15 plants had the non-waxy character of *caudata*. However 3 plants lacked the dominant gene for non-waxy. As the genome-type of B_1 should be AABBDDC, it was assumed that crossing-over between the non-waxy *caudata* chromosome and one *vulgare* chromosome took place when they were paired at prophase in the F_1 but later separated into two univalents.

The results were reported in detail at Xth International Congress of Genetics in Montreal.

13. *Ecogenetical Studies in Agropyron II.*

(By Sadao Sakamoto)

(1) F_1 hybrid between an early ecotype and the common type of *A. tsukushiense* var. *transiens* Ohwi.

Artificial hybrids between a strain of the early ecotype described in a previous report* and a strain of the common type were easily obtained. The growth of the F_1 plants was very vigorous. Of the 19 observed characters of the F_1, 14(74%) were intermediate between the parents (Table 1). At meiosis of the F_1 hybrid, the frequency of tetravalent conjugation

Table 1. Characters of F_1 hybrids between an early ecotype and the common type of *A. tsukushiense* var. *transiens* (1958).

Characters \ Strains (Cult No.)	Early ecotype (58107)		F_1 (5881)		Common type (58109)	
Upper side of rosette leaf	Pubescent		Pubescent		Non-pubescent	
Plant height (cm)	67.22	1.67	85.85	3.41	97.80	3.55
First internode from top (cm)	27.85	0.53	40.16	1.83	39.70	1.23
Flag leaf (cm)	10.29	0.41	11.75	0.40	11.21	0.39
Spike (cm)	11.12	0.03	14.96	0.44	18.11	0.40
No. of spikelets	10.02	0.07	14.29	0.45	18.65	0.41
No. of florets per spikelet	10.54	0.15	8.86	0.16	6.68	0.21
Empty glume Length (cm)	0.93	0.08	0.85	0.10	0.80	0.14
Empty glume Width (cm)	0.22	0.03	0.20	0.01	0,16	0.01
Lemma with awn Length (cm)	3.41	0.38	3.43	0.27	3.61	0.27
Lemma with awn Width (cm)	0.21	0.02	0.19	0.02	0.17	0.06
Palea Length (cm)	1.28	0.08	1.15	0.07	1.03	0.14
Palea Width (cm)	0.15	0.03	0.13	0.02	0.12	0.02
Seed Length (cm)	0.72	0.04	0.64	0.04	0.53	0.02
Seed Width (cm)	0.20	0.01	0.18	0.02	0.16	0
Seed Weight (mg/100 grains)	890.40	31.68	647.20	31.60	443.60	5.04
Color of anther	red-brown		red-brown		yellow	
Average date of first heading	26 April		7 May		22 May	
Average date of first flowering	28 April		9 May		24 May	

amounted to 14% of the observed 163 PMCs. Pollen- and seed-fertilities of the F_1 were lower than in either parent but fairly good and the growth of the F_2 seedlings was very good.

* S. Sakamoto (1957) Ann. Rep. Nat. Inst. Genet. Japan 8:34-36.

(2) Natural hybrids between *A. tsukushiense* var. *transiens* and *A. ciliare* Franch.

A. tsukushiense var. *transiens* and *A. ciliare* are both common perennial plants in Japan. The former is hexaploid (2n=42), growing in fields and along road-sides, and the latter is tetraploid (2n=28), mainly occurring in the hilly regions.

In the sympatrical habitats of both species in the suburbs of Misima, 11 clones of a natural hybrid between those two were found in the hills and on river-dikes. The natural and artificial hybrids were morphologically similar and somewhat resembled *A. tsukushiense* var *transiens*, but the lemmata were pubescent which is a specific characteristic of *A. ciliare*. Both hybrids were vigorous but completely sterile.

Chromosome conjugation in the meiosis of the artificial hybrid showed the configuration $9_{II}+17_{I}$ to $15_{II}+5_{I}$ (mode: $14_{II}+7_{I}$). Similar results had been reported by MATSUMURA (1941),

14. *Cytotaxonomic Studies in Poaceae, VI.*

(By Tuguo TATEOKA)

1. In 1958, the chromosome numbers of the species listed in Table 1 were determined.

2. To examine the systematic relationship between *Garnotia* and *Arundinella*, their leaf structure was examined. In both genera unique features were found which suggest that the two are related, but since their spikelet structure is quite different, *Garnotia* may be treated as an independent tribe, Garnotieae, tentatively placed near the Arundinelleae.

Table 1.

Species	2*n*
Leptaspis coohleata Thw.	24
Streptogyna crinita Beauv.	24
Arundinella villosa Arn. ex Nees	14
A. leptochloa Hook. f.	20
Garnotia scoparia Thw.	20
Perotis patens Gand.	40
Spinifex littoreus Mear.	18

15. *Glutinous and Non-glutinous Isogenic Lines in Rice*

(By Hiko-Ichi OKA)

Taichung No. 65 is a representative Horai variety of rice from Taiwan. It is non-glutinous. In order to obtain a glutinous line of this variety, a strain was crossed with a Japanese glutinous variety, Kinoshita-mochi, and back-crosses were repeated seven times using the same strain of Taichung No. 65 as the recurrent parent. Then a heterozygote for the glutinous gene was selfed, and 22 non-glutinous and 22 glutinous homozygous plants were selected from the progeny.

The 44 descendant lines, together with the strain used as the recurrent parent, were tested in a randomized-block experiment with three replications, at Taichung, Taiwan, in the first and second crops in 1958. There were 135 plots in each crop, each consisting of 30 two-plant-hills. The results of this experiment showed that the average yield of the glutinous lines (351.2 kg/ha in the first crop. and 221.0 kg/ha in the second crop) was slightly lower than that of non-glutinous lines (357.3 kg/ha in the first crop, and 222.3 kg/ha in the second crop). The yield ratio of glutinous to non-glutinous was 98.3% in the first crop, and 99.4% in the second crop. But variance analysis of the data showed that this slight difference was insignificant, as indicated below.

Table 1. Variance analysis of the yield of glutinous and non-glutinous lines of Taichung No. 65.

Variation due to	d.f.	Mean square	F	Signi. level	
				5%	1%
Replication	2	14,824.0	15.52**		
Class (Gl.: Non-gl.)	1	923.0	0.97	3.84	6.63
Lines (within class)	43	1,114.7	1.17	1.49	1.59
Crop	1	1,188,428.0	1243.78**		
Class × Crop	1	391.0	0.41		
Line × Crop	43	1,210.4	1.27		
Error	178	955.49			

** Beyond 1% level of significance.

As shown in the above table, it was also found from the variance analysis that the differences among lines were insignificant. The degree of freedom for error variance in this experiment was as large as 178, and there was no reason for suspecting that this experiment was inferior to others in its accuracy. It may then be assumed that all of the lines tested have

practically the same genotype, except that the glutinous and non-glutinous lines differ in the glutinous gene and possibly some minor genes closely linked with it.

It is known, as pointed out by MORINAGA (1943), that glutinous rice varieties generally show lower yields than non-glutinous ones; the difference is usually about 5%, and has been suspected to be attributable to the biochemical nature of glutinous starch itself. The results of the present experiment seem to suggest, however, that the glutinous gene may have no such yield-lowering effect. If this is true, it is conceivable that the generally low yield of glutinous varieties may be due to the effect of certain genes linked with the glutinous gene, which linkage might have been established in the course of origin of glutinous varieties.

16. *A Systematic Study of Oryza by Statistical Methods** *(a preliminary report)*

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

This report deals with an attempt to find systematic relationships among *Oryza* species by non-subjective techniques. The statistical method explored by SOKAL and MICHENER (1958) which deals with unweigted measurements of numerous characters was adopted for the study of 12 *Oryza* species.

First, correlation coefficients between species were computed from measurements of 20 characters, each of which had been normalized to have a standard deviation unit. Characters used are 1) grain length, 2) grain width, 3) length × width of grain, 4) length/width ratio of grain, 5) apiculus hair length, 6) empty lemma length, 7) ligule length, 8) awn length, 9) anther length, 10) pollen grain diameter, 11) weight of 100 grains, 12) grain number per panicle, 13) panicle length, 14) number of first rachises, 15) stoma size, 16) plant height at the seedling stage, 17) index of alkali-test, 18) germinating capacity (hulled seeds, 90 days after heading), 19) number of days of germination, 20) seed fertility. Correlation coefficients thus found between species ranged from 0.81 (*O. sativa spontanea—O. perennis*) to −0.67 (*O. sativa—O. eichingeri*).

The matrix of correlation coefficients was then constructed from the data, and the two species between which the correlation was highest in both the row and column to which they belong were chosen to be the "nucleus" of the group to be formed. Then, the third species showing the highest average correlation with members of the nucleus was added, then the fourth species, and so on. The limit of a group was assumed

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to be determined by a drop of more than 0.20 in correlation coefficient. When the limit has been reached, a second matrix was constructed for regrouping the resultant assemblage. Correlations between groups were computed by SPEARMAN's sum of variables method. The same method of grouping as in the first correlation matrix was applied and matrices were formed up to the fifth to obtain a single group of the 12 species. The resultant relationships are shown in a treelike diagram (Fig. 1.), in which the magnitude of correlation coefficient between any two joining stems can be read on the abscissa.

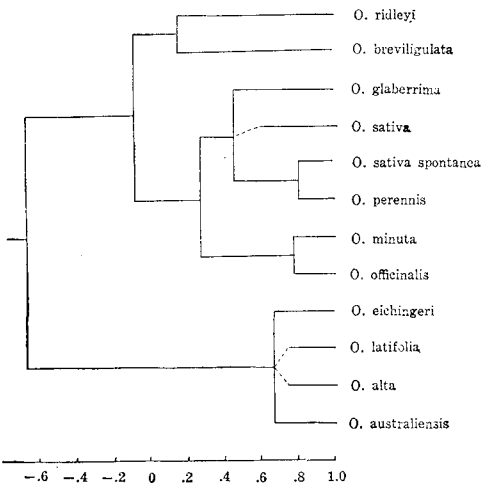

Fig. 1. Diagram showing interspecific relationships in *Oryza*, in terms of correlation coefficients.

We have some reservations about the results obtained, however, because the number of characters used does not seem to be large enough, and they might not be fully indicative of the entire spectrum of potential variation among these 12 species. As Fig. 1 shows, however, the treelike diagram thus obtained is not very far from the previously established classification of the genus *Oryza*, and may throw some new light on phylogenetic relationships within the genus. For example *O. minuta* and *O. officinalis* (r=0.79) are rather nearer to the group of cultivated species and its relatives than to *O. latifolia* and *O. alta*, which form another group together with *O. australiensis* and *O. eichingeri*. *O. ridleyi* which has been thought to stand apart from the others, was found to be correlated with *O. breviligulata* which some workers have considered to be the ancestral from of *O. glaberrima*, though the correlation coefficient, r=0.19, between these two species is too low to evaluate the relationship between them.

17. *Discrimination between Oryza sativa and O. glaberrima by Morphological Characters**

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

A group of cultivated rice varieties grown in various regions of West Africa has been regarded as belonging to a particular species, *Oryza glaberrima* Steud., which is characterized by glabrous glumes, short ligules with a roundish tip, stiff panicles and other traits. However, it is in many other characters similar to *Oryza sativa* L., and the phylogenetic relationship between these two species is not well known. To inquire into the relationship between them, many rice varieties supposedly belonging to one or the other of these species were grown in Misima, and a number of morphological characters were investigated. 20 typical varieties of *O. glaberrima* and 30 varieties of O. sativa were chosen, and a discriminant function for classifying the two species was constructed using six morphological characters as given below. The linear function to maximize the ratio of variance between the two groups to that within the group was found to be:

$$X=x_1-0.76x_2+0.42x_3-0.22x_4+0.48x_5+2.70x_6\ ,$$

where x_1 represents length × width of grain (mm^2), x_2, length/width ratio of grain, x_3, empty lemma length (mm), x_4 number of first rachises, x_5, panicle length (cm) and x_6 ligule length (mm). The length of the apiculus hair was not included in this calculation since most strains supposedly belonging to *O. glaberrima* were hairless, and the species name "glaberrima" itself implies glabrousness. It was found that the F value for the between-group variance of the composed character X was higher than the highest value given by a single character (ligule length). The above function would then be effective for discriminating between these two

Table 1. Frequency distribution of the score given by a discriminant function for classifying *O. sativa* and *O. glaberrima*.

Group	Score									Total number of varieties
	10	15	20	25	30	35	40	45	50	
Hairless varieties	3	19	5		3	1		2		33
Hairy varieties			1	2	7	15	13	12	1	51
Total	3	19	6	2	10	16	13	14	1	84

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

species. The distribution of the score X found in a number of varieties of the two species is given in Table 1, in which the varieties are classified into hairy and hairless.

The data in the table show that the two species can be classified by the score into two groups, though a few varieties appear to be intermediate. Some varieties described in our collection-list as *O. glaberrima* were found to be near to *O. sativa* according to the score of this discriminant function. A few varieties of *O. sativa* were hairless, but their scores were within the range of that species.

18. *A Preliminary Note on Investigation of Wild and Cultivated Rice Strains Collected from the Mountain Region of Orissa State, India**

(By Hiko-Ichi Oka, Wen-Tsai Chang and Takashi Narise)

The mountain region in the western part of Orissa State, India, comprises about 10,000 square miles called the Jeypore Tract, which has never been influenced by modern civilization. The greater part of this area is under forest, but in valleys and some other places, rice is grown by the native people in their "shifting" farms or in terraced paddies. It has been found that this area contains an extremely rich variation in both cultivated and wild rice forms, and the Indian Council of Agricultural Research has been since 1955 sending collecting parties to this area for survey and collection of rice strains. Through the kindness of Dr. S. Govindswamy of the Central Rice Research Institute, Cuttack, India, who is in charge of this project, one of the writers (Narise) could join the collecting party and visit some parts of this area in November, 1957, when he was staying in Cuttack with the senior writer. They were allowed to use the materials for their studies.

About 200 rice strains collected from the Jeypore Tract were tested by the first two writers in Taiwan Provincial Agricultural College, Taichung, Taiwan. Seeds were sown on May 29, 1958, and five plants per strain were raised in the experimental paddy. Investigation were made on an individual plant basis regarding degree of grain shedding(S), grain weight (W), germinating capacity of grains 50 days after heading(G), resistance to potassium chlorate(P), length-width ratio of grains(L), length of hairs at apiculus(H), and many other characters.

Variations in these characters among the Jeypore strains were as large or larger than those found among rice varieties widely collected from

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

various Asian countries. In some cases, however, it was difficult to judge from the appearance in the field whether the strains were cultivated or wild forms. In order to classify the wild and cultivated forms of rice as clearly as possible, a discriminant function (X_1) was constructed based on the variations in characters S, G and W among 21 Indian strains of *Oryza perennis* and *O. sativa spontanea*, showing typical characteristics of those species, and 21 cultivated varieties, including both the "Indica" and "Japonica" types. It was found that

$$X_1 = S - 1.857G - 0.006,28W .$$

The distribution of the score given to the Jeypore strains by this formula is shown in Table 1.

Table 1. Distribution of the score X_1 for discriminating wild and cultivated forms of rice.

Population	Score								No. of strains
	−1.8	−1.4	−1.0	−0.6	−0.2	0.2	0.6	1.0	
Cultivated vars.	3	12	4	2					21
Indian wild rice								21	21
Jeypore strains									
Group 1*				1	2	2	9	21	35
Group 2**		8	10	8	15	36	43	1	121

* Those considered by the collector to be wild.
** Those considered to be under human control.

The data in the table show that, although the 21 cultivated varieties and the 21 wild forms can be clearly discriminated by the score, most of the Jeypore strains are either intermediate between the cultivated and wild forms or nearer the wild form, and only a few of them are truly the cultivated type.

Secondly, in order to investigate the differentiation of the Indica (continental) and Japonica (Insular) types, a discriminant function (X_2) which may maximize the difference between the two types was constructed from the senior writer's old data (Oka 1957, Jap. Jour. Breed. 6), using characters P, L and H. It was found to be

$$X_2 = P - 0.237L + 0.567H .$$

Distribution of the score X_2 is given in Table 2.

The data in the table indicate that a greater part of the strains from the Jeypore tract, as well as of wild strains (*O. perennis* or *O. sativa spontanea*), are intermediates between typical Indica and Japonica varieties,

Table 2. Distribution of the score for discriminating the Indica and Japonica types.

Population	Score								No. of strains
	−1.0	−1.5	−2.0	−2.5	−3.0	−3.5	−4.0	−4.5	
Cultivated vars.									
"Indica"				4	4	3	22	7	40
"Japonica"	2	12	20	1					35
Jeypore strains									
Group 1*				5	1	10	13		29
Group 2*		1	3	2	31	40	40	3	120
Indian wild rice**		3	4	9	5	2	7		30

* Explanation in Table 1.

** *Oryza perennis* or *O. sativa spontanea*.

while a few of them are of the Japonica type. The writers are engaged in a more detailed study.

19. *A Study of the Flowering Time in Wild Rice*

(By Kan-Ichi SAKAI and Takashi NARISE)

The flowering time of wild rice collected in India and Ceylon was studied. The collection was made between November of 1957 and February of 1958 at various spots in those countries. Seeds were germinated in petri dishes in May of 1958 and young plants grown in pots were transplanted to a field at Peradeniya, Ceylon, in June. The flowering time was measured on an individual plant basis by the number of days from sowing of seeds to the first day of flowering. Locations where the materials were collected are as follows:

Species	Notation of population	Location
Oryza rufipogon	B	Chinsurah, West Bengal, India
(*O. sativa spontanea*)	P	Cuttack, Orissa, India
	F-b	Cuttack, Orissa, India
	T-(1–5)	Samalkot, Andhra, India
	Ill	Illuppaiyadichchenai, East coast of Ceylon
	PP	Pottuvil Periphery, East coast of Ceylon
	PT	Pottuvil Town, East coast of Ceylon
Oryza perennis	H-1	Cuttack, Orissa, India
	Vey	Veyangoda, West inland of Ceylon
	Yag	Yagoda, West inland of Ceylon

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

Table 1. Flowering time of various populations of wild rice species.

Species	Population	No. of plant observed	111–115	116–120	121–125	126–130	131–135	136–140	141–145	146–150	151–155	156–160	161–165	166–170	171–175	176–180	181–185	186–190	191–195	196–200	201–205	206–210	211–215	216–220	Non flowered	Mean
Oryza rufipogon	B	21	2	8	5	3	2	1																	0	122.76
	P	11					5	2	2	2															0	138.55
	F-b	58			1	15	22	15	2	2		1													0	134.21
	T-1	76						1	5	18	12	10	11	12	4	2	1								0	157.92
	T-4	76							3	13	13	14	13	5	7	8									0	159.87
	T-5	78					2	8	15	19	9	9	5	5	5		1								0	151.28
	I11	53				1	2	3	3	12	16	7	7	1	1										0	152.34
	PP	26				2	2	8	6	6	2														0	141.04
	PT	44	1	6		2	2	6	8	15	2	1		1											0	140.91
Oryza perennis	H-1	84									2	4		2		6	1								69	—
	Yag	319								2	1	4	12	10	9	70	119	64	15	7	3	1	1	1	0	182.63
	Vey	156								2		24	21	17	9	14	20	17	20	5	4	1		2	0	177.47

The flowering time of different populations of wild rice is presented in Table 1.

Table 1 shows that among various populations of *Oryza rufipogon* (*O. sativa spontanea*) native to India, there was a general tendency for plants from higher latitudes to flower earlier than those from lower latitudes. *O. perennis* flowered, in general, later than *O. rufipogon*. In this connection, it is noteworthy that most of the plants of the H–1 population of *O. perennis* collected in Orissa, India, did not reach anthesis within the duration of this experiment, while all plants of *O. rufipogon* flowered before the end. This indicates that the H–1 race may have become so sensitive to a special photoperiodic cycle that they fail to flower under the conditions of Ceylon.

20. *Observations on Flowering and Seed Development in a Wild Rice Species, Oryza rufipogon (O. sativa spontanea) in Ceylon.**

(By Takashi NARISE)

Flowering and seed development in wild *Oryza* species have never been observed in detail, so far as the writer knows. Wild rice plants belonging to *O. rufipogon* (*O. sativa spontanea*), collected from natural habitats at Illuppaiyadichchenai and Pottuvil, Batticalo District, Ceylon, were grown in pots in the Botanical Garden, Peradeniya. At the same time several cultivated varieties were also grown in pots, and their flowering and seed development were observed for comparison. Investigations were made on (1) how long the flowers remain open, (2) time of flower opening, and (3) daily increase in kernel weight after fertilization.

Results of observations on the duration of the open condition of flowers are given in Table 1. As the data in Table 1 show, *O. rufipogon* and the cultivated variety indigenous to Ceylon showed not much difference in this respect, while the flowers of varieties introduced from foreign countries remained open longer.

Secondly, variations in the time of flower opening in *O. rufipogon* and a cultivated variety, Mas M-24, observed on fine days and on rainy or cloudy days, are given in Table 2. It is interesting to note that the wild species showed a marked delay in flowering on rainy or cloudy days, while the cultivated varieties did not depend so much on the weather.

Thirdly, the daily increase in fresh as well as dry weight of kernels in *O. rufipogon* was observed. As the data in Table 3 show, it was found that the development of the kernels may be completed about two weeks

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Table 1. Variation in the duration of flower opening in a wild rice species and five cultivated rice varieties.

Variety	Origin	Duration in minutes											No. of spikelets	Mean value
		35	40	45	50	55	60	65	70	75	80	85		
*O. rufipogon**	Ceylon	3	4	12	13	12	4						50	48.88 ± 6.13
Murugan Samba**	Ceylon	1	6	22	10	1							50	43.88 ± 4.71
Mas M-24**	Indonesia	1	7	5	9	11	10	3	3		1		50	53.32 ± 9.44
FAO No. 5817**	Italy						1	2	9	16	14	8	50	76.30 ± 5.49
FAO No. 5818**	Italy						4	9	9	10	18		50	72.34 ± 6.39
FAO No. 5820**	Italy						5	19	14	8	3	1	50	68.24 ± 5.24

* Wild rice collected at Illuppaiyadichchenai.
** Cultivated variety.

Table 2. Variation in flowering time in a wild and a cultivated rice.

Variety	Weather	10^h				11^h				12^h				13^h				14^h		No. of spikelets	Mean value
		0′–15′	–30′	–45′	–0′	–15′	–30′	–45′	–0′	–15′	–30′	–45′	–0′	–15′	–30′	–45′	–0′	–15′			
Mas M-24	Fair	23	7	9	16	168	24	14	0	7	2	0	0	0	0	0	0	0		270	$11^h04'$
	Cloudy or rainy	0	0	0	1	66	68	2	10	6	9	2	1	0	0	0	0	0		165	$11^h24'$
	Total	23	7	9	17	234	92	16	10	13	11	2	1	0	0	0	0	0		435	$11^h12' \pm 20.30'$
Oryza rufipogon	Fair	8	28	10	5	46	47	35	16	19	18	5	6	2	7	0	2	1		255	$11^h24'$
	Cloudy or rainy	0	0	0	0	1	3	1	4	42	43	15	6	5	5	4	2	0		131	$12^h25'$
	Total	8	28	10	5	47	50	36	20	61	61	20	12	7	12	4	4	1		386	$11^h48' \pm 49.35'$
Average temperature (°C)		26.2	27.4	28.2	28.9	28.8	28.8	28.7	29.3	29.2	29.4	28.5	28.6	28.4	28.9	29.0	28.7	28.9			

Table 3. Daily increase in fresh weight, dry weight and water content of developing kernels in wild rice.

No. of days after flowering	0	1	2	3	4	5	6	7	8
Fresh weight (mg)	5.8	6.0	4.9	6.0	7.7	9.0	12.3	14.1	11.8
Water content (%)	45	38	33	38	43	36	46	40	35
Dry weight (mg)	3.2	3.4	3.3	3.7	4.4	5.8	6.7	8.4	7.7
Ratio of dry to fresh weight	.55	.57	.67	.62	.57	.64	.54	.59	.55
No. of days after flowering	9	10	11	12	13	14	15	16	
Fresh weight (mg)	14.6	14.6	17.7	18.5	16.4	18.4	18.2	18.5	
Water content (%)	31	27	30	30	36	24	24	24	
Dry weight (mg)	10.0	10.7	14.2	13.1	10.5	14.0	13.9	14.0	
Ratio of dry to fresh weight	.68	.73	.70	.70	.64	.76	.76	.76	

after anthesis. The water content of kernels seemed to decrease with the increase in weight. The color of the glumes changed to brown about two weeks after anthesis.

21. *Photoperiodic Responses of Oryza Species**

(By Tadao KATAYAMA and Hiko-Ichi OKA)

The materials used in this experiment are strains of various *Oryza* species collected from India and other foreign countries. This report is a preliminary note on the results of experiments on photoperiodic responses of those species. For the strains from tropical countries, five automatically controlled short-day fields were constructed in this Institute with the aid of a grant from the Rockefeller Foundation. Three plots were set for 11, 12 and 13 hour-days. For the remaining two plots, special time-controlling clocks called Astrodial were used, with a "latitudinal control" which brings about the natural change of day-length at a given latitude. During 1958 the control was set for 35°N and the installations were operated so as to advance the calendar one month for one plot and two months for the other. Seeds were sown on May 19 and seedlings were transplanted to the short-day field on June 23.

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In addition to this experiment, the same strains were grown in Taichung, Taiwan. They were sown on May 22 and June 21 and grown under conditions of natural day-length (see paper 22 by OKA et al. in this annual report), and photoperiodic responses, measured by the differences in the

Table 1. Interspecific variations in photoperiodic sensitivity and critical day-length.

	Taichung		
	No. of strains	Sensitivity in degree	Critical day-length
O. australiensis	1	10°	13h20′
O. breviligulata	2	83°–90°	14h02′–14h09′
O. eichingeri	1	90°	13h23′
O. glaberrima	10	75°–90°	12h40′–13h23′
O. latifolia	4	52°–70°	13h19′–14h08′
O. minuta	2	78°–90°	13h27′–13h30′
O. officinalis	3	12°,40°–69°	13h45′–13h58′
O. perennis	3	83°–90°	12h59′–13h20′
O. sativa	163*	0°–90°	12h45′–14h30′
O. sativa v. *spontanea*	4	38°–70°	13h11′–13h57′
O. ridleyi	1	55°	13h16′
	Misima		
	No. of strains	Sensitivity in degree	Critical day-length
O. alta	1	6°	
O. australiensis	1	48°	12h16′
O. eichingeri	1	58°	12h15′
O. glaberrima	9	56°–90°	12h00′–13h00′
O. latifolia	1	60°	13h00′
O. minuta	1	3°	12h58′–13h00′
O. officinalis	2	84°–86°	12h58′–13h00′
O. perennis	5	27°–67°	11h00′–13h19′
O. sativa	6	4°–68°	12h00′–13h30′
O. sativa v. *spontanea*	7	12°–76°	12h00′–13h00′
O. ridleyi	1	non-heading	

* From OKA 1953.

length of the growing period, were recorded for the groups of plants sown in May and in June.

The sensitivity to day-length was measured by the method formerly used by the junior writer (OKA 1953, 1958), i.e. by the ratio of shortening of the growing period to a unit shortening of day-length at the time of flower initiation (assumed to be 30 days before heading). The day-length at which the ratio was largest was regarded to be the "critical day-length", and the sensitivity to that day-length was expressed in terms of the arc tangent of the largest ratio.

It is known that cultivated rice varieties (*O. sativa*) can be classified into sensitive and insensitive strains. Almost all of wild rice material tested, however, including many strains of *O. perennis* and *O. sativa* v. *spontanea*, were found to be photoperiodically sensitive, except that an insensitive strain was found in each of *O. alta*, *O. officinalis* and *O. minuta* (Table 1). All tested varieties of *O. glaberrima* (cultivated) were also sensitive, as shown in Table 1. This seems to suggest that photoperiodic sensitivity favors the wild rice plants with some selective advantage.

It was found further that the critical day-length for wild rice strains was 12 to 13.5 hours. The relation found between the critical day-length and the latitude of the place of collection is given in Table 2. The data in this table show the tendency for critical day-length to become shorter in low latitudinal countries. This may be due to the fact that heading time is primarily determined by latitude in these species.

Table 2. Geographical variation in critical day-length among photoperiodically sensitive strains.

Latitude (N)	Critical day-length							Total of strains
	12h00′	12h15′	12h30′	12h45′	13h00′	13h15′	13h30′	
10°–11°	3							3
13°–15°	3	1			1			5
19°–22°	4	2			6			12
24°–27°					7	1		8
35°–37°							2	2

22. *Variation in Photoperiodic Sensitivity among and within Populations of Oryza perennis and Other Wild Oryza Species*

(By Hiko-Ichi OKA, Chao-Hwa HU and Wen-Tsai CHANG)

A number of populations of wild rice belonging to *Oryza perennis* Moench., *O. sativa spontanea* and other species, which had been collected in India and Thailand by the senior writer, and in Taiwan by the junior writers, were grown in an experimental paddy in Taiwan Provincial Agricultural College, Taichung, Taiwan. The seeds were soaked in water for germination on May 22 and June 21, 1958. Heading was observed ranging from early September to early November, except for a few plants which were very late. The average temperatures of growing periods of different populations are mostly between 26°C and 28°C, and the difference found between May and June seedings in the number of days from seeding to heading can be attributed to the difference in the day-length to which they have responded.

Photoperiodic sensitivities of those populations were shown, by the method formerly adopted by the senior writer (OKA 1953, 1958), by the angle between the abscissa and the line showing the shortening of growing period (in days) in response to shortening of the day-length 30 days before heading (in minutes). Further, the day-length at the mid-point of the line was considered to be the "critical day-length". Thus, the mean sensitivity and mean critical day-length of each population were determined. Their standard deviations which show the extent of within-populational variation were computed from the standard deviations of the heading date as follows: First, twice the standard deviation of the heading date was subtacted from, or added to, the mean heading date to estimate the early or late marginal points of the distribution. Then, the early or late marginal dates for each of the two groups of plants belonging to a population, seeded in May and in June, were compared by the above-mentioned method to find the sensitivity and critical day-length of the earliest or latest plant. Thus, a set of three measurements for earliest, average and latest plants were found for each population.

The results of this calculation for each of the populations tested are set out in Fig. 1, where the range of variation in photoperiodic sensitivity or critical day-length is shown by a line connecting the values for the earliest and latest plants. It is recognized in Fig. 1 that some populations are quite heterogeneous for photoperiodic sensitivity, while a few are uniformly highly sensitive. It seems that populations of *O. sativa spontanea*

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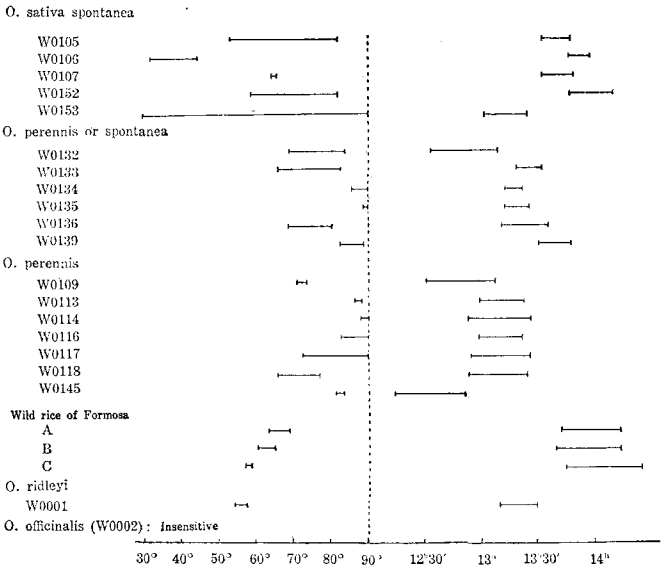

Fig. 1. Variations in photoperiodic sensitivity and critical day-length among polulations of *O. perennis* and other wild *Oryza* species.

generally show a wide range of variation in photoperiodic sensitivity, while *O. perennis* is relatively uniformly sensitive and its variation in heading date is chiefly due to variation in the critical day-length. It was found further that *O. ridleyi* was as sensitive to day-length as *O. perennis*, and *O. officinalis* (a strain from Bangkok, Thailand) was insensitive.

23. *Susceptibility of Wild and Foreign Cultivated Rice to Blast Fungus, Piricularia oryzae**

(By Keizô KATSUYA)

In order to examine the susceptibility of the genus *Oryza* to blast, 14 strains of 9 wild species, 10 foreign strains of cultivated rice belonging to *O. sativa* and 3 varieties of Japanese cultivated *O. sativa* were tested in a greenhouse with 2 strains of *Piricularia oryzae*, namely P-2 and 54-04, obtained from the National Institute of Agricultural Sciences. As to pathogenicity, the P-2 strain is comparatively virulent while the 54-04 strain is less virulent. The plants were kept in pots to which ammonium sulphate, calcium superphosphate and potassium sulphate were added. They

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were inoculated by spraying an aqueous spore suspension of the fungus on the third or the fourth leaf, and by injecting it into the still folded fourth to sixth leaf with a hypodermic syringe. The spores were cultured on boiled barley. The degree of susceptibility was determined by the types of lesions formed on the leaves. Blast readings were made on infected leaves 5 to 11 days after inoculation. Sheath inoculation (SAKAMOTO 1951, TAKAHASHI 1957) was used for comparison. These tests were repeated 2 to 4 times.

The experimental results in general showed that the injection method effected a more severe pathogenicity than spraying. Among the wild species, *O. australiensis* (W 0008) was very susceptible to the two strains of blast fungus. *O. officinalis*? (W 0012), *O. eichingeri*? (W 0015), *O. latifolia* (W 0019) and *O. breviligulata*? (W 0028) exhibited resistance to the P-2 strain when sprayed and resistance or moderate susceptibility when injection was used; they were resistant to the strain 54-04 when inoculated by injection. *O. subulata* (W 0510) was moderately susceptible to the P-2 strain and resistant to the 54-04 strain.

O. perennis, *O. sativa* var. *fatua* and other foreign cultivated rice varieties of *O. sativa* exhibited considerable differences according to the strain employed.

24. *Susceptibility of Chlorophyll Mutants of Einkorn Wheat to Stem and Leaf Rust, Puccinia graminis and Puccinia triticina*

(By Keizô KATSUYA)

In order to examine the susceptibility of chlorophyll mutants of *Triticum monococcum flavescens* to stem and leaf rusts, the mutants *chlorina*, *basiviridis* II, *virido-albina* and *albina* were tested in the phytotron (20°C in daytime, 15°C at night) at the first leaf stage with *Puccinia graminis* f. sp. *tritici* 17 and *P. triticina* 21 B. Both filter paper and brushing inoculation methods were used. Rust readings were made 7 to 16 days after inoculation. The degree of susceptibility was determined by the types of lesions formed on the leaves. These tests were repeated 2 or 3 times.

Susceptibility of chlorophyll mutants to stem and leaf rusts is shown in Table 1. The mutant strain "*chlorina*" whose chlorophyll content was about 50% of that of the normal plants was not much different from the normal plants in susceptibility to both rusts. However it showed a tendency to earlier formation of uredosori. The mutant strain "*albina*" differed from the normal plants in susceptibility to both rusts exhibiting resistance. No uredosori were found, since *albina* dies from 7 to 12 days after inoculation. Both kinds of rusts formed uredosori on the white parts,

Table 1. Susceptibility of seedlings of chlorophyll mutants to stem and leaf rust of wheat.

Plants inoculated		Type of Infection	
		P. graminis f. sp. *tritici* 17	*P. triticina* 21B
Normal		S	R-MR
Chlorina		S	R-MR
Basi-viridis II	green parts	S	
	light green parts	S	
Virido-albina	green parts	S	MR-S
	white parts	S	MR-S
Albina		R	R

R: Resistant, MR: Moderately Resistant, S: Susceptible

having no chlorophyll, and also on the green parts of leaves of *virido-albina*, having chlorophyll. The susceptibility of this mutant to leaf rust was higher than that of normal plants. The mutant strain "*basi-viridis* II" was susceptible to stem rust.

C. CYTOLOGY AND GENETICS OF *NICOTIANA* AND SOME OTHER PLANTS

25. *Cytogenetic Studies of the Genus Nicotiana XI*

(By Yô TAKENAKA)

The reduction divisions in PMC's were studied in 3 interspecific hybrids: *N. knightiana*×*N. tabacum*, 4x *N. tabacum*×*N. benavidesii* and 4x *N. tabacum*×*N. langsdorffii*.

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

So far as I know, no report on this hybrid has been published. At MI in PMC's of this hybrid, the number of bivalents ranged from 0 to 5, with the mode at 0. The frequency of PMC's with one bivalent followed that of PMC's with only univalents, and amounted to 30% of the total number of cells observed. The frequencies of PMC's with 2 bivalents and 3 bivalents were 20% and 10% of the total number, respectively. PMC's with 4 bivalents were very rare. Considering the small number of bivalents, it is difficult to determine whether the chromosomal affinities are

allosyndetic between the genomes of the parents or autosyndetic between the two genomes of *N. tabacum* which is of amphidiploid origin.

2) F_1 of 4x *N. tabacum* (n=48)×*N. benavidesii* (n=12).

This hybrid was produced for the purpose of introducing a gene or genes for immunity against the common mosaic disease from *N. benavidesii* into cultivated tobacco.

At MI in PMC's of this hybrid, the total number of bivalents and trivalents per cell was 24. The number of trivalents ranged from 0 to 6, with the mode at 2. PMC's with 5 or 6 trivalents were very rare.

At meiosis of the F_1 of 2x *N. tabacum*×*N. benevidesii* GOODSPEED (1954) observed the range of chromosome pairs to be from 0 to 6, with the mode at 3. Considering that in my study hybrid plants with two chromosome sets of *N. tabacum* and one chromosome set of *N. benevidesii* have been used, it may be said that the results of my observations are generally in agreement with GOODSPEED's data, because the affinity between homologous chromosomes may in this hybrid be saturated by the presence of two chromosome sets of *N. tabacum*.

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

The purpose of producing this hybrid was to introduce a gene or genes for immunity to mildew and a gene or genes for resistance to black root rot from *N. langsdorffii* into cultivated tobacco.

At MI in PMC's of the F_1 of 4x *N. tabacum*×*N. langsdorffii*, the total number of bivalents and trivalents per cell was 24. The number of trivalents ranged from 0 to 5, with the mode at 1. PMC's with 4 or 5 trivalents were very rare. In my laboratory, Mr. HU (1956) observed meiosis of the hybrid between *N. tabacum* and *N. langsdorffii*, and observed a range from 5 to 12 chromosome pairs with the mode at 11. Considering his data, more trivalents than those observed by myself may be expected in the F_1 of 4x *N. tabacum*×*N. langsdorffii*. But the small number of trivalents found in this hybrid may be due to saturation of the affinity between homologous chromosomes by the two chromosome sets of *N. tabacum* present in this hybrid.

26. *Cytological Studies in the Genus Euphorbia III*

(By Shôhachi SHIMOYAMA)

(1) Somatic chromosomes of *Euphorbia Lathyris* L.

Euphorbia Lathyris is a native of South Europe and Southwest Asia and it is cultivated in Japan as a medical plant.

The materials for the present study were supplied by the Botanical Gardens of Technical University, Netherlands; the Botanical Gardens of

München, Germany; the Botanical Institute of the University of Liege, Belgium and the Botanical Gardens of the University of Tokyo.

Somatic chromosomes in root-tip cells were observed by the acetic orcein squash method. The chromosome number was without exception 2n=20, in agreement with the observation of PERRY (1943). According to my observations the chromosome set of this species consists of 18 V-shaped chromosomes and two J-shaped chromosomes with a satellite on the short arm. Tho karyotype can be expressed as follows:

$$K(2n)=20=2A^{m}+2B^{m}+2^{t}C^{st}+10D^{m}+4E^{m} .$$

The basic chromosome numbers of *Euphorbia* are considered to be 6, 7, 8, 9 and 10 (PERRY 1943, TISCHLER 1950, DARLINGTON and WYLIE 1955). The chromosomes of plants with the basic number 10, including *E. Lathyris*, are usually larger than those of plants with other numbers.

(2) Karyotypes of *Euphorbia Sieboldiana* Morr. et Decne. and *E. Sieboldiana* var. *idzuensis* Nakai.

Euphorbia Sieboldiana is distributed in Southern Kurile, Sachalin (southern part), Hokkaidô, Honsyû, Sikoku, Kyûsyû and Korea (?). This species is very polymorphic and presents a troublesome problem for the taxonomist.

E. Sieboldiana s. str., collected at Tazimagahara, Prov. Musasi, has 20 somatic chromosomes; 14 with a median centromere, four with a submedian centromere and two with a subterminal centromere. The karyotype can be expressed as follows:

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

E. Sieboldiana var. *idzuensis* is endemic in Izu-Peninsula, Izu-Osima and Prov. Kazusa. In this variety, differences in the karyotype between habitats were found. The material obtained at Misima, Heda-misaki and Irôzaki in Prov. Izu were similar to *E. Sieboldiana* in their karyotype, but the plants collected at Izu-Osima had a satellite on the short arm of the C-chromosomes. The karyotype of the latter is represented as follows:

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

E. Sieboldiana includes various varieties other than *idzuensis*; *miyazimensis* Hurusawa, *montanus* Tatewaki, *ohsumiensis* Hurusawa and *Shikokiana* Hurusawa. They are now being studied from the cytogenetical point of view.

(3) Chromosome variations in *Euphorbia pekinensis* Rupr. and allied species.

Euphorbia pekinensis is perennial and widely distributed in Japan, Korea, Manchuria and China. In Japan, it is grown in Honsyû, Sikoku and Kyusyû. Typical localities of this species are found in North China.

This form is very polymorphic and may be divided into many subspecies, varieties and forms. Moreover, there are several species whose distinction from *E. pekinensis* is sometimes obscure.

In a previous paper, chromosome numbers of 2n=28 and 56 were reported for this species; plants coming from mountainous regions had the former chromosome number, while the latter number was found in specimens collected on flat ground.

In 1958, one variety and one species closely allied to *E. pekinensis* were examined cytologically.

E. pekinensis var. *ibukiensis* Hurusawa 2n=52

E. adenochlora Morr. et Decne. 2n=26

E. pekinensis var. *ibukiensis* occurs at a high elevation on Mt. Ibuki in Prov. Ômi: the plants are low, herbaceous and fasciated. *E. adenochlora* grows gregariously on wet land and is distributed in Hokkaidô, Honsyû and Kyûsyû.

Perry (1943) reported 2n=24 for *E. pekinensis* from material of unknown origin. It seems that in this species-group of Euphorbia both polyploidy and aneuploidy have been effective in speciation.

(4) Chromosome numbers of some *Euphorbia* species native to foreign countries.

In 1958 the chromosomes of six species native to foreign countries were examined. The results are given in Table 1, together with the sources of materials.

Table 1. List of chromosome numbers.

Species	2n	Source
Euphorbia Peplus	16	Jardin Botanique de la Ville de Nancy, France; Jardin Botanique de l'Université et de la Ville, Besançon, France; Jardin Botanique de l'Université de Lisboa, Portugal
E. bulbolina	20	Botanischer Garten der Universität Göttingen, Deutschland
E. cyparissias	20	〃
E. palustris	20	Hortus Botanicus Academiae Scientiarm RSS Turcomaniae Ashkhabas USSR
E. stricta	20	〃
E. marginata	56	〃

27. *Cytotaxonomic Studies in Veronica and Related Genera, I*

(By Takashi YAMAZAKI and Tuguo TATEOKA)

As part of a taxonomic investigation, the chromosomes of *Veronica* and related genera have been examined by the junior author. In 1958 the following results were obtained.

Veronicastrum sibiricum Pennell var. *zuccarinii* Hara	2n=34
V. sibiricum var. *japonicum* Hara	2n=68
Veronica schmidtiana Regel ex Fr. Schmidt var. *bandaina* Makino form. *senanensis* Yamazaki	2n=34
V. routunda Nakai var. *subintegra* Yamazaki	2n=33*
V. kiusiana Furumi subsp. *miyabei* Yamazaki var. *villosa* Yamazaki	2n=34
V. kiusiana subsp. *maritima* Yamazaki var. *maritima* Yamazaki	2n=68
V. ornata Monjuschko	2n=68
V. nipponica Makino ex Matsumura	2n=18
V. onoei Franch. et Savat.	2n=36
V. hederaefolia Linn.	2n=54
V. repens Clar.	2n=14
*V. sp.***	2n=16

28. *Development of Seeds and Embryos in Three Crosses between Diploids and Artificial Tetraploids*

(By Kazuo FURUSATO)

In general, when artificially produced tetraploids are crossed with the original diploids as pollen contributors, the seeds ripen and germinate well. But the reciprocal crosses, diploid×tetraploid, usually give non-viable seeds only. The development of seeds and embryos in the latter crosses has been investigated in *Nicotiana tabacum*, *Citrullus vulgaris* and *Citrus sp.* with the following results.

1. *Nicotiana tabacum*

At self-fertilization and in crosses between diploid strains approximately

* This unexpected number may be derived through the loss of one chromosome from 2n=34. The material used in this observation was only one individual.

** The seed sample of this species was supplied by the Edinburgh Botanic Garden. According to a label attached to the sample, the species was obtained from Tibet.

one day after pollination the male nucleus divides and two nuclei are found in the pollen tube. After another day they enter the embryo-sac and perform double fertilization. One or two days after fertilization, the egg cell starts to divide and in the course of the next 5–7 days the differentiation into cotyledons enclosing the pulmula and radicula is perfected. From then on the further development takes its regular course, and about 20 days following pollination the seeds are ripe.

But when the pollen of a colchicine- produced tetraploid is dusted on the stigma of the original diploid, the germination of the pollen grains is considerably slower than in the reciprocal combination. Moreover some of the grains show abnormal growth, and quite often twisted pollen tubes can be seen. After fertilization the egg cell starts to divide but this process is very slow if compared with the 2x×2x-combination. No differentiation of the embryo can be detected. Even after 30 days only a cell mass of globular shape is found which finally shrinks away in the immature seed. At the same time, the endosperm nucleus undergoes a number of divisions and in the first 4–5 days following fertilization free nuclei can be observed. Their number is much smaller than when the pollen is furnished by a diploid tobacco. It seems that poor development of the endosperm is one of the reasons for the poor growth of the embryo.

2. *Citrullus vulgaris*

When a diploid is pollinated by another diploid the seeds ripen well 25–30 days after pollination. But when a colchicine-produced tetraploid furnishes the pollen dusted on a diploid stigma, the same phenomena can be observed as in tobacco, and the cell mass representing the embryo remains undifferentiated. However, when the mother is the diploid *Citrullus colocynthis* and the pollen is furnished by the tetraploid *C. vulgaris*, the development of seed and embryo is normal and viable seeds are produced. The cause of this exceptional behavior is not clear. Perhaps the firm flesh of colocynthe protects the developing seeds from drying out.

3. *Citrus sp.*

In pollinations between diploids, seeds are easily produced, in mono- as well as in polyembryonic varieties. But when the pollen derives from a tetraploid, the monoembryonic varieties produce aborted seeds only and the fruits are practically seedless. In polyembryonic varieties a few small nucellar embryos are developed and the seeds are undersized. The poor growth of the nucellar embryos may be caused by the deficient development of the endosperm.

29. *Frequency of 3x Seedlings in Several "triploid" Varieties of Sugar Beets*

(By S. MATSUMURA, A. MOCHIZUKI and M. NEZU)

The somatic chromosome numbers of the offspring were determined in the best combination, No. 4398 (4x)×No. 162 (2x). When 2x and 4x seeds were sown mixed in the ratio of 1 : 5, the frequency of 3x seedlings was the highest, namely 46.5% (Ann. Rep. No. 7, p. 49). In order to produce

Table 1. Frequency of 2x, 3x and 4x obtained from European "triploid" varieties.

Variety		2x	3x	4x	Total
Hilleshög-St-poly (Sweden)		49	43	7	99
		14	18	13*	45
	Total	63	61	20	144
	%	43.75	42.36	13.89	100
Hilleshög-R-poly (Sweden)		44	43	14	101
		9	19	8	36
	Total	53	62	22	137
	%	38.69	45.25	16.06	100
KL-Cercopoly (Germany)		73	100	32	205
		5	13	7*	25
	Total	78	113	39	230
	%	33.91	49.13	16.96	100
Polyrave (Netherlands)		27	40	6	73
		0	37*	6	43
	Total	27	77	12	116
	%	23.28	66.38	10.34	100
Trirave (Netherlands)		10	40	0	50
		20.00	80.00	0	100
Beta-1 (Hungary)		26	74	28	128
		0	2	14	16
	Total	26	76	42	144
	%	18.05	52.78	29.17	100
Beta-3 (Hungary)		27	35	5	67
		4*	11*	3*	18
	Total	31	46	8	85
	%	36.47	54.12	9.41	100

* incl. heteroploid

"triploid" seeds on a commercial scale, promotion of the percentage of 3x plants should be attempted. Recently "triploid" varieties have been brought to market by Sweden, Germany, Denmark, Netherlands and Hungary. Table 1 shows the frequencies of 2x, 3x and 4x seedlings in several European "triploid" varieties. There is no significant difference in the percentage of 3x plants between our combination, No. 4398×No. 162, and the Swedish or German varieties. The Dutch varieties show the best results. A new Dutch triploid variety, "Trirave", has been advertised recently as a triploid obtained by using 2x male sterile plants. Our experiments show beyond doubt that in sugar beets 3x seeds can be obtained through natural pollination even from 2x mother plants, when 2x and 4x beets are planted. If 2x mother plants are male sterile, almost all seeds obtained from them must be 3x.

In another experiment, attempts have been made to induce tetraploidy by colchicine treatment in several resistant American varieties (GW 602, GW 674 etc.) so as to use them as tetraploid parents.

30. *Heterostyly and Pollen Grain Number in Buckwheat*

(By Yukio DOIDA)

According to the number of pollen grains formed in a pollen sac, five types of plants can be distinguished in *Polygonum*. They are: pollen sacs with (1) 8, (2) 16, (3) 32, (4) 128 and (5) 256 pollen grains (DOIDA, 1957). In 1958, the pollen grain formation of long-styled flowers of buckwheat was examined and compared with that of short-styled flowers.

It was found that both long-styled and short-styled flowers have different average numbers of pollen grains formed in a pollen sac (Table 1); the mode for the long-styled flowers was at 48 while that for the short-styled was at 32. Moreover, the variance for the long-styled flowers was clearly

Table 1. Number of pollen grains in a pollen sac (*P. fagopyrum*).

		Number of pollen grains														Total	Mode
		less than 23	24	28	32	36	40	44	48	52	56	60	64	68	more than 69		
No. of cases	Short-styled flowers		3	47	176	89	22	2								339	32
	Long-styled flowers				10	31	52	76	96	51	16	11	8	1		352	48

larger than for the short-styled flowers.

It is well known that in plants with heterostyly the size of the pollen grains is different in long- and short-styled flowers. This is true of buckwheat too. Moreover, the long- and short-styled flowers of this species are characterized by a different average number of pollen grains, as Table 1 shows.

31. *Developmental Studies in the Genus Polygonum*
I. Microsporogenesis in Polygonum persicaria

(By Yukio DOIDA)

The number of pollen grains per pollen sac is a constant trait in *Polygonum* species. In a previous paper (DOIDA, 1957), the author distinguished five types, based on different numbers of pollen grains per pollen sac. It was found in 1958 that *P. persicaria* does not fall into any of the five types. Usually this species has only four pollen grains in a pollen sac.

The development of the pollen mother cell starts with the differentiation of a large cell from the hypodermal cell layer of the anther primordium. This cell is soon clearly distinguishable by its size from the surrounding cells. It continues to grow and increase in size but does not undergo mitotic division and becomes in this condition a pollen mother cell. On the other hand, the cells surrounding the pollen mother cell divide and form four cell layers. Regular meiotic division takes place in the pollen mother cell, and a tetrad and subsequently four pollen grains are produced. Following the meiotic stage, the inner two of the four cell layers surrounding the pollen mother cell begin to degenerate. When an anther reaches maturation, its peripheral zone consists of two cell layers.

Rarely a mitotic nuclear division of the sporogenous cell takes place and eight pollen grains are formed in a pollen sac. Dwarf-pollen grains are seldom found. Therefore it is assumed that meiosis is very regular. This assumption was confirmed by direct observation. Pollen sacs having no pollen grains are also found very rarely. It was histologically confirmed that in such cases the sporogenous cell usually differentiates but the peri-

Table 1. Number of pollen grains in a pollen sac (*P. persicaria*).

	No. of pollen grains				Total	Mode
	4	8	12	3+2m*		
No. of cases	248	20	0	1	269	4

* m: micro-pollen

pheral zone of the anther, especially the tapetal cells, do not develop satisfactorily. The degeneration of the pollen mother cell may be caused by insufficient supply of nutrition which ensues from insufficient development of tapetal tissue.

32. *Developmental Studies in the Genus Polygonum*
II. Effects of Gibberelline on Microsporogenesis of Polygonum fagopyrum

(By Yukio DOIDA)

The author has examined the action of gibberelline upon the development of microspores in *Polygonum fagopyrum*. This material was chosen because of the small number of pollen grains formed in a pollen sac. The concentrations of gibberelline used were 0–100 p.p.m.

The results are as follows:

(1) The higher the concentration of gibberelline, the smaller is the number of pollen grains per sac. At concentrations higher than 10 p.p.m. pollen formation was markedly inhibited, as Fig. 1 shows.

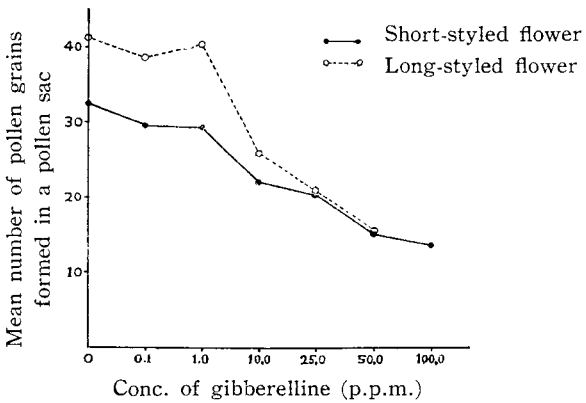


Fig. 1

(2) The diameter of pollen grains remained unchanged but the anthers became shorter with increasing concentrations.

(3) The surface area of the epithelial cells was considerably reduced after treatment with 50 p.p.m. in comparison with the control. The surface area of the epithelial cells was 1.58 ± 0.19 in the control and 1.236 ± 0.189 after gibberelline treatment. The figures represent relative values obtained from planimeter measurements. The difference was significant ($p<0.05$).

From the above results it is clear that gibberelline had an inhibitory action on the microsporogenous tissue of *Fagopyrum*. The same tendency was observed in some flower organs.

33. *Further Studies on Cherry-red Leaf in Tobacco*

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

The writers have formerly reported that in the "Bright Yellow" tabacco variety, strains markdly differ in the grade of expression of cherry-red leaf color, and that genes for high cherry-redness might be dominant, as the results of investigations of F_1 and F_2 plants have shown. This year, F_3 lines, derived from one of the already reported crosses between "Bright Yellow" strains, were observed. As in the years before, in order to obtain a formula which gives proper weightings to the index-numbers for the grades of cherry-redness, 18 different strains were grown in addition to the F_3 lines. The F_3 test comprised plots of 22 F_3 lines, parental strains and F_1's of reciprocal cross combinations, each consisting of a row of 15 plants. The plots were randomized with three replications. The 18 strains were grown in rows of 12 plants, with two replications.

At maturity, the five upper leaves were harvested and after having been dried, were graded into five classes with the index-numbers 0 to 4. Then, the average cherry-redness of each plant was calculated from the data by using the weighting formula.

1) Test of strains: From the data of the strain test, the following formula was obtained maximizing the ratio of variance among plants to that within a plant, i.e.,

$$X=\frac{1}{5}(0x_0+0.144x_1+0.384x_2+0.723x_3+1.0x_4)\ ,$$

where x_0 to x_4 are the number of leaves falling in the respective classes, and X represents the average grade of cherry-redness of a plant. Variance analysis of the score X showed that the variation among strain means was significant. Having computed from the results the variance components for individual plants and for strains, the heritability value was found to be as high as 0.50 for a plant, and 0.89 for a strain.

Further, variance analysis was made of the data for the four years, 1955–1958. The results showed, as given in Table 1, that the variation among strains was highly significant, and the interaction between strain and year was insignificant. It may be concluded, therefore, that the grade of cherry-redness is essentially a genotypically controlled character.

2) Test of F_3 lines: Variance analysis of the data showed that the

Table 1. Variance analysis of the data of strain test for cherry-redness.

Variation due to	d.f.	Mean square
Year	3	.0681*
Strain	17	.2488**
Strain × Year	51	.0220
Error	90	.0159

* Between 5% and 1% levels.
** Above 1% level of significance.

differences between lines were significant, as given in Table 2. It was also found that the correlation between the F_2 measurements and the F_3 line means was as high as 0.727. Besides the high heritability value as already given, this may also indicate that selection for this character would be successful. However, the number of effective genes could not be estimated.

Table 2. Variance analysis of the data of F_3 test.

Variation due to	d.f.	Mean square
Replication	1	.1228**
Line	21	.1277**
Error	42	.0095

** Above 1% level of significance.

3) Relation between cherry-redness and quality of leaves: An insignificant correlation coefficient $r=0.234$ was found between the grade of cherry-redness and the quality of leaves.

34. *Sexuality in Rumex hastates*

(By Yô TAKENAKA)

Rumex hastatus is a gynodioecious species consisting of females and androhermaphroditic plants. The latter flower have 5 perfect anthers and an undergrown pistil.

In the offspring of the females, 79♀ and 80♂ were found. From the pistil of the androhermaphroditic plants, 35♀ and 92♂ were obtained. If we designate the gene effecting male expression *M* ("Realisator"), the genotypes would be *mm* for the females and *Mm* for the androhermaph-

roditic plants. The cross $mm \times Mm$ should give females and males in the proportion of 1:1, as it was observed to do. In the offspring of *Mm* we should have $1mm:2Mm:1MM$, i.e. 1♀:3♂, a proportion which was actually found. One third of the males, those which are *MM*, should be pure males. Moreover, from crosses between those pure males and the females we should expect 100% androhermaphroditic males, *Mm*.

A detailed investigation of this interesting problem is under way.

D. BIOMETRICAL GENETICS AND PROBLEMS OF BREEDING

35. *Inheritance of Competitive Ability in Rice*

(By Hiko-Ichi OKA and Kan-Ichi SAKAI)

It was demonstrated early in this series of competition studies that the competitive ability of a strain is a genetically determined character. It is known that varieties of the Indica type of rice generally show higher competitive ability than those of the Japonica type (Annual Report No. 6: 83–84). This report deals with the inheritance of competitive ability observed in F_2 and F_3 lines of a cross between two rice varieties, Kinoshita-mochi (Japonica) and U-koh (Indica, from Taiwan). In this cross, though the parental varieties were distantly related, the F_1 was almost completely fertile in both pollen and seed setting.

Design of experiment: Plots, each consisting of a row of 15 plants, belonging to the parental varieties, F_2 and 40 F_3 lines were randomized with four replications. The interval between rows was as large as 50 cm so as

Table 1. Variance components for the increments or decrements in a tester strain due to competition with hybrid or parental plants of a cross between two rice varieties.

	Panicle number		Plant weight	
	Observed	Expected	Observed	Expected
V_{F_2}	12.47	12.57	347.3	348.8
$V_{\overline{F}_3}$	3.20	3.20	30.8	30.8
$\overline{V}_{F_3}$	12.02	11.79	344.7	341.6
E_1	10.90	11.01	332.9	334.4
E_2	1.72	1.70	21.2	20.6
D		2.96±1.065		17.7±14.57
H		0.33±2.922		22.1±40.01

to avoid competition between adjacent rows. In each row, the plants were spaced at 25 cm, and between them, two plants of a "tester" strain were inserted, reducing the distance between plants to about 8 cm. As a tester, Yaeho, a rice variety common in Sizuoka Prefecture was used. It was assumed that the two tester plants standing on each side of a plant belonging to the hybrid or parental population would show the effect of competition. The sums of the measurements of each pair of tester plants were recorded and used for variance analysis. Panicle number and plant weight were the characters investigated.

Results: Partitioning of variance components was made by MATHER's method. The results are given in Table 1.

The data in the table show that the inheritance of competitive ability can be analysed by the same method as used for ordinary quantitative characters, if the experiment is designed as in the present study. This suggests that competitive ability is controlled by Mendelian factors. However, the number of effective factors could not be estimated because the variance in the F_3 lines was too great.

36. *Effect of Soil Fertility on Competitive Ability in Upland Rice Varieties*

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

It has been shown before by OKA and SAKAI (Annual Report No. 6) that competitive ability in lowland rice varieties is affected by soil fertility and other environmental conditions. In this paper, the results of an experiment on the effect of fertilizers on competitive ability of an upland rice variety are reported. Competitive ability (p) was computed based on the change in the proportion of genotypes after one generation of mix-planting, by using the formula (presented by the senior writer in 1954),

$$p=\frac{a_1-a_0+(1-a_1)a_0q}{a_0(1-a_0)}.$$

A Japanese upland rice variety, Norin-mochi No. 1, and the so-called "Red Rice" (an Indica-type rice occurring in upland rice farms in some localities in Japan, which is strong in competition with ordinary upland varieties) were used. Seeds of these two strains were mix-sown, the initial proportion of "Red Rice" (a_0) being 5%. The experimental plots had five fertilizer levels, i.e., 4, 1, 1/2, and 1/4 of the normal dosage and a non-fertilized plot. The normal dose consisted of N 6.0 kg/ha, P_2O_5 7.5 kg/ha and K_2O 3.75 kg/ha of ammonium sulphate, calcium superphosphate and potassium sulphate.

At the same time, plots of each variety were raised. From the number of seeds harvested from these plots, the propagation rates ($1-q$) of "Red Rice" relative to Norin-mochi No. 1 at different fertilizer levels were estimated, as given in Table 1. Competitive ability (p) at each fertilizer level was computed from the proportion of "Red Rice" seeds in the mix-planted plots (a_1), as also given in Table 1.

Table 1. Relative propagation rate ($1-q$), proportion of seeds in mix-planted plots (a_1) and competitive ability (p) for "Red Rice". ($a_0=0.05$)

Fertilizer dose	$1-q$	a_1 Observed	a_1, Expected without competition	p
4	0.746	0.035	0.038	−0.063
1	0.870	0.077	0.044	0.683
1/2	0.907	0.105	0.046	1.243
1/4	0.908	0.130	0.046	1.766
0	1.123	0.149	0.056	1.966

The data in the table show that when four times as much fertilizer as the standard dose was applied, "Red Rice" was no longer a strong competitor against Norin-mochi No. 1, but the competitive ability of "Red Rice" tended to increase with the decrease of fertilizer. This suggests that the propagation of "Red Rice" mix-growing in upland rice farms would be promoted by poor soil fertility.

37. *Studies on the Breeding Behavior of Wild Rice (Oryza rufipogon and Oryza perennis)**

(By Kan-Ichi SAKAI and Takashi NARISE)

A biometrical method for the estimation of the percentage of outcrossing in plants by using polygenic characters has been reported by SAKAI and IYAMA (1957). In the present study, this method was adopted for two species of wild rice, *O. rufipogon* (*O. sativa spontanea*) and *O. perennis*. The habitats of the material were as follows:

Species	Notation	Location
O. rufipogon	Chi	Chinsurah, West Bengal, India
	Ill	Illuppaiyadichchenai, East coast of Ceylon
	PP	Pottuvil Periphery, East coast of Ceylon
	PT	Pottuvil Town, East coast of Ceylon
O. perennis	Vey	Veyangoda, West inland of Ceylon
	Yag	Yagoda, West inland of Ceylon

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

Seeds were collected at random in wild populations on individual plant basis. Young plants grown in pots for about one month were transplanted into the field at Peradeniya, Ceylon on the 24th of June, 1958. After harvesting, the width of spikelets was measured. With regard to details of the estimation, the reader is referred to the 7th issue of the Annual Report (page 53).

The analysis of variance, expected components of mean squares and percentage of outcrossing are given in Table 1.

Table 1.

Source of variation	Mean square						Expectation of mean square
	Chi	Ill	PP	PT	Vey	Yag	
Between strains	0.1914	0.3093	0.3041	2.0100	1.2509	0.6467	$\sigma_e^2+k_1\sigma_o^2+k_2\sigma_p^2$
Between individuals within strain	.0325	.1228	.1210	.3184	.3978	.1864	$\sigma_e^2+k_1\sigma_o^2$
Within individual within strain	.0005	.0046	.0065	.0057	.0062	.0062	σ_e^2
σ_e^2	.0005	.0046	.0065	.0057	.0062	.0062	
σ_o^2	.0032	.0118	.0115	.0313	.0392	.0180	
σ_p^2	.0046	.0554	.0652	.3978	.1192	.0489	
Percentage of outcrossing (%)	50.67	20.00	31.60	7.41	26.51	22.42	

Table 1 shows that percentages of outcrossing in *O. rufipogon* were on an average 25–30% although they varied from population to population, *i.e.* from 50% in Chi to 7% in PT population. Similar values were also found in two populations of *O. perennis*.

From these results, we may be right to conclude that wild rice plants, *Oryza rufipogon*, growing in their native habitats are partially allogamous, the percentage of outcrossing being roughly estimated as 30%. The percentage of outcrossing may not be the same among different populations.

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

(By Kan-Ichi SAKAI and Takashi NARISE)

Analysis of genetic variability in wild populations of *Oryza perennis* and *O. rufipogon* (*O. sativa spontanea*) was carried out in 1958 with materials collected from different spots in India and Ceylon. Regarding the method of investigation, reference should be made to the last issue, No. 7, of this Annual Report (page 67). Localities where collection was made are listed below:

Species	Notation of population	Locality	No. of individuals collected
Oryza perennis	CH	Chinsurah, West Bengal, India	50
	JA	Jagatapur, Orissa, India	30
	CT-A	Cuttack, Orissa, India	50
	CT-B	Cuttack, Orissa, India	50
	CT-C	Cuttack, Orissa, India	50
	BG	Baliguda, Orissa, India	50
	PH	Phagad, Orissa, India	30
	SM	Samalkot, Andhra, India	50
O. rufipogon	CH	Chinsurah, West Bengal, India	21
	CN	Canning, West Bengal, India	50
	JA	Jagatapur, Orissa, India	40
	F-b	Cuttack, Orissa, India	58
	PH	Phagad, Orissa, India	36
	T-1	Samalkot, Andhra, India	75
	T-4	Samalkot, Andhra, India	75
	T-5	Samalkot, Andhra, India	72
	TR	Trichur, Kerala, India	50
	Pt	Puttalam, West coast of Ceylon	24
	SD	Sadulgona, East coast of Ceylon	50
	LT	Laphugala Tank, East coast of Ceylon	41

Analysis of variance for length and width of spikelets is presented in Table 1, and the mean values for those characters are shown in Table 2.

The data in Table 1 show that the variations between species, between populations of the same species, and between individuals within a population are all statistically significant. In Table 2, we find that *O. rufipogon* has, in general, slightly longer and wider spikelets than *O. perennis*.

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

Table 1. Analysis of variance of spikelet length and width in wild rice populations.

Source of variation	d.f.	Mean square	
		Spikelet length	Spikelet width
Between species	1	284.836**	63.013*
Between populations within species	18	26.527**	9.236**
Between individuals within populations	932	1.880**	0.269**
Within individuals	8,568	0.216	0.012

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

Table 2. Mean value of spikelet length and width in wild rice populations.

Species	Population	Spikelet length	Spikelet width
O. perennis	CH	8.04mm ±0.267	2.39mm ±0.106
	JA	7.64 ±0.428	2.75 ±0.025
	CT-A	8.36 ±0.362	2.51 ±0.191
	CT-B	8.10 ±0.202	2.79 ±0.086
	CT-C	7.86 ±0.620	2.59 ±0.250
	BG	8.17 ±0.402	2.75 ±0.265
	PH	7.95 ±0.318	2.58 ±0.173
	SM	7.88 ±0.369	2.33 ±0.255
	Mean	8.00 ±0.248	2.58 ±0.162
O. rufipogon	CH	8.59 ±0.287	2.70 ±0.150
	CN	8.40 ±0.360	2.79 ±0.071
	JA	8.24 ±0.409	2.77 ±0.116
	F-b	7.75 ±0.251	2.63 ±0.116
	PH	8.30 ±0.298	2.82 ±0.084
	T-1	8.50 ±0.102	2.77 ±0.091
	T-4	8.60 ±0.352	2.86 ±0.069
	T-5	8.81 ±0.299	2.84 ±0.102
	TR	8.47 ±0.365	2.75 ±0.159
	Pt	8.42 ±0.330	2.63 ±0.070
	SD	8.02 ±0.296	2.71 ±0.200
	LT	8.43 ±0.450	2.46 ±0.146
	Mean	8.38 ±0.267	2.73 ±0.121

Populations of *O. rufipogon* collected in India were investigated to find whether or not the length and width of spikelets differ according to locality. No geographical cline was found, however.

Further, the magnitude of genetic variability in these wild rice species as shown by the heritability values of spikelet length and width was investigated. The values of heritability (the amount of genetic variance in percent of total variance) thus found are presented in Table 3.

Table 3. Heritability values in wild populations of two *Oryza* species.

Species	Population	Heritability value	
		Spikelet length	Spikelet width
O. perennis	CH	0.7536	0.7580
	JA	.3312	.1310
	CT-A	.5299	.1037
	CT-B	.5828	.4098
	CT-C	.6160	.8048
	BG	.5314	.9124
	PH	.1386	.3630
	SM	.5536	.9929
O. rufipogon	CH	.4419	.9375
	CN	.5813	.3530
	JA	.1824	.6149
	F-b	.4488	.6176
	PH	.2823	.2485
	T-1	.6249	.5837
	T-4	.9466	.6227
	T-5	.9780	.5688
	TR	.7981	.4945
	Pt	.2660	.4454
	SD	.6041	.5710
	LT	.7231	.9328

Fig. 1 shows the geographical distribution of heritability values for the average of the values for the two characters. It is noticeable in Table 3 that wild rice populations are generally highly heterogeneous. It seems that populations collected from Orissa, India, have lower heritability values though they may differ according to localities.

Genetic as well as environmental correlations between spikelet length and width are shown in Table 4.

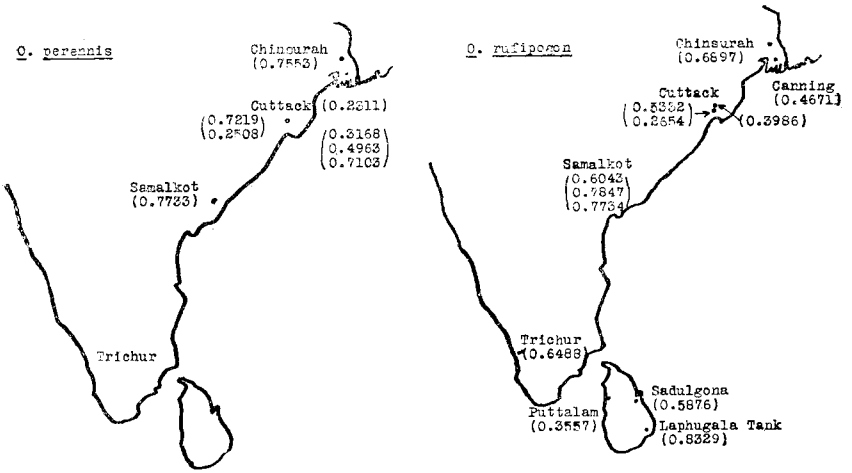


Fig. 1. Geographical distribution of the average heritability values of spikelet length and width.

Table 4. Environmental (r_e) and genetic (r_g) correlations between spikelet length and width.

Species	Population	r_e	r_g
O. perennis	CH	0.1477	0.0549
	JA	.0116	.5415
	CT–A	.0914	.4787
	CT–B	.1864	.6887
	CT–C	.0155	.8410
	BG	.3979	.3080
	PH	.0577	−.2027
	SM	.1175	.2079
O. rufipogon	CH	.2896	.1928
	CN	.8409	−.0335
	JA	.0108	.6634
	F–b	.0030	.0688
	PH	.0133	.4973
	T–1	.1274	.3378
	T–4	−.7568	.3733
	T–5	.0550	.3307
	TR	.1166	.5441
	Pt	.0260	−.2310
	SD	.4359	.5104
	LT	.1916	.3988

Table 4 shows that the environmental correlation between the two characters is generally insignificant except for a few populations, while genetic correlations vary from population to population.

39. *Variation in Anthesis of Wild Rice Populations (Oryza rufipogon and Oryza perennis)**

(By Kan-Ichi SAKAI and Takashi NARISE)

Progeny lines of plants collected from three populations of *O. rufipogon* (*O. sativa spontanea*) and two of *O. perennis* were investigated with regard to the date of heading. The places where this material was collected are as follows:

Species	Notation of population	
O. rufipogon	Ill	Illuppaiyadichchenai, East coast of Ceylon
	PT	Pottuvil Town, East coast of Ceylon
	PP	Pottuvil Periphery, East coast of Ceylon
O. perennis	Vey	Veyangoda, West inland of Ceylon
	Yag	Yagoda, West inland of Ceylon

The progeny lines were sown in pots and after four weeks transplanted to an experimental field on June 24, 1958. The mean number of days per line from sowing to flowering is presented in Table 1.

Table 1. Frequency distribution of number of days from sowing to flowering in five populations of wild rice.

Species	Population	No. of lines	No. of days from sowing to flowering								Average
			121 ~ 130	131 ~ 140	141 ~ 150	151 ~ 160	161 ~ 170	171 ~ 180	181 ~ 190	191 ~ 200	
O. rufipogen	Ill	15		2	4	8	1				150.88
〃	PT	10	2	0	7	1					149.00
〃	PP	8			4	4					141.02
O. perennis	Vey	22					3	11	7	1	177.73
〃	Yag	24					1	8	24	1	182.62

In Table 1, we find that the anthesis of *O. rufipogon* occurs markedly earlier than that of *O. perennis*. The results of analysis of variance and estimates of components of variances are presented in Table 2.

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

Table 2. Analysis of variance of days from sowing to heading in progeny lines of five wild rice populations.

Variation due to	d.f.	Mean square	Expectation of m.s.
Species	1	121,897.34**	$\sigma_w^2+k_1\sigma_s^2+k_2\sigma_p^2+k_3\sigma_A^2$
Polulations within species	3	2,323.63**	$\sigma_w^2+k_1\sigma_s^2+k_2\sigma_p^2$
Strains within population	84	244.35**	$\sigma_w^2+k_1\sigma_s^2$
Plants within strain	510	98.26	σ_w^2
σ_A^2		608.02	k_1 6.4624
σ_p^2		21.93	k_2 98.8147
σ_s^2		21.59	k_3 196.6611
σ_w^2		98.26	

** Above 1% level of significance.

In Table 2, σ_A^2 represents the component of variance between species, σ_p^2 that between populations within species, σ_s^2 that between strains within populations, and σ_w^2 that between individuals within a strain. By using the values of components, the magnitudes of variation in heading date are shown in terms of intraclass correlation, and those between populations and between species are compared with each other. They are as follows:

Variability between strains within populations:

$$\frac{\sigma_s^2}{\sigma_w^2+\sigma_s^2}=0.2198$$

Variabiiity between populations within species:

$$\frac{\sigma_p^2}{\sigma_w^2+\sigma_s^2+\sigma_p^2}=0.1547$$

Variability between species:

$$\frac{\sigma_A^2}{\sigma_w^2+\sigma_s^2+\sigma_p^2+\sigma_A^2}=0.8109$$

These values show that so far as anthesis is concerned, interspecific variability was the highest, within each species, however, neither inter-population nor especially interstrain (within the same population) variability was negligible, which indicates that wild populations are genetically heterogeneous with respect to anthesis.

40. *Genetic Studies on Seed Size in Rice Hybrids**

(By Kan-Ichi SAKAI and M. E. R. PINTO**)

The present paper deals with the inheritance of length and width of paddy-grown rice seeds from six F_2 populations. The parental varieties and the number of plants examined are as follows:

Cross No.	Female parent	Male parent	Number of plants investigated
1	Podiwi B-8	Mas M-24	136
2	Ptb-16	Mas M-24	137
3	Bengawan B-27	Mawi B-11	142
4	Murungakayan 3	Mawi B-11	129
5	Ptb-16	Sigadis	90
6	N-48	Mas M-24	141

Ten seeds from each plant were collected at random and their length and width were measured. Analysis of variance of the data obtained was made and the within-individual and between-individual variance components were estimated. The former component was taken as the estimate of environmental variance, and genetic variance was estimated from the between-individual variance component. Environmental and genetic covariance components were estimated in a similar way.

Heritability and genetic as well as environmental correlation computed for the six F_2 populations are presented in Table 1.

Table 1. Heritability (h^2) and genetic (r_G) and environmental (r_E) correlations of length and width of seeds in six F_2 populations of rice hybrids.

Cross number		1	2	3	4	5	6
h^2	Seed length	0.9092	0.6819	0.7556	0.4615	0.0693	0.6082
	Seed width	0.4711	0.3714	0.5927	0.6160	0.0845	0.4449
Length × Width	r_G	−0.0964	0.1826	0.1107	−0.1218	0.0186	0.0394
	r_E	0.1792	0.2420	0.3280	0.1593	0.4933	0.2351

* This investigation was carried out in Division of Botany, Department of Agriculture, Peradeniya, Ceylon.

** Agricultural Instructor, Department of Agriculture, Ceylon.

From Table 1, we find that: (1) heritability values for seed length as well as width are on an average as high as around 50%, those for length being generally higher than those for width. (2) Among parental varieties used in the crosses, Ptb–16, Sigadis and Mas M–24 are very similar in seed length and width. Very low heritability values obtained for both characters in Ptb–16×Sigadis (cross No. 5) and rather high ones in the cross Ptb–16×Mas M–24 (cross No. 2) suggest that Ptb–16 and Sigadis must be very similar in their genetic make-up, while Mas M–24 must be genetically different from Ptb–16, although phenotypically they appear alike. (3) The genetic correlation between seed length and seed width is found to be as low as 0 or 10–20% in the plus or minus direction, while the environmental correlation between these two characters is rather high and always in the plus direction.

A number of useful suggestions have been deduced from the result of this experiment.

41. *Inheritance of Size and Shape of Grains in Rice*

(By Shin-ya IYAMA, Hiroko MORISHIMA and Hiko-Ichi OKA)

The size of rice grains is known to be a character affected relatively slightly by environmental agents. In view of its usefulness for a survey of genetic variability in rice populations, its inheritance in two varietal crosses was investigated by statistical-genetic methods in order to obtain fundamental knowledge for population studies. The two crosses used are Kinoshita-mochi (Japonica)×U-koh (Indica, from Formosa), and Norin-mochi No. 18 (Japonica, upland)×"Red Rice" (Indica).

The parental varieties and the F_2 and F_3 lines of each cross were grown in the experimental field of this Institute, according to a randomized block

Table 1. Variances and covariances of grain length, observed and expected in Kinoshita-mochi × U-koh.

Population	Observed			Expected
	I	II	Mean	
$V_{F_2}=1/2D+1/4H+E_1$	.0856	.0871	.0864	.0882
$V_{\bar{F}_3}=1/2D+1/16H+E_2$	.0744	.0699	.0722	.0702
$W_{F_2/F_3}=1/2D+1/8H$	.0434	.0425	.0430	.0459
$\overline{V}_{F_3}=1/4D+1/8H+E_1$	.0731	.0646	.0689	.0634
E_1	.0381	.0318	.0349	.0386
E_2	.0392	.0091	.0241	.0261

design with two replications. The unhulled grain length and width of 10 seeds were measured in each plant. Variances in the F_2 and other populations, and the covariance between the F_2 and F_3 lines were calculated for length, width and length-width ratio, as shown in Table 1. The components of variances were partitioned by MATHER's method. The results are given in Table 2.

Table 2. Additive genetic variance (D), non-fixable genetic variance (H) and environmental variances (E_1, E_2) of grain characters.

Cross 1: Kinoshita-mochi × U-koh
Cross 2: Norin-mochi No. 18 × "Red Rice"

Character	Cross	D	H	E_1	E_2	Among vars.*
Length	1	.0845±.0207	.0294±.0662	.0386±.0056	.0261±.0057	.5926
	2	.1388±.0367	.1013±.1179	.0220±.0111	.0180±.0101	
Width	1	.0204±.0095	.0203±.0304	.0107±.0026	.0046±.0026	.1254
	2	.0379±.0138	.0042±.0443	.0110±.0038	.0048±.0038	
Lth/Width	1	.0197±.0018	.0129±.0056	.0035±.0005	.0030±.0005	.1895
	2	.0177±.0080	.0535±.0256	.0071±.0022	.0061±.0022	

* Variances among more than 600 varieties from various Asian countries.

As the data in Table 2 show, the observed variances gave a good fit with the expected ones in cross 1 (standard deviations are relatively small), but in cross 2, deviations between observed and expected variances were larger. The results of scaling tests proved that a convenient scale (in millimeters) could be used in both crosses. It seems that the larger deviations in cross 2 might be due to changes in the segregation ratio caused by gametic selection, because the F_1 plants were almost completely fertile in cross 1, but showed semisterility (pollen fertility being about 40%) in cross 2.

It is found in Table 2, further, that the additive genetic variances due to the crosses were one tenth to one fifth of the variance found among a large collection of varieties from various Asian countries.

In the process of computation, a slight effect of linkage among relevent genes was pointed out for the length-width ratio in cross 1, and for grain length in cross 2. The number of effective genes could not be successfully estimated.

The variances of grain characters within the same panicle were derived from the data, in addition to the above computation. Based on those values, components of environmental variances due to grains (σ^2_{gr}), plants (σ^2_{pl}) and polts (σ^2_{plot}) were computed. The results are given in Table 3, together with heritability values, the ratio of H/D and other values

Table 3. Heritability value, ratio of H/D, and components of environmental variances found from the data.

Character	Cross	h^2_{Fr}	H/D	$10E_2/E_1$	Environmental variance		
					σ^2_{gr}	σ^2_{pl}	σ^2_{plot}
Length	1	.479	.348	6.76	.0538	.0332	.0232
	2	.594	.730	8.18	.1217	.0099	.0158
Width	1	.392	.995	4.30	.0119	.0095	.0034
	2	.619	.111	4.36	.0931	.0017	.0037
Lth/Width	1	.556	.655	8.57			
	2	.302	3.023	8.59			

derived from the above computation. The data in Table 3 show that significant environmental variation in grain size due to the effects of plants and plots occurs, in addition to the variation within a plant; in other words, the environmental variances found within a panicle, between plants and between plots are not of the same order. This should be considered when we deal with genetic variations of this character.

42. *Polygenic Mutation, Induced by X-rays, in Biometrical Characters of Drosophila melanogaster**

(By Yukio YAMADA and Osamu KITAGAWA)

The dominant marked multi-chromosomal inversion technique which was proposed originally to establish iso-homozygous lines was used in this study, with the exception of the fourth chromosome.

We treated each sample of five males, taken at random from an iso-homozygous line extracted by the above technique from a long inbred line, as follows: (A) control, which was not irradiated, (B) 2000 r and (C) 4000 r of X-rays; 36, 47 and 30 homozygous lines were obtained, respectively. In each line 40 females and 40 males were scored with respect to the number of abdominal (4th and 5th sternites) and sternopleural (right and left sides) micro-hairs. This score, pooled for females and males, was used for the analysis. The results obtained so far are given in Table 1.

As the table shows, the means of three treatments agreed well with each other for both abdominals and sternopleurals. However, variances among the lines within treatments increased considerably in both B and C, as compared with the control, A. Variance in A could possibly result from spontaneous mutation and rarely occurring recombinations during isogenisation, and/or sampling by chance, but it could still be used to test the

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

Table 1. Mean and variance of bristle numbers.

Treatments	No. of sperm sampled	Abdominals		Sternopleurals	
		Mean	Variance	Mean	Variance
A. Control	36	31.2903	0.271378	15.1881	0.439861
B. 2000 r	47	31.1801	0.924382**	15.1165	0.903444*
C. 4000 r	30	31.1736	0.940674**	14.9538	0.374808

* Significantly different from control at 5% level.
** Significantly different from control at 1% level.

variances of B and C. The increment of variance in those two treatments, therefore, should be ascribed to polygenic mutation induced by X-rays. An increment in variance per unit dose was calculated by linear regression; it amounts to 0.000167 for abdominals, but it is negative for sternopleurals, although the variance in B is significantly larger than in A. A possible explanation for the relatively lower variability between lines in C could be sought in their lowered viability caused by the high dose of irradiation. If mutations in the character analysed are impairing fitness, the line will be eliminated during isogenisation. Evidences of lower hatchability and viability in the lines treated with a high dose support this possibility.

Taking the increment in variance due to spontaneous mutation to be 0.00475, which is only an average of the values reported by CLAYTON and ROBERTSON (1955) and PAXMAN (1956), the tentatively calculated doubling dose in abdominal bristles was 56.89, or approximately 60 r.

43. *The Effects of X-ray Irradiation on Selection Response**

(By Osamu KITAGAWA)

It has been shown in *Drosophila melanogaster* that the response to selection for a varying number of bristles was increased after X-ray irradiation (SCOSSIROLI, 1953; CLAYTON and ROBERTSON, 1955).

The purpose of this experiment was to determine the effect of X-rays on the acceleration of artificial selection. Selection was conducted for high and low numbers of chaetae on the fourth and fifth abdominal plates of flies in an isogenic strain, originating from the Oregon-R strain and designated the P line, and also in a hybrid between two isogenic strains, namely Oregon-R and Samarkand, which was designated the C line. Selected parents were treated at each generation with X-rays of 1500 r just before mating. The high and low selected lines were classified in four

* This work was supported partly by Grant RF 57178 from the Rockefeller Foundation.

lots according to whether the treatment included: (1) both sexes, (2) only females, (3) only males, or (4) neither sex. The selection intensity was 20% (6 out of 30 in each sex), except that no selection could be found in some later generations because of high sterility due to the irradiation.

After the twentieth generation, lots (2) and (4) of the P line showed little or no response whereas some response (a few units in chaetae counts) was obtained in lots (1) and (3). In the C line, a response was observed in all lots, an especially large response being obtained in lot (1). Lot (2) showed a response nearly equal to lot (3). These results seem to indicate that the remarkable effects of X-rays on the induction of new mutations in polygenic systems and on the increase of recombinations especially in females might induce the new variations. The response in lot (2) of the C line was strengthened by both, while in lot (3) the response was induced mainly by the former effect. Furthermore, the mutation rate in the polygenic system investigated under the influence of X-rays was thought to be higher in the male than in the female.

44. *Breeding Structure of Poultry Flock which Maximizes the Genetic Progress by Selection*

(By Yukio YAMADA)

A typical poultry flock consists of offspring from a number of sires, each mated to several dams. It is assumed that selection is from a population of sdn female offspring originated from s sires each mated to d dams, each mating producing n daughters.

Very recently OSBORNE (1957) derived selection indices for a sex-limited trait, by which optimum gains may be obtained when individual, dam family, and sire family averages are available for females but only dam and sire family averages for males. Selection based on these indices is theoretically possible to achieve the largest progress compared with any other type of selection, e.g. the combination selection of individual and full-sib averages proposed by LERNER (1950).

Genetic gains per generation are generally expressed as

$$\Delta\hat{G}=\frac{z}{p}h\sigma_G \quad \text{or} \quad \frac{z}{p}r_{GX}\sigma_G \qquad (1)$$

where z/p is the intensity of selection and h or r_{GX} is the correlation of genotypes and phenotypes. In the case of index selection r_{GX} is thus the correlation between the genotype and the selection index used. As was already reported by the author (1958), the genetic gain can be partitioned into several parts according to the selection actually applied. If different selection indices were applied for males and females, the expected gain would be

$$\Delta\hat{G}=\frac{\Delta S+\Delta D}{2}=\frac{1}{2}\left\{\frac{z_1}{p_1}R_{GI_1}+\frac{z_2}{p_2}R_{GI_2}\right\}\sigma_G \qquad (2)$$

where R_{GI_1} and R_{GI_2} are multiple correlations of genotypes and indices which were given by OSBORNE. Subscripts 1 and 2 refer to males and females, respectively.

Assume that the population size of females is $N=sdn$ and the selection intensities for males and females are fixed in every generation, and that at least one male is retained for each dam family until they reach the breeding age. Only one male for each selected family can, however, be used as the breeder for the next generation so as to prevent inbreeding progress, even though several males are available for selection in each selected family. Thus the selection intensities are in turn

$$p_1=\frac{s}{sd}=\frac{1}{d} \quad \text{for males and} \quad p_2=\frac{sd}{N}=\frac{1}{n} \quad \text{for females}$$

and hence,

$$d=\frac{1}{p_1}, \quad n=\frac{1}{p_2} \quad \text{and} \quad s=Np_1p_2 \ (\because N=sdn).$$

After substituting these into (2), we obtain another expression of $\Delta\hat{G}$ in terms of p_1, p_2, z_1, z_2, h^2, N, and σ_G. In order to get the maximum $\Delta\hat{G}$ we differentiate it with p_1 and p_2, and obtain a set of simultaneous equations. p_1 and p_2 which satisfy the eqations, would be the optimum selection intensities for males and females of the breeding flock for a given h^2 and N. Consequently optimum numbers of families and daughters will be determined. Details will be given elsewhere with numerical illustrations.

45. *Performance Test and Selection Efficiency Involving Different Environments*

(By Yukio YAMADA and Toshitaka ITO)

The present report deals with the performance tests of birds of similar genotypes under different environments. Tests of this nature are important from the point of view of estimating the magnitude of testing station effects (confounding with management) and also for the purpose of selecting birds when the family members are tested at several different places. It is fairly common in this country to test the performance of birds for breeding purposes under such conditions, mainly due to the small facilities available to single breeders. In such cases, several breeders cooperate with each other in testing and selecting birds on the basis of average family records, little attention being paid to their individual performance. However, if there were significant interactions between genotypes and

environments, the method used would not be appropriate for the purpose of selecting birds of superior genotypes under a specific environment.

The data were collected in 1957 from three breeding groups of Barred Plymouth Rocks totalling 1580 birds. Chicks were hatched at the main station, distributed to a number of branch stations within a small district and reared under different management and housing. Hen housed egg production of 500 days of age and the time of sexual maturity were analysed. Because of non-orthogonal data the usual method of analysis of variance is not adequate to test the variances corresponding to specific effects. Therefore, "Method 1" described by HENDERSON (1953) was employed.

The model used here was.

$$Y_{ijkl}=\mu+f_i+s_{ij}+l_{ik}+(sl)_{ijk}+e_{ijk}+e_{ijkl}$$

where f, s, l, sl and e stand for breeding groups, sires, locations, interactions between sires and locations and errors, respectively. All factors other than the mean were assumed to be random variables. The sum of squares and coefficient of each variance component in these expectations are presented in Table 2.

Table 1. Statistics of the population analysed.

Groups	Sires	Offspring	No. of testing locations involved	Sexual maturity	Egg production	Egg weight
A	14	771	21	199.68	196.55	57.80
B	9	276	11	195.05	181.71	54.10
C	9	533	13	200.65	198.27	57.46
Total	32	1,580	45	199.23	194.31	56.96

Table 2. Sums of squares and coefficients of variance components.

Source of variation	μ^2	σ_f^2	σ_s^2	σ_l^2	σ_{sl}^2	σ_e^2	Sexual maturity	Egg Production
T-CT		975.76	1521.57	1471.85	1566.72	1579	1568,735.00	5862,689.73
F-CT		975.76	101.53	211.56	27.39	2	6,045.44	55,925.44
S-F			1420.03	47.64	326.68	29	94,577.75	120,387.67
L-F			85.17	1260.29	204.47	42	275,611.50	303,500.18
SL-S-L+F			−85.17	−47.64	1018.18	258	158,422.23	886,994.93
T-SL						1248	1034,078.68	4495,881.51

The solution to these equations is $\sigma_s^2=52.66$, $\sigma_l^2=194.21$, $\sigma_{sl}^2=-41.28$ and $\sigma_e^2=828.59$ for sexual maturity and $\sigma_s^2=15.03$, $\sigma_l^2=125.41$, $\sigma_{sl}^2=-34.90$

and $\sigma_e^2=3602.47$ for egg production. These resulted in $h^2=0.204$ for sexual maturity and $h^2=0.016$ for egg production. So far as relative magnitude of variance components is concerned, the variances of interaction were both negative and thus the interaction would be unimportant, at least under these conditions. Variances of station differences in both traits were fairly large but did not greatly affect the efficiency of selection. The error variance of egg production was exceedingly large, this would be ascribable to a variety of micro-environments within locations.

It is concluded that the method of testing birds under such conditions is still valid but sources of environmental variation such as housing and management should be unified or standardized as well as possible, to lessen the environmental variation, and accordingly increase the efficiency of selection.

46. *Evidence of Significant Interaction between Sire Family and Date of Hatching in Egg Production of Chickens*

(By Yukio YAMADA)

The data utilized in the present report were obtained from the records of 15 sire groups of Barred Plymouth Rocks. Comparison of the influence of different hatching dates was made on the basis of three wide periods only; these extended from the 13th of February to the 10th of March, from the 11th of March to the 10th of April, and from the 11th of April to the 29th of May. Parents used in the analysis were represented by their offspring in each period.

A preliminary analysis has shown that the variance of sub-classes was highly significant. Further analyses of variance, using the method of fitting constants to correct for disproportionality in subclasses, and the method of weighted squares of means, were carried out. The results, presented in Table 1, clearly indicate the significance of interaction between sire family and the date of hatching for hen-housed egg production until

Table 1. Analysis of variance for interaction and main effects by the method of weighted squares of means.

	d.f.	M.S.
Hatches	2	25,606.21**
Sires	14	911.63
Interaction	28	4,596.18**
Error	925	2,670.69

** Significant at 1% level.

500 days of age. The same analysis was carried out in the case of sexual maturity; however no significant interaction in this trait could be detected.

If the situation encountered here is a common one for prolonged hatching periods, the correction of egg production for the date of hatching, the estimation of heritability and improvement by selection, and the method of multiple shift in poultry are not appropriate. Research on this kind of interaction is now under way and a discussion of its significance in poultry breeding will be given after the completion of the experiment.

47. *A Comparative Analysis of Body Weight in Two Purebreeds and Their Reciprocal Crossbreeds in the Domestic Fowl*

(By Takatada KAWAHARA and Shun ICHIKAWA)

During the three years from 1956 to 1958, intra- and inter-breed matings involving White Leghorns (WL) and Barred Plymouth Rocks (BPR) were practiced. Average coefficients of inbreeding were 3.4% and 9.2% respectively in WL and BPR. Data were collected from 762 purebreed and 586 crossbreed females originating from 16 sires and 141 dams in WL and 16 sires and 119 dams in BPR. Mating systems were such that these purebreeds and crossbreeds were half-sibs. Measurements of body weight were taken at 0, 4, 8, 12 and 18 weeks of age. The mean body weights and their standard deviations for all surviving birds are presented in Table 1.

Table 1. Mean body weights and their standard deviations.

Breed or cross	No. started	Body weight (g)				
		0 week	4 weeks	8 weeks	12 weeks	18 weeks
WL	435	35.9 3.98	216.6 39.79	560.1 95.39	861.9 146.00	1174.7 151.3
BPR	309	34.6 3.41	219.3 36.29	625.7 112.30	1013.6 242.90	1466.0 222.00
WL ♀ × BPR ♂	331	35.4 4.24	231.9 32.31	616.4 77.15	959.1 145.80	1302.1 164.20
BPR ♀ × WL ♂	255	33.2 3.20	230.0 31.92	620.0 81.94	995.0 143.00	1393.5 120.50
Total	1348					

The significance of differences between mean body weights is given in Table 2. A heterotic effect on body weight appeared definitely at the age of 4 weeks; afterwards the body weight of crossbreeds approached that of

Table 2. Significance test of differences between means.

Comparison	*t* values				
	0 week	4 weeks	8 weeks	12 weeks	18 weeks
WL vs. BPR	4.73**	0.98	8.40**	10.10**	19.52**
WL♀×BPR♂ vs. BPR♀×WL♂	7.04**	0.70	0.55	2.90**	7.14**
WL vs. WL♀×BPR♂	1.69	5.69**	2.76**	11.00**	10.59**
WL vs. BPR♀×WL♂	9.42**	4.54**	8.27**	11.19**	18.57**
BPR vs. WL♀×BPR♂	2.66**	4.58**	3.73**	3.37**	10.11**
BPR vs. BPR♀×WL♂	5.11**	3.62**	0.66	1.04	4.40**

** Significant at the 1% level.

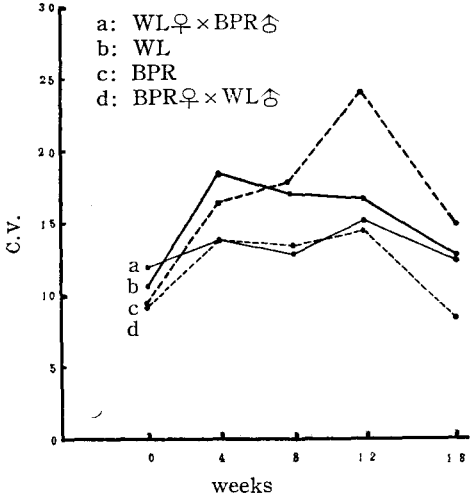


Fig. 1. Coefficients of variation of body weight in pure breeds and their reciprocal hybrids at various stages.

the mid-parents. The most interesting result is the finding of differences in body weight between reciprocal F_1 hybrids at the age of 12 and 18 weeks. The "t" test shows that the BPR♀×WL♂ cross gives significantly heavier offspring than the reciprocal cross.

Trends in variability of body weight in terms of coefficients of variation (C.V.) are illustrated in Fig. 1. During growth, the variability of purebreeds is remarkably higher than in crossbreeds, especially in the period from 4 to 12 weeks, which suggests that a more effective homeostasis is operating on body growth in the F_1 hybrids, at least from the 4th to 12th week of their development.

48. *Influence of Heterosis on Viability of F_1 Hybrids between Two Breeds of the Domestic Fowl*

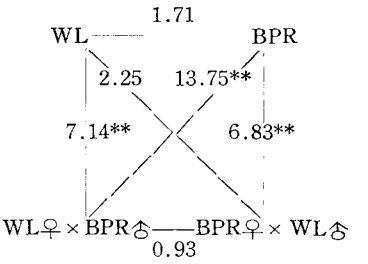
(By Takatada KAWAHARA)

A mortality analysis with data given in a previous report, excluding the T2 group (KAWAHARA 1957) and accidentally dead birds, was undertaken.

The birds were maintained without any conscious culling up to eighteen weeks of age. A summary of data with a significance test based on the chi-square method is given in Table 1. The mortality of hybrid chicks was 5.7% less than that of purebreeds, and this difference was significant statistically at the 1% level. The mortality of BPR was 3.2% greater than that of WL (11.5% vs. 8.3%), and the mortality of F_1 hybrids of a BPR♀×WL♂ cross was 1.7% greater than that of the reciprocal (4.9% vs. 3.2%). However, these differences in mortality were not significant statistically.

Table 1. Mortality of various mating types up to eighteen weeks of age.

Breed or cross	No. tested	Mortality		χ^2
		No.	%	
WL	362	30	8.3	
BPR	253	29	11.5	
WL♀×BPR♂	281	9	3.2	
BPR♀×WL♂	227	11	4.9	
Pure (WL / BPR)	615	59	9.6	20.90**
Hybrid (WL♀×BPR♂ / BPR♀×WL♂)	508	20	3.9	



** Significant at the 1% level.

E. MATHEMATICAL GENETICS

49. *A Maximum Principle in the Genetical Theory of Natural Selection*

(By Motoo KIMURA)

FISHER's "Fundamental theorem of natural selection" states that the rate of change of population fitness, measured in Malthusian parameters, is equal to the additive genetic variance in fitness. However it does not

specify how gene frequencies change in the process of natural selection acting on genotypes.

The present report is an attempt to formulate a law of change in gene frequencies in the course of natural selection. Let A_1, A_2, A_3, $\cdots$, A_n be a series of n alleles with respective frequencies of x_1, x_2, $\cdots$, x_n in a population. Under the assumption of random mating and constant fitness of individual genotypes, it is possible to prove the following theorem: For a given short time interval δt, natural selection causes gene frequency changes δx_1, δx_2, $\cdots$, δx_n, in such a way that the increase of population fitness shall be at maximum under the restriction

$$\sum_{i=1}^{n} \frac{(\delta x_i)^2}{x_i} = \frac{1}{2} V_g (\delta t)^2 ,$$

where V_g is the additive genetic variance in fitness.

The theorem can easily be extended to any number of loci. Furthermore, it may be extended to cover quite a general situation from which the above restrictions of random mating and constant fitness of genotypes are removed.

The most remarkable feature of this theorem is that the set of differential equations describing the law of change in gene frequencies by natural selection can be derived from it. It may be called "a maximum principle in the genetical theory of natural selection", analogous to the principle of least action in physics.

(cf. Kimura, M. 1958. Heredity 12: 145–167)

50. *A Gene Frequency Cline Determined by Selection and Migration*

(By Motoo KIMURA)

Consider a population which is forming effectively a continuum on a linear habitat, where dominant individuals (*AA* and *Aa*) are advantageous on one side and recessive individuals (*aa*) are advantageous on the other side. If the range of migration of each individual per generation is very small as compared with the total range of distribution, a gene frequency cline may be formed. For mathematical treatment, it may be convenient to express a position on the habitat as a point on an one-dimensional coordinate and take the neutral position as origin. We assume that the dominants are advantageous on the negative or left side of the coordinate axis while the recessives are advantageous on the positive or right side (Fig. 1).

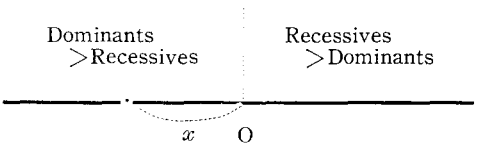


Fig. 1.

Let $u(x)$ be the frequency of the dominant gene A at the point x and assume that the selective advantage (or disadvantage) of the dominants over the recessives at any point is proportional to the distance of that

Table 1. Numerical solution of $u''=xu(1-u)^2$ with boundary conditions: $x=-\infty$ $u=1$, $x=+\infty$ $u=0$.

x	u	x	u	x	u	x	u
0	0.40115	1.0	0.17294	2.0	0.04839	3.0	0.01009
0.1	0.37597	1.1	0.15515	2.1	0.04172	3.1	0.00868
0.2	0.35103	1.2	0.13858	2.2	0.03585	3.2	0.00754
0.3	0.32620	1.3	0.12324	2.3	0.03072	3.3	0.00664
0.4	0.30191	1.4	0.10913	2.4	0.02627	3.4	0.00595
0.5	0.27821	1.5	0.09624	2.5	0.02241	3.5	0.00546
0.6	0.25525	1.6	0.08452	2.6	0.01909	3.6	0.00517
0.7	0.23314	1.7	0.07394	2.7	0.01625		
0.8	0.21200	1.8	0.06443	2.8	0.01384		
0.9	0.19190	1.9	0.05593	2.9	0.01180		

x	u	x	u	x	u	x	u
0	0.40115	−2.9	0.87133	−5.8	0.96518	−8.7	0.98580
−0.1	0.42633	−3.0	0.87790	−5.9	0.96648	−8.8	0.98611
−0.2	0.45129	−3.1	0.88407	−6.0	0.96671	−8.9	0.98640
−0.3	0.47614	−3.2	0.88987	−6.1	0.96889	−9.0	0.98668
−0.4	0.50052	−3.3	0.89533	−6.2	0.97001	−9.1	0.98694
−0.5	0.52441	−3.4	0.90047	−6.3	0.97107	−9.2	0.98720
−0.6	0.54769	−3.5	0.90530	−6.4	0.97208	−9.3	0.98745
−0.7	0.57033	−3.6	0.90984	−6.5	0.97305	−9.4	0.98770
−0.8	0.59223	−3.7	0.91412	−6.6	0.97397	−9.5	0.98794
−0.9	0.61334	−3.8	0.91815	−6.7	0.97484	−9.6	0.98818
−1.0	0.63362	−3.9	0.92196	−6·8	0.97568	−9.7	0.98840
−1.1	0.65306	−4.0	0.92556	−6.9	0.97647	−9.8	0.98861
−1.2	0.67163	−4.1	0.92894	−7.0	0.97723	−9.9	0.98881
−1.3	0.68934	−4.2	0.93214	−7.1	0.97795	−10.0	0.98900
−1.4	0.70618	−4.3	0.93517	−7.2	0.97864	−10.1	0.98918
−1.5	0.72217	−4.4	0.93803	−7.3	0.97930	−10.2	0.98954
−1.6	0.73732	−4.5	0.94073	−7.4	0.97992	−10.3	0.98950
−1.7	0.75166	−4.6	0.94329	−7.5	0.98052	−10.4	0.98964
−1.8	0.76521	−4.7	0.94570	−7.6	0.98109	−10.5	0.98977
−1.9	0.77800	−4.8	0.94798	−7.7	0.98163	−10.6	0.98989
−2.0	0.79006	−4.9	0.95014	−7.8	0.98214	−10.7	0.99000
−2.1	0.80143	−5.0	0.95219	−7.9	0.98263	−10.8	0.99009
−2.2	0.81213	−5.1	0.95412	−8.0	0.98310	−10.9	0.99018
−2.3	0.82220	−5.2	0.95595	−8.1	0.98355	−11.0	0.99026
−2.4	0.83169	−5.3	0.95769	−8.2	0.98397	−11.1	0.99033
−2.5	0.84062	−5.4	0.95934	−0.3	0.98438	−11.2	0.99038
−2.6	0.84902	−5.5	0.96090	−0.4	0.98476	−11.3	0.99044
−2.7	0.85692	−5.6	0.96239	−0.5	0.98512	−11.4	0.99050
−2.8	0.86435	−5.7	0.96381	−0.6	0.98547		

point from the origin. Then it is possible to show that, by choosing a proper unit of length to measure x, $u(x)$ at equilibrium satisfies the following second order differential equation

$$\frac{d^2u}{dx^2}=xu(1-u)^2$$

with boundary conditions

$$x=-\infty \qquad u=1$$
$$x=+\infty \qquad u=0\,.$$

Because of the non-linearity of the above equation, it would seem to be very difficult to obtain an analytical solution. So numerical analysis was employed to get values of $u(x)$ correct to five decimal places, from $x=-11.4$, by intervals of 0.1, to $x=3.6$. The values are tabulated in Table 1 and are also plotted on a solid line in Fig. 2. I owe the numerical analysis to Dr. Shôzô

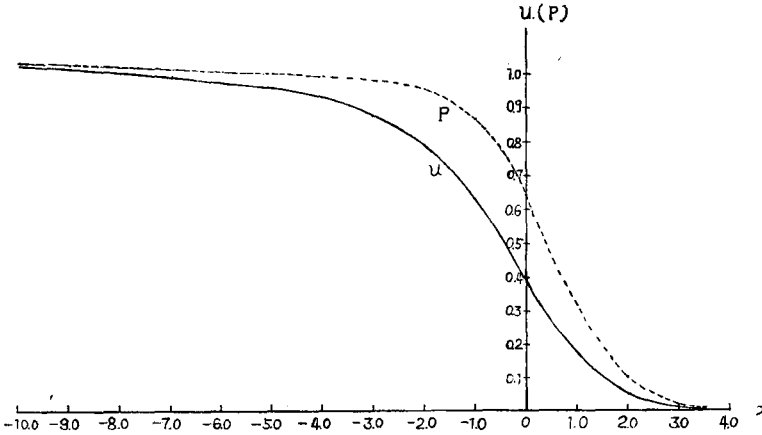


Fig. 2. A graph showing the gene frequency cline (solid line) and the phenotype frequency cline (dotted line) determined by selection and migration when a pair of alleles with complete dominance are involved.

SHIMADA operating the HIPAC 1 (Hitachi Parametron Automatic Computer 1) at the Hitachi Central Research Laboratory, to whom I wish to express my deep appreciation for his help. The value of $u(x)$ and its slope $u'(x)$ at the origin are as follows:

$$u(0)=0.401152$$
$$u'(0)=-0.252046\,.$$

The frequency of the dominant individuals may be calculated by

$$P=2u-u^2\,,$$

values of which are plotted on a dotted line in Fig. 2.

51. *Conflict between Self-fertilization and Outbreeding in Plants*

(By Motoo KIMURA)

It has long been known, and was even noted by DARWIN, that seedlings produced by crossing two different individuals of the same species are usually more vigorous than the ones produced by self-fertilization.

On the other hand, there are certain circumstances in which self-fertilization is favored by natural selection. For example, where the density of individuals is very low, self-fertilizing devices such as cleistogamy are certainly of advantage as they ensure seed-production. Also, the formation of amphidiploids more vigorous than their parental species is known to be one of the important means of species formation with which plants are specially endowed and since the capacity of self-fertilization is essential for the establishment of new amphidiploid species, such ability will be promoted or at least preserved in the course of evolution.

A more interesting factor promoting self-fertilization may be the occurrence of genes which enable a plant to be fertilized by its own pollen without losing its power of fertilizing other plants. Let A_1 and A_2 be a pair of alleles with respective frequencies of x and $1-x$ in the population, and let P_{11}, $2P_{12}$ and P_{22} be the respective frequencies of A_1A_1, A_1A_2 and A_2A_2. We assume that in A_1A_1 individuals, ovules are fertilized by pollen grains randomly taken from the entire population, while in A_2A_2 individuals they are self-fertilized with probability S and out-crossed with probability 1—S. In the heterozygous individuals (A_1A_2), the probability of self-fertilization is assumed to be Sh where h represents the degree of dominance of A_2 gene. If D is the amount of inbreeding depression of the seeds produced by self-fertilization such that $1-D$ is the relative viability of the selfed seeds as compared with the seeds produced by the out-cross, then it can be shown that the rate of change of the frequency of A_1 per generation may be given by

$$\Delta x=\frac{S\left(D-\frac{1}{2}\right)\left\{2hP_{12}\left(x-\frac{1}{2}\right)+P_{22}x\right\}}{\bar{w}},$$

where

$$\bar{w}=1-SD(2hP_{12}+P_{22})$$

is the average selective value of the population. If h lies between 0 and 1, the term in the braces of the above equation is non-negative and since S and $\bar{w}$ are both positive, the right side of the equation is positive or negative according as

$$D-1/2$$

is positive or negative. Thus, if

$$D<1/2\ ,$$
$$\Delta x>0$$

unless $x(1-x)=0$ and an intermediate gene frequency will lead to $x=1$. Namely the self-fertilizing gene A_2 will eventually be lost from the population. On the other hand, if

$$0\leqq D<1/2\ ,$$

Δx is negative and the gene A_2 will be fixed in the population. If $D=1/2$, however, an intermediate gene frequency will be held at neutral equilibrium.

It is interesting that these conclusions depend only on the value of D and not at all on S and h. Since on the average half of the heterozygous loci become homozygous after one generation of selfing, the condition

$$D=1/2$$

will be realized if the population contains recessive deleterious genes amounting to one lethal equivalent per gamete. Thus we may say that an outbreeding plant species is immune to the attack of self-fertilizing genes if it contains recessive deleterious genes in the amount of more than one lethal equivalent per gamete or two per zygote.

52. *Inverse Approach to the Estimation of Genetic Load Discolosed by Inbreeding*

(By Motoo KIMURA)

The method which I propose in this report for the estimation of the genetic load through a consanguinity study may be called the inverse or retrospective approach, in the sense that the distribution of various types of inbreeding is investigated retrospectively after a sample of affected individuals has been collected. On the other hand, in the usual approach, which may be called the direct or forward approach, incidences of abnormalities or mortality are investigated among individuals produced from different degrees of consanguinity. It is this forward approach which was used recently by MORTON, CROW and MULLER to assess the genetic load in terms of lethal equivalents. They estimated parameters involved in the regression of mortality on the coefficient of inbreeding. Their contribution is outstanding, especially in bringing to light the genetic implication of such parameters.

The inverse or retrospective approach is based on the rather obvious consideration that consanguinity would be higher among affected than un-

affected individuals, and by studying the amount of difference one could asses the hidden genetic load involved.

In what follows, I will explain, using neonatal death as an example, how the parameters relating to the genetic load may be estimated by this approach, using the method of maximum likelihood.

We assume that, out of the total (T) births registered, M cases of natural neonatal death are reported, and M' of them are available for the study of consanguinity, among which m_i' cases are found to be the results of marriages between individuals of relationship f_i ($i=0, 1, 2, \cdots$).

$$M'=m_0'+m_1'+m_2'+\cdots .$$

Suppose also that we extract a random sample of size N from T births to investigate the distribution of the inbreeding coefficients and find that n_i ($i=0, 1, 2, \cdots$) of them have the inbreeding coefficients f_i, where

$$f_0=0 , \quad f_1=1/16 , \quad f_2=1/32 , \quad \text{etc.}$$

Let P_i be the rate of neonatal deaths among babies with the inbreeding coefficient f_i such that

$$P_i=A+Bf_i ;$$

then the joint likelihood of getting the above samples is

$$L=\text{Const.}\times\{\prod_{i=0}(C_iP_i)^{m_i'}\}\overline{P}^{M-M'}(1-\overline{P})^{T-M}\{\prod_{i=0}C_i^{n_i}\} ,$$

where $\overline{P}$ is the rate of neonatal death in the general population with the inbreeding coefficient $\bar{f}$, i.e.

$$\overline{P}=A+B\bar{f} ,$$

and C_i is the proportion of marriages with the relationship f_i, such that

$$\bar{f}=\sum_{i=0}C_if_i .$$

After eliminating the unnecessary parameters of C_i's, we obtain the following set of equations to give the maximum likelihood estimates of A and B.

$$\begin{cases} \displaystyle\sum_{i=0}\frac{m_i'}{A+Bf_i}=\frac{TM'}{M} \\ \displaystyle A+B\sum_{i=0}\frac{(m_i'+n_i)f_i}{N+T(M'/M)(A+Bf_i)}=\frac{M}{T} , \end{cases}$$

The above equations may be solved by successive approximation. It may be convenient to use

$$A=\frac{MNm_0}{M'Tn_0} \quad \text{and} \quad B=\frac{16NM(M'n_0-Nm_0')}{Tn_0M'(N-n_0)}$$

as the starting values, which may be obtained by neglecting consanguineous marriages other than first cousin marriages.

The error variances σ_A^2 and σ_B^2 and the error covariance σ_{AB} of the above estimates may be obtained by the following equation:

$$\begin{bmatrix} F_A^2 & 2F_AF_B & F_B^2 \\ F_AG_A & F_AG_B+F_BG_A & F_BG_B \\ G_A^2 & 2G_AG_B & G_B^2 \end{bmatrix} \begin{bmatrix} \sigma_A^2 \\ \sigma_{AB} \\ \sigma_B^2 \end{bmatrix} = \begin{bmatrix} K_1 \\ K_2 \\ K_3 \end{bmatrix},$$

where

$$F_A = -\sum_{i=0} \frac{u_i'}{P_i^2}, \qquad F_B = -\sum_{i=0} \frac{u_i' f_i}{P_i^2},$$

$$G_A = 1 - BpM' \sum_{k=0} \frac{(m_k' + n_k) f_k}{(Np + M' P_k)^2},$$

$$G_B = \frac{P-A}{B} - BpM' \sum_{k=0} \frac{(m_k' + n_k) f_k^2}{(Np + M' P_k)^2},$$

$$K_1 = \frac{1-P}{P^2 M} - \frac{1}{M' p^2} + \frac{1}{M'} \sum_{k=0} \frac{u_k'}{P_k^2},$$

$$K_2 = \frac{G_p(1-p)}{M} + \frac{B}{M'} \sum_{k=0} \left(\frac{p}{P_k} - 1 \right) \frac{m_k' f_k}{Np + M' P_k},$$

$$K_3 = G_p^2 \frac{p(1-p)}{T} + B^2 p^2 \sum_{k=0} \frac{(m_k' + n_k) f_k^2}{(Np + M' P_k)^2} - \frac{B^2 p^2}{M'} \left(\sum_{k=0} \frac{m_k' f_k}{Np + M' P_k} \right)^2 - \frac{B^2 p^2}{N} \left(\sum_{k=0} \frac{n_k f_k}{Np + M' P_k} \right)^2,$$

in which

$$u_i' = \frac{m_i'}{M'}, \quad p = \frac{M}{T} \quad \text{and} \quad G_p = -\frac{A}{p} - BpN \sum_{k=0} \frac{(m_k' + n_k) f_k}{(Np + M' P_k)^2}.$$

F. BIOCHEMISTRY OF PIGMENTS AND OTHER SUBSTANCES IN PLANTS

53. *Biochemical Genetics of Flower Color in Swiss Giant Pansies, Viola × Wittrockiana Gams*

(By Toru ENDO)

1. Chemistry of anthocyanins

In the common garden varieties of Swiss Giant Pansies, flower colors ranging from red to blue are caused by at least six anthocyanins which

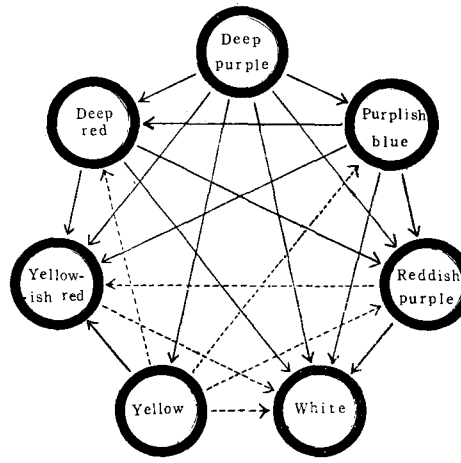
are arbitrarily designated as aC_1, aD_2, C_3, D_4, C_5 and D_6. They were separated from each other by mass paperchromatography in a comparatively pure state. Their aglycone, sugar and organic acid components, and sugar residues were studied chromatographically after complete as well as partial hydrolysis, and also after saponification with alkali. The results are summarized as follows:

aC_1: cyanidin-?-*p*-coumarylglycoside,
aD_2: delphinidin-3:5-*p*-coumarylglucorhamnoside,
C_3: cyanidin-3-glucorhamnoside (keracyanin),
D_4: delphinidin-3-glucorhamnoside (tulipanin),
C_5: cyanidin-3:5-glucoglucorhamnoside,
D_6: delphindin-?-glucorhamnoside.

Among these anthocyanins, the major pigment in reddish varieties is C_3 and the pigment in bluish varieties is aD_2 which is not violanin (dephinidin-3-*p*-coumarylglucorhamnoside) but a new anthocyanin, as mentioned above. The others are minor pigments. Anthocyanins in blotched parts of the flower petals are chiefly aD_2 together with a small amount of aC_1 (Bot. Mag. **72**: 10–19).

2. Dominance relations in F_1 hybrids, with special reference to flower color and anthocyanin pigment constituents

Seven varieties with different flower colors were crossed and approximate dominance relations were observed as follows:



Anthocyanins in the varieties and their F_1 hybrids were paperchromatographically analysed. The results show that (1) the pigments in cyanic varieties reappear as a group in F_1 hybrids between cyanic and acyanic varieties: (2) the genetic background responsible for the production of

a major anthocyanin, aD_2, is dominant over that of another major anthocyanin, C_3: (3) some anthocyanins are quantitatively increased, decreased or inhibited in F_1 hybrids among the cyanic varieties: (4) on the chromatograms, every one of the anthocyanins found in the F_1 hybrids corresponds to one present in the parental varieties. (Jap. J. Genet. **34**: 116–124).

54. *Qualitative and Quantitative Analyses of Bitter Substances in the Ripening fruit of Citrullus colocynthis*

(By Yoshito OGAWA and Kazuo FURUSATO)

The results of qualitative and quantitative analyses of bitter substances found in the ripening fruits of *Citrullus colocynthis* are reported.

The flesh of the fruits was mashed and treated with methanol 5, 10, 15, 20 and 30 days after flowering. After the evaporation of the solvent, remaining dark green oil was treated with chloroform by which the bitter substances were completely absorbed. Then they were subjected to paper-chromatography by the ascending method with water-saturated ethyl-acetate at 20°C. All of the materials were fractionated into 41 parts. All factions were dissolved in water and their taste threshold was examined by the same method as used in the study on *Swertia chirata* by KORTE (1955).

In ripe fruits, only one bitter substance was detected. This substance was identical with "Citbittol", formerly isolated by the writer (1957). In

Table 1. Variation in the amount of bitter substances in the ripening fruits of *Citrullus colocynthis*, Schrad.

(Bitter value)

Days after flowering	Citbittol								
	A 0.90*	B 0.70*	C 0.61*	D 0.54*	E 0.44*	F 0.38*	G 0.30*	H 0.15*	Total
5	191.6 (17.2**)	2.4	3.0	2.4	0.0	0.6	0.2	0.0	200.2
10	59.0 (9.4**)	2.4	3.6	0.4	0.6	0.2	0.2	0.2	66.6
15	27.8 (10.3**)	0.2	1.2	0.4	0.0	0.0	0.0	0.0	29.6
20	14.6 (10.9**)	0.0	0.4	0.0	0.0	0.0	0.0	0.0	15.0
30***	1.0 (1.0**)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0

* R_f of paper-chromatography with water-saturated ethyl-acetate by ascendence method at 20°C.

** Amount of Citbittol A per fruit.

*** Ripened.

unripe fruits, seven new bitter substances were found in addition. The former name "Citbittol" was then changed to "Citbittol A", and the newly found bitter substances were called "Citbittol B, C, D, E, F, G and H". Citbittol A, however, formed over 90% of the total of bitter components.

55. *Bitter Substances in the fruits of F_1 Hybrids between Watermelon and Colocynthe*

(By Yoshito OGAWA and Minoru SHIMOTUMA**)

Research on the bitter substances contained in the fruits of the F_1 hybrids between *Citrullus vulgaris* Schrad ($2x$, $4x$) and *Citrullus colocynthis* Schrad ($2x$) was carried out by the same method as was used in the experiment with *Citrullus colocynthis*.***

The methanol extract of the fruits of the hybrids was analysed by paper-chromatography with water saturated ethyl-acetate and the bitter-value was calculated.

Table 1. Bitter substances in the ripe fruits of F_1 hybrids between watermelon and colocynthe. (Bitter value)

Cross combination	Citbittol					
	A 0.90*	B 0.70*	C 0.61*	D 0.54*	E 0.44*	Total
C. vul. (Otome, $2x$) ×*C. colo.* (No. 1, $2x$)	74.0	1.4	1.8	0.4	0.2	77.8
C. vul. (Fumin, $2x$) ×*C. colo.* (No. 1, $2x$)	33.0	0.0	0.0	0.0	0.0	33.0
C. vul. (Yamato-cream, No. 2, $2x$) ×*C. colo.* (No. 1, $2x$)	106.8	3.8	0.6	0.0	0.0	111.2
C. vul. (Ashi-yamato, $4x$) ×*C. colo.* (No. 1, $2x$)	85.0	1.8	0.6	0.6	0.2	88.2
C. vul. (Yamato, $4x$) ×*C. colo.* (No. 1, $2x$)	42.0	1.8	0.0	0.0	0.0	43.8
C. vul. (Fuken, $4x$) ×*C. colo.* (No. 1, $2x$)	56.4	0.6	0.4	1.0	0.2	58.6
C. colo. (No. 1, $2x$) ×*C. vul.* (Asahi-yamato, $2x$)	81.0	0.4	1.4	0.2	0.0	82.0
C. colo. (No. 1, $2x$) (Standard)	1.0	0.0	0.0	0.0	0.0	1.0

* R_f of paper-chromatography with water-saturated ethyl-acetate by ascending method at 20°C.

** Kihara Institute for Biological Research, Yokohama.

*** Refer to Report No. 54.

The concentration of "Citbittol A" in the ripe fruits of the hybrids is generally higher than in *Colocynthe*. The bitter substances of the hybrids are different and their concentrations vary according to the variety of watermelon examined as shown in Table 1.

The watermelon has no bitter substance. The bitter substances detected in the fruits of the hybrids have been already identified in the Colocynthe parent.*

56. *Anti-cancer Activity of "Citbittol A"*

(By Yoshito OGAWA)

LAVIE et al. (1957) reported that "Elatericin B", a glycoside isolated from Cucurbitaceae (*Ecballium elaterium* and *Citrullus colocynthis*), showed at 4 gamma per 1 g. body weight a good tumor controlling effect on Sarcoma 37 transplanted into mice. "Elatericin B" (kindly supplied by Dr. LAVIE) is very bitter to the taste.

In the present study, the anti-cancer activity of another Cucurbitaceae component was tested against an ascites tumor, Yoshida sarcoma, transplanted into a strain of rat, Wistar (two months old). The Cucurbitaceae component tested was "Citbittol A", a bitter substance isolated by the writer (1957) from the fruit of *Citrullus colocynthis* Schrad.

Immediately after the transplantation of the sarcoma, Citbittol A (50 gamma or 5 gamma per 1 g. body weight of rat) was injected at the axilla subcutaneously, and the effect of this substance on the sarcoma was observed, especially in the following three points:

1) Mitotic activity in sarcoma cells,

2) Systemic symptoms of the rat bearing the sarcoma (appetite, ascites icterus and metastasis),

3) Prolongation of the host's life.

In the mitotic activity of sarcoma cells, a transient decrease (significant at 5% level) was observed 48 hours after injection. As to the systemic symptoms of the host rat, no difference was recognized between the treated and the control groups in the first seven days. But 10 days after injection, the treated group showed a remarkable recovery and the animals remained alive for more than 100 days after transplantation of the sarcoma cells, except for one rat which died 17 days after injection. The control group was extinct on the 10th to 13th day. No toxicity of "Citbittol A" in the above mentioned doses was observed either at the injected site or in systemic symptoms.

* Refer to Report No. 54.

This experimental result may reaffirm the anti-cancer activity of Cucurbitaceae components. Biochemical and pharmacological comparisons between "Citbittol A" and "Elatericin B" are now being made.

G. GENETICS AND BIOCHEMISTRY OF MICROORGANISMS

57. *Curly Flagellar Mutants in Salmonella*

(By Tetsuo IINO)

A mutant strain, SW577, of *S. typhimurium* R (FREIWER & LEIFSON, 1952) produces curly flagella in phase-1, i-phase bacteria, and normal flagella in phase-2, 1.2-phase organisms. The cells with curly flagella rotate in liquid media and aggregate. On semi-solid media, however, they cannot swarm. In contrast with paralyzed mutant cells, they are sensitive to chi-phage. The strain reverts rarely and then produces cells with normal flagella in both phase-1 and phase-2. The antigen types in each phase are identical with those of SW577.

Transduction was carried out from the phase-2 culture of the diphasic strains to the phase-1 culture of SW577 mediated by phage P22. Phase-1 cells with normal flagella were selected on semi-solid medium containing anti-1.2 serum. The normal type transductional clones obtained manifest the antigen type of the donor in phase-1 and that of the recipient in phase-2. That is, the gene which controls the curl of flagella in phase-1 is not separable from H_1 by recombination. In transduction from SW577 to the strains with Ah_1^- (SW1061, SW629 and SW547; see the Annual Report, 1957), recombinants between Ah_1^- and the curl controller were obtained.

A parallel type of mutant in phase-2 was isolated from *S. abortus-equi* SL23. The mutant strain, SJ30, produces curly flagella in phase-2. The curly character is transduced linked to H_2.

From these results, together with the results obtained previously (the Annual Report, 1957), it is inferred that H_1 and H_2 are the primary templates of flagellar protein (flagellin) in phase-1 and phase-2 respectively; mutations in H_1 or in H_2 produce the altered configuration of flagellin, resulting in a change in antigen type, modification of the flagellar morphology or the inhibition of flagellar morphogenesis.

58. *Cistron Test of Motility Genes in Salmonella*

(By Tetsuo IINO)

The trail phenomenon in the transduction of motility (STOCKER, 1956; LEDERBERG, 1956) was applied as a test of the cis-trans position effect between the motility genes of *Salmonella*. In sixteen non-motile mutants which were obtained from *S. typhimurium* TM2, the production of swarms and trails in reciprocal transductions was examined on semi-solid media.

The strains, SL499, SL478, SW1153 and SJ60, are paralyzed types; that is, they are flagellated but non-motile. Transductions between them produce swarms but no trails at all, indicating that they are mutants in a cistron. The cistron will be given the symbol *Mot*. The remaining 12 strains are non-motile and non-flagellated. Two of them (SL482 and SJ35) are mutants in the Fla_1 cistron, which is linked to H_1. Each of the four strains, SL480, SL481, SW1154 and SJ31, produced both swarms and trails in transductions to and from any other strains tested. The results of reciprocal transductions among the remaining six mutants are summarized in Table 1. Under the hypothesis that the mutant genes in these six strains

Table 1. Reciprocal transductions between six non-motile mutants of *S. typhimurium* TM2.

Recipient / donor	SJ33	SJ34	SL488	SL490	SJ36	SL488
SJ33	0	1	0	1	2	3
SJ34	1	0	1	1	2	3
SL488	0	1	0	0	1	2
SL490	1	1	0	0	1	2
SJ36	2	2	1	1	0	2
SL483	3	3	2	2	2	0
TM2 (Fla^+)	3	3	3	3	3	3
—	0	0	0	0	0	0

0: neither swarm nor trail. 1: swarms. 2: swarms and a small number of short trails. 3: swarms and trails.

are complementary but the complementarity declines as the distance between the two mutant sites increases, the sequence of the mutant sites is inferred to be as follows: SJ34-SL488-SJ36-SL483; SL488 is a mutant which includes the sites of both SL33 and SL490.

59. *Pteridine Metabolism in Aspergillus oryzae*

(By Saburo NAWA)

A purple fluorescent substance produced by a strain of *Aspergillus oryzae* was characterized as a derivative of 2-amino-4, 7-dihydroxypteridine (I) from its ultraviolet absorption spectrum and chemical nature. When *A. oryzae* was grown on Czapek-Dox's medium supplemented with extra 2-amino-4-hydroxypteridine (II), two fluorescent compounds were isolated as metabolites of (II). One of these compounds, which is bright blue fluorescent in alkaline solution, had an ultraviolet absorption spectrum very similar to that of 2, 4-dihydroxypteridine (III). The second purple fluorescent substance was identified as 2, 4, 7-trihydroxypteridine (IV), paperchromatographically and spectrophotometrically. It was observed that the rapid deamination of (II) to (III) occurs in homogenates of the mycelium. Enzymes, partially purified by precipitation with acetone and dialysis, were also capable of catalyzing the reaction. Furthermore, (III) was oxidized to (IV) at very slow rate in both homogenates and enzymes. Therefore, it seems that *in vivo* (II) is first deaminated to (III) by pterine deaminase and ultimately oxidized to (IV) by pterine oxidase.

It was also found that the pterine oxidase is capable of oxidizing (II) to (I) and that (I) can not be acted upon by the deaminase. Therefore, the evidence that no trace of (I) could be detected in metabolites of (II) suggests that the activity of the deaminase is much higher than that of the oxidase. Derivatives of (II) substituted in the 6-position were also converted to substituents of (III) by the deaminase.

From these observations, there are the two following possibilities; i) A naturally occurring pteridine in *A. oryzae* carries some substituents which prevent attack by the deaminase, assuming that the pteridine is derived through an oxidation pathway of the (II) family. ii) Otherwise, the pteridine is synthesized by different pathways from oxidation of the (II) family, in case of the absence of such substituents.

H. RADIATION GENETICS IN ANIMALS

60. *Radiation Mutagenesis in the Silkworm*
*IV. Independence of Radiation Intensity as Shown by Induced Sterility of Spermatogenic Cells**

(By Yataro TAZIMA)

Silkworm spermatogenic cells show a hyper-sensitivity to radiation, as revealed in terms of excessive sterility, on about the second day of the fifth larval stadium, a few days earlier than the peak stage of radiation-induced mutability. The existence of separate sensitive periods for the two types of radiation response suggests that the cause of excessive sterility is different from that of induced mutation.

Moreover, the hatchability curve for pigmented eggs, plotted against stage of irradiation, remains almost constant at the same dose of X-ray irradiation with the exception of the excessively sterile stage. This result shows that the factor causing excessive sterility must be different from the factor inducing dominant lethals which are presumably produced by gross structural changes in the chromosomes.

Therefore, physiological damage of the cell as a whole was assumed as a possible explanation of the hyper-sensitivity of irradiated cells at this particular stage (TAZIMA, 1958).

According to this hypothesis, radiation would affect germ-cells through multiple hits at this stage, eventually killing them. The validity of this assumption was tested by irradiation experiments with different intensities. An experiment was carried out with 0.3 r/min γ-rays and 62 r/min X-rays, keeping the total dose delivered the same (800 r).

The γ-rays from ^{60}Co were delivered to the germ-cells of fifth instar larvae from the ages of 11 to 56.5 hours for 45 hours, except for several short breaks for feeding. X-ray irradiation was carried out at one of the three checking stages, i.e. at the ages of 11, 33.6 and 56.5 hours. The duration of the X-ray treatment was 12.9 minutes.

Two of the X-rayed groups were affected less than the one irradiated at 33.6 hours, whereas in this group the effect of acute irradiation was found to be of about the same degree as that of the γ-irradiated group, even though the radiation intensity differed by a ratio of 207:1.

These results show that the effect of radiation on sterility of germ-cells is independent of intensity, and is cumulative with the doses delivered. This result is in contradiction to the expectation from the multiple hit

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

hypothesis. The above working hypothes is being disproved, it could be suggested that radiation-induced disturbance in the synthesis of basic cellular constituents, such as DNA, may be the cause of the excessive sterility of the germ-cells.

61. *Radiation Mutagenesis in the Silkworm*
*V. Further Data on Radiation-induced Mutation Rates in the Silkworm**

(By Yataro TAZIMA and Kimiharu ONIMARU)

It was reported previously (TAZIMA, 1958) that visible mutation rates at $+^{pe}$ and $+^{re}$ loci of the silkworm were markedly higher than the mutation rates found in *Drosophila*. The mean mutation rates obtained at these loci were about 7 times as high in the mature sperm and 28 times as high in spermatogonium as the rates reported by ALEXANDER (1954) for *Drosophila*.

In calculating mutation rates in *Drosophila*, ALEXANDER excluded all phenocopies, partial expressions, sterile variants and dead before completion of the test, while in the silkworm most of these categories were not excluded from the calculation. It must also be noted that in the silkworm the mutation rates were obtained for mutant characters expressed in the egg stage, in contrast to those obtained for adults in *Drosophila*.

Survival of egg color mutants was, therefore, examined in comparison with normals in the same lots. The survival indices of *pe* and *re* mutants from females irradiated with 2000 r were 0.78 and 0.56 respectively, giving 0.67 as an average. Even if we take 0.5 as the most conservative estimate of survival rate and 0.5 as the fraction covering phenocopies and others, which are not transmitted to the offspring, we still get higher mutation rates for the silkworm than for *Drosophila*.

Hence, another experiment was carried out using *chocolate*, a body color mutant for newly hatched larvae, as a marker. The results are shown in Fig. 1 and Table 1.

The mutation rates according to stage are in this experiment more or less similar to those obtained in the previous experiment with egg color mutants, even though they are lower.

The rates obtained are still 3.5 times as high in spermatogonium and 6.7 times as high in mature sperms as those in *Drosophila*. The survival index for the detected mutants was higher than for *pe* and *re*, and approximated 0.80.

* This work was supported partly by Grant RF 57178 from the Rockefeller Foundation.

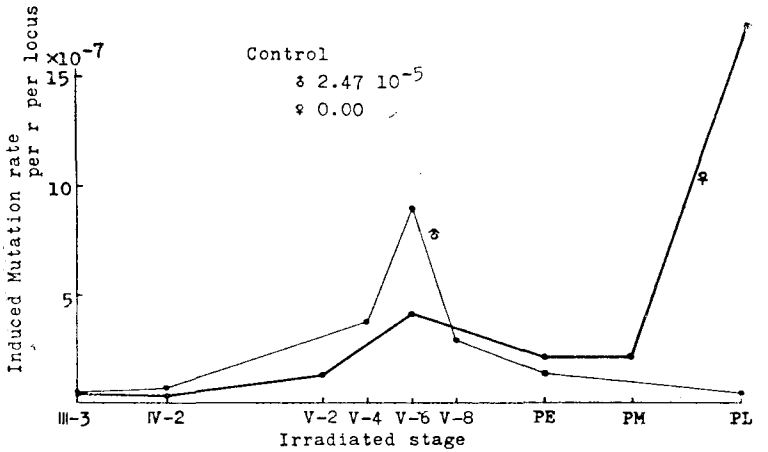


Fig. 1. Changes in radiation induced visible recessive mutation rate at $+^{ch}$ locus with the development.

Table 1. Spontaneous and radiation-induced mutation rates per r at $+^{ch}$ locus.

Sex	Spont. mutation rates	Radiation-induced mutation rates	
		Gonium	Matured egg or sperm
Female	0.00×10^{-5}	0.42×10^{-7}	17.5×10^{-7}
Male	2.47	0.53	4.0

However, it is not as yet conclusively determined whether or not mutation rates are higher in the silkworm than in *Drosophila*.

62. *Histological Study of Radiation Sensitivity of Spermatogenic Cells of the Silkworm (a Preliminary Note)**

(By Toshihiko SADO)

In a recent report by TAZIMA (1957) it was demonstrated that the incidence of radiation-induced sterility in the silkworm differs with sex and age of the irradiated insect, and that an extremely sensitive stage occurs early in the fifth stadium of the male. However, no cytological study of the cause of these phenomena had been made. Therefore, the present experiment was undertaken.

Various doses of X-rays (100 r, 500 r, 1000 r and 4000 r) were given to silkworm larvae at the early fifth stadium with a dose rate of 147.7r/min (180 kV, 25 mA, 1.0 mm Al-filter, 50 cm distance), but some younger stages also were subjected to X-rays. Testes from irradiated animals were fixed at definite intervals after irradiation, and examined following the usual histological methods.

Several hours after irradiation pycnotic or necrotic cells were observed in the gonial region, increasing in number from the time of irradiation up to 24 hours, when the highest incidence was observed (Fig. 1). The incidence of these degenerating cells was raised with increasing doses. Later, the necrotic cells rapidly liquefied and reduced the number of spermatogonia in the gonial region of the irradiated testes.

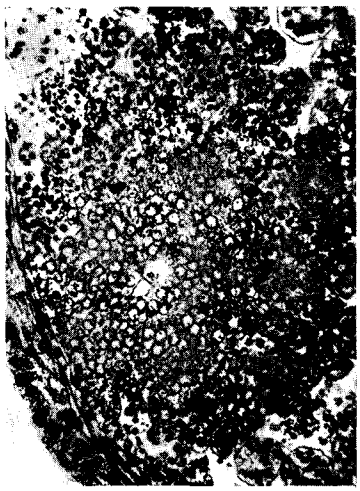


Fig. 1. Necrotic cells observed in spermatogonial region 24 hours after X-irradiation at 1000 r on the 2nd day of the 5th instar. Most of the late spermatogonia are necrotic.

In a testis weakly irradiated at 100 r, recovery was observed in the depleted gonial region, while in testes heavily irradiated at 4000 r the ability to recover was reduced to such a great extent that scarcely any spermatogonia were observed around the apical cells, even 11 days after irradiation. In moderately irradiated groups, regeneration of spermatogonia was found seven days after irradiation.

The most sensitive of the irradiated spermatogenic cells were late spermatogonial stages.

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Spermatocytes were less sensitive to X-rays than were spermatogonia, throughout the experiment. Cells irradiated at the former stage underwent apparently normal meiotic division without showing any necrotic changes, but as they turned into spermatozoa they showed nuclear abnormalities.

The apical cells located at the blind end of each testicular follicle were found to be resistant to radiation.

The reduced fertility of irradiated animals appears to be partly due to a lack of sperms as a result of spermatogonial depletion and partly to the formation of abnormal sperms which were not able to perform fertilization.

63. *Studies on Mutation Rates after Chronic Irradiation in Mice. A Preliminary Report on Sex Ratio.*

(By T. SUGAHARA, K. TUTIKAWA and T. TANAKA)

Male mice of a multiple recessive stock were irradiated with gamma-rays for three successive generations as proposed by J. B. S. Haldane, in order to obtain animals which would accumulate recessive mutations. The adult male mice were reared in the irradiating room, exposed to gamma-rays from ^{60}Co at a dose of 7.8 to 5 r, 22 hours per day for 30 to 45 days. The mating system and irradiating conditions are shown in Fig. 1. Irradiated animals are marked with asterisks. *G* denotes the dominant wild stock and *g* the multiple recessive one.

					Total dose in r	Period in days
P_4	$gg♀ \times gg♂*$	$gg♀ \times gg*$	$gg♀ \times gg♂*$	$gg♀ \times gg♂*$	167	30
P_3	$gg♀ \times gg♂*$		$gg♀ \times gg♂*$		167	32
P_2	$gg♀ \times gg♂*$				225	45
P_1	$GG \times gg$				0	
F_1	$Gg \times Gg$				0	

In this report, the sex ratio in each generation was studied for three to six months after the end of irradiation. The multiple recessive stock mice belonged to the NH strain, and those of the wild type were CBA.

The number of litters obtained, mean litter size, and sex ratio in each generation are shown in Table 1. The increase in the proportion of males in the first generation and its gradual decrease in the succeeding generations are considerable, although the statistical errors are rather too

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large to make the data statistically significant because of the small number of animals used. Variations in the mean litter size were not always parallel with changes in the sex ratio. The reason for the discrepancy is not clear.

Table 1.

	Control	P_4	P_3	P_2	P_1 ♂	P_1 ♀
No. of litters	47	22	41	45	17	29
No. of progeny	253	88	188	189	77	187
Mean litter size	5.4	4.0	4.6	4.2	4.5	6.4
Sex ratio	0.538	0.570	0.547	0.505	0.506	0.427
Probable error	±0.031	±0.054	±0.036	±0.038	±0.056	±0.036

The deviations of the sex ratio may be interpreted as follows, on the assumption of sex-linked dominant and recessive lethals induced by irradiation. The mutation rate for sex-linked dominant lethals is denoted by μ_1/r, and that for sex-linked recessive lethals by μ_2/r, according to the hypothesis that a linear relation exists between dosage and mutations. Radiation doses for each generation are denoted by D_1, D_2, and D_3. The deviations of the sex ratio from that of the controls in P_4, P_3, and P_2 were Δ_1, Δ_2, and Δ_3 respectively, while in P_1 the difference in sex ratio between *gg* males and *gg* females was Δ_4. Then, we have as a good approximation ;

$$\mu_1 D_1 = 4\Delta_1$$

$$\mu_1 D_2 - \frac{1}{2} D_1 \mu_2 = 4\Delta_2$$

$$\mu_1 D_3 - \left(\frac{1}{4} D_1 + \frac{1}{2} D_2\right)\mu_2 = 4\Delta_3$$

$$-\left(\frac{1}{8} D_1 + \frac{1}{4} D_2 + \frac{1}{2} D_3\right)\mu_2 = 4\Delta_4$$

From the above equations, using the method of least squares for our experimental results, we have

$$\mu_1 = 7.1 \times 10^{-4}$$

$$\mu_2 = 2.0 \times 10^{-5}$$

The Δ_s observed and those calculated with these values for μ_1 and μ_2 are in good accord with each other in the range of the statistical errors as shown in Table 2. Further study of the sex ratio is now under way in order to examine the correctness of our assumption.

Table 2. Deviation of sex ratio

	P_4	P_3	P_2	P_1 ♂	P_1 ♀
Observed	.032	.009	−.033	/	−.079
Calculated	.030	−.009	−.018	/	−.081

64. *Effect of Radiation on Living Cells in Tissue Cultures*

(By Masakatsu HORIKAWA* and Tsutomu SUGAHARA)

It is well known that the primary effect of radiation on *Drosophila* larvae is not killing of the irradiated larvae, but a delay of pupation and a decrease of pupation and emergence percentages over a wide range of radiation doses.

Furthermore, it has been observed by the authors that the body weight of irradiated larvae, whose pupation was delayed, was considerably higher than that of the control larvae. In the present investigation, the mechanism of this phenomenon has been studied in detail using several organs and discs cultured in a synthetic medium after irradiation.

Four wild strains (*Oregon-R*, *Canton-S*, *Kochi*, and *Samarkand*) and several eye-color mutants (*bw*, *w*, *v*, *cn*, *v bw*, *cn bw*, *Bar*, *bar-3*, and *Dp*/*In*(*3L*)*P*, *In*(*3R*)*C*, *Sbel*(*3*)*e*) of *Drosophila melanogaster* were used as material. Third-instar larvae grown under sterile conditions were irradiated with various doses of X-rays (160 kVp, 25 mA, 370 r/min with a 1 mm Al filter, at a distance of 30 cm.)

The larvae were dissected and various organs or tissues were taken out (cephalic complexes, wing discs, eye discs, leg discs, testes, ovaries, salivary glands, fat bodies, and blood cells) and single cells from these organs or discs were isolated under a binocular microscope in a sterile glass chamber.

The isolated cells were cultured in a normal synthetic medium, and in the same medium containing a radioactive isotope (0.5 $\mu c^{32}P/ml$ and 1 μc Thymine-$^{14}C/ml$). The sensitivity of the cultured organs and cells to irradiation was determined by observing their growth and differentiation and by measuring the amount of radioactive ^{32}P and thymine-^{14}C incorporated into them.

When the irradiated eye discs, leg discs, and salivary glands were cultured together with the normal cephalic complexes (from ten larvae), they showed pronounced growth and differentiation. The incorporation of radioactive phosphorus into these discs or glands was comparable to

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that in the non-irradiated discs or glands.

When the non-irradiated eye discs, leg discs, and salivary glands were cultured together with the irradiated cephalic complexes (from ten larvae), they showed pronounced growth, but no differentiation.

Since the cephalic complex is known to secrete a hormone controlling growth and metamorphosis, it would appear that the normal activity of the cephalic complex on differentiation of organs and discs is destroyed after irradiation, even with low doses.

As mentioned above, the eye discs, the leg discs, and the salivary glands were not affected even by irradiating with high doses, whereas the wing discs and the ovaries were affected by low doses.

These results coincide with those showing the incorporation rate of thymine-^{14}C into isolated single cells. The difference between the sensitivities of various organs and discs to irradiation could be elucidated by the sensitivities of cells isolated from them.

As described above, the metamorphic hormone secreted from the cephalic complex was not affected, even after irradiation with high doses.

The results obtained in this experiment show that the delay in pupation and the decrease in emergence percentage seem to be due to damage of the cephalic complexes, especially of the ring glands, by irradiation. In the *bar-3* strain which, among the thirteen strains used in this experiment, showed the highest sensitivity to whole body irradiation the cephalic complex seems to have a higher sensitivity to radiation damage than in the other strains.

65. *Unsuccessful Attempt at in vitro Cultivation of Spermatogenic Cells of the Silkworm**

(By Toshihiko SADO)

An investigation of *in vitro* cultivation of spermatogenic cells of the silkworm, *Bombyx mori*, was undertaken in order to explore a technique which might be useful for the study of the sensitivity of germ-cells to radiation at successive developmental stages.

Spermatogonia and/or spermatocytes from early 5th instar larvae of *Bombyx mori* were cultured in blood medium prepared from larvae and pupae of the same species.

As was demonstrated by SCHMIDT and WILLIAMS (1953) in their tissue culture of male sex cells of the *Cecropia* silkworm, growth and dif-

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ferentiation hormones are needed for the normal completion of spermatogenesis. Taking this fact into consideration, the blood of the following stages was prepared as a culture medium: 3rd, 5th, and 7th day of the 5th instar larvae, larvae at spinning, prepupae and midpupae.

In order to inhibit tyrosinase activity in the blood, either of the following methods was used: i) adding a few crystals of phenylthiourea and centrifuging, or ii) maintaining a temperature of 56°C for five minutes and centrifuging. Though the latter method was superior to the former in respect to the cleanness of the medium, the former was used as a routine procedure throughout this experiment with a few exceptions, because heat treatment seemed to modify to a great extent the nature of blood.

The individuals used were sterilized with 0.1 per cent aqueous solution of mercuric chloride for about 30 minutes, washed in sterile tap water three times, and then placed on a filter paper in a sterile Petri dish until dry.

A number of cysts containing spermatogonia or spermatocytes were suspended in a hanging drop of the culture medium, and then incubated at 27°C.

The attempts at cultivation have, however, been unsuccessful, showing only degenerative figures. Fifteen to thirty hours after the suspension was made, depending on the stage from which the blood medium was prepared, a large number of minute bubbles appeared on the surface of the cysts. Then the cells gradually became abnormal and after five days or more some follicles were broken off and the cells were liberated into the surrounding medium.

As is well known, the physico-chemical nature of silkworm blood, i.e., pH, viscosity, osmotic pressure, and chemical constituents, etc., undergoes considerable change with age at and after the fifth stage, owing to the drastic change in the formation and in the secretion of silk substance; during this period normal spermatogenesis proceeds. The same may be true for the metamorphosing hormones.

It seems very difficult to control these dynamic changes in the properties of blood *in vitro*, although it may not be impossible.

66. *Influence of Radiation on Mitochondrial Function in Cellular Metabolism**

(By Saburo NAWA and Bungo SAKAGUCHI)

It is well known that various cytoplasmic granules are bounded by

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

semi-permeable membranes. The work to be reported here was initiated in an attempt to evaluate the effect of radiation on the mitochondrial function controlling the accessibility of the enzymes, substrates and cofactors.

The mitochondria were prepared from mouse liver and suspended in 0.25 M sucrose. The mitochondrial suspensions were put in ampoules, kept in an ice-bath and exposed to varying doses of X-rays. After irradiation, the activities of acid phosphatase and DNase II were measured in both intact and fully activated mitochondria. Alternative freezing and thawing was used for the activation of mitochondrial enzymes. The results are shown in Tables 1 and 2.

Table 1. Effect of X-rays on mitochondrial acid phosphatase activity.

Dose of X-rays (r)	Activity			Ratio	
	Total*	0.025M**	0.25M**	0.025M/Total	0.25M/Total
0	100	82	65	82	65
10,000	95	80	58	86	62
50,000	90	85	58	95	66
100,000	85	83	51	97	61

*) Activation by freezing and thawing.
**) Final sucrose concentration of the reaction mixture.

Table 2. Effect of X-rays on mitochondrial DNase II.

Dose of X-rays (r)	Activity		Ratio
	Total	0.25M	0.25M/Total
0	100	27	27
10,000	110	29	27
50,000	132	54	41
100,000	128	77	60

The ratios of acid phosphatase activity of intact mitochondria in isotonic sucrose to total activity were not markedly changed by increasing dosages of X-rays. The total activity of acid phosphatase in activated mitochondria slightly decreased in the course of a series of irradiations. On the contrary, the ratios of activity of intact mitochondria in hypotonic sucrose to total activity increased under these conditions.

The mitochondrial swelling (Table 3) caused by suspension in a hypotonic medium, was measured by a decrease in optical density, and the degree of swelling increased with an increasing dosage of X-rays. Therefore,

the alteration of mitochondrial membranes seems to have been caused by the X-rays.

Table 3. Effect of X-rays on mitochondrial swelling.

Dose of X-rays (r)	Decrease in optical density (%)
0	100
10,000	116
50,000	150
100,000	176

In the case of DNase II, the activated mitochondria increased in activity with increasing dosages of X-rays. The activation of DNase II in mitochondria may be not completely achieved by this procedure but the increased rates of activation were assumed to be caused by X-rays. The activity of intact mitochondria increased obviously with increasing dosages of X-rays. These results suggest that the reaction between DNase II and DNA through mitochondrial membranes might be accelerated by X-rays. In the cell, biochemical reactions are generally controlled by the localization of the enzymes and substrates. It is probable that one of the direct effects of X-rays is a removal of intracellular barriers between enzyme and substrate. The damage may be intensified in the many biochemical processes following, even though the actual change in the subcellular particles is very slight.

67. *Studies on Genetic Variation in Response to Radiation in Mice* (I) *Effect of Single Doses of Total-body X-irradiation on the Leukocyte Values of the Peripheral Blood.**

(By Kiyosi TUTIKAWA, Masaaki ONOUE and Kotoyo TUTIKAWA)

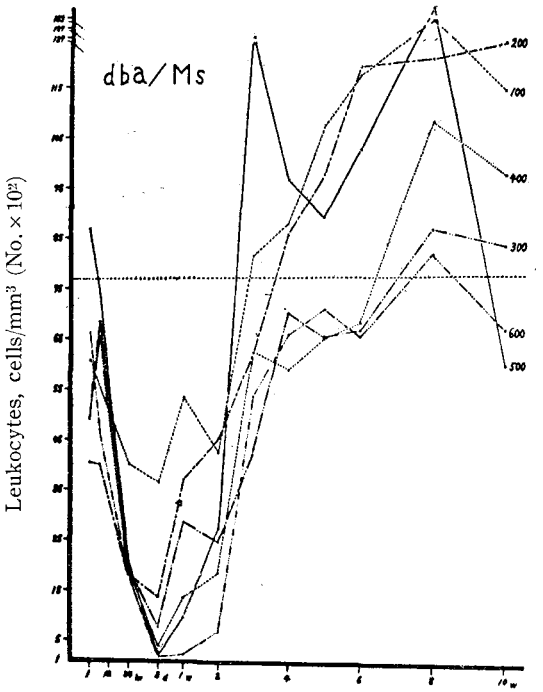
In mammals, it has been shown that the blood-forming tissues are among the most sensitive to ionizing radiations. After total-body exposure, manifestations of injury to the blood-forming tissues may appear in the peripheral blood even in absence of detectable change in the bone marrow or lymphatic tissues. In rabbits, it has been recognized that lymphocyte values fall below the control level with doses of 25 r.

The present report deals with the peripheral blood changes produced by X-ray dosages ranging from 100–600 r.

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

The mouse stocks used mainly in this study were SM/Rr and dba/Ms. The former stock was originated by selection for small body size and these mice also have inherited leukopenia. In this experiment, the normal leukocyte counts averaged 3418 in the SM/Rr strain and 7694 in the dba/Ms strain.

Irradiation conditions were : at 160-kVp, 25mA with HVL of 0.3mm Cu and 0.5 mm Al ; target distance 50 cm to the mid-point of the turn table. Each irradiation group consisted of five animals which were placed on the turn table and they were irradiated at 9 A.M. These procedures were repeated in this experiment. Blood samples were obtained between 9 and 12 A.M. from the tails of mice 60 days old. We used CHAI's method in which, before the blood was taken, the mice were placed in a battery jar and heated for approximately 5 to 8 minutes under a 60-watt light. The white blood cells were counted in the usual manner.



Time after exposure
hr; hour, d: day, w: week

Fig. 1. Effect of total-body X-irradiation with 100-600 r on the leukocyte values of the peripheral blood of mice (strain : dba/Ms).

The blood was examined for any change in leukocyte values at 1, 6, 12, 24 hours, 3 days, and 1 to 10 weeks, following single acute doses of 100, 200, 300, 400, 500 and 600 r. With all dosages as shown above, the total leukocyte values of the peripheral blood reflected the rapidly changing status during the first 24 hours after whole-body exposure. A mild leukocytosis occurred within 6 hours and a maximum depression was reached 3 days after irradiation (Fig. 1). The degree of this depression

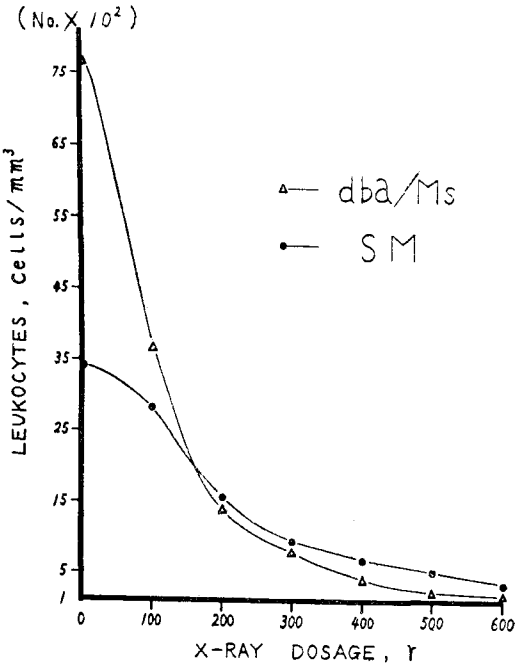


Fig. 2. Relation between X-ray dose and degree of depression of leukocyte value at 3 days after exposure in two strains of mice.

became more severe with increasing doses (Fig. 2). The leukocyte values 3 days after exposure, however, were higher in SM/Rr than in dba/Ms mice with dosages of 200 r or above, in spite of the fact that normal mean counts in SM/Rr were lower than in dba/Ms mice. We conducted, therefore, additional experiments using six other inbred strains or stocks with differential normal leukocyte numbers. However, no specific correlation with the normal leukocyte values could be seen from the counts of the leukopenic state.

Recovery of the leukocyte value followed, and an absolute increase or a normal level was reached between the third and fourth weeks after exposure of the mice to 100 r or more. The pattern of change in the peripheral leukocytes following exposure is generally in agreement with HENSHAW's (1944) pioneering research.

68. *Studies on Genetic Variation in Response to Radiation in Mice* (II) *Strain Differences in Mice in the Response of Leukocyte Values to X-irradiation**

(By Kiyosi TUTIKAWA and Kotoyo TUTIKAWA)

It has been previously reported that the maximum depression of peripheral leukocyte values was reached 3 days after a single total-body X-irradiation with doses ranging from 100 to 600 r, and no specific correlations with the normal leukocyte values could be seen from the counts of the leukopenic state. During the course of this study, it was discovered that differences exist among strains of mice in the degree of maximum depression of leukocytes 3 days after total-body exposure with doses of 500 r. This report presents the evidence on the genetic variation of this condition.

The mouse stocks used in this study were C57BL/6, dba/Ms, SM/Rr and LG/Rr. Other inbred strains, BALB/c, C58/LwMs and DBA/2Jax, were added in the course of this experiment. The irradiation conditions and the procedures of blood sampling have been previously reported (I).

(1) Age, sex and strain differences in the response of leukocyte values to X-irradiation.

In a preliminary experiment using dba/Ms mice 60 and 30 days old, an age difference was found; leukocyte counts in older and younger animals were 179 ± 67 and 111 ± 52, respectively. Subsequently we used only mice 60 days old in this study.

The mean leukocyte values and their standard deviations under normal conditions and 3 days after exposure to doses of 500 r are given for four inbred strains in Table 1. The mean value of the leukocytes was computed by pooling the count in each strain, since an insignificant difference between sexes was found except in the case of strain C57BL/6. To test whether the variation in leukocyte value is normal within a strain, the data were plotted on probability scale paper, and the points fell along a straight line. From the magnitudes of the standard deviations, it can be seen that the variation in leukocyte values seems to depend upon the mean counts.

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

Table 1. Mean counts of leukocytes, in normal condition and 3 days after X-irradiation with doses of 500 r in four strains.

Strain	Number of mice	Total number of leukocytes, cells/mm³			
		Normal		3 days	
		Mean	σ	Mean	σ
C57BL/6	50	8598	2009	177	61
dba/Ms	42	6449	2409	179	67
SM/Rr	50	3876	985	372	102
LG/Rr	50	5802	1553	550	166

The difference in mean counts of leukocytes 3 days after exposure to doses of 500 r between strains C57BL/6, SM/Rr and LG/Rr is statistically significant, but the difference between C57BL/6 and dba/Ms is insignificant.

The results of the present investigation lead, therefore, to the conclusion that strain differences can be seen in the response of leukocyte values to radiation.

(2) Genetic analysis of radiation response to leukocyte values

Three inbred strains, dba/Ms, SM/Rr and LG/Rr, which show different radiation responses in leukocyte values, were used for further genetic analysis of this sort of response. This report presents only the results obtained from six genotypic groups including two inbred strains and their hybrids. Maternal influence within each hybrid group was roughly balanced by making reciprocal matings between the two parental genotypes.

Table 2. Means and standard deviations of leukocyte values, in normal condition and 3 days after X-irradiation with doses of 500 r in each genotypic group of mice.

Generation	Number of mice	Total number of leukocytes, cells/mm³			
		Normal		3 days	
		Mean	σ	Mean	σ
P_S (SM/Rr)	50	3876	985	372	102
P_L (LG/Rr)	50	5802	1553	550	166
F_1	34	5152	1553	486	127
F_2	108	4756	1456	437	180
B_S	48	3830	1255	297	114
B_L	40	5095	1640	505	138

The mean leukocyte values of each genotypic group and their standard deviations are given in Table 2 for normal mice and those tested 3 days after exposure to doses of 500 r. The leukocyte values of the F_1 and F_2 generations in normal condition and in the leukopenic state were closer to those found in P_L(LG/Rr) than in P_S(SM/Rr). The values of the backcrosses to SM/Rr ; B_S were shown to be approaching in magnitude those of the P_S, and the values of the backcrosses to LG/Rr ; B_L were shown to be approaching those of the P_L. The rate of approach in the B_S, however, was slightly higher than that in the B_L. These results are in agreement with CHAI's study (1957) on the leukocyte values of inherited leukopenia in normal conditions. The present evidence indicates that radiation response, as shown by the magnitude of the leukocyte value 3 days after exposure, is genetically controlled and possibly determined by a small number of genetic factors.

The mean counts of F_1 and F_2 mice 3 days after exposure were quite similar, but the variance of the F_2 was the largest among six genotypic groups. Assuming that the variance of the F_1 is an estimate of the environmental variation and that the variance of the F_2 is environmental variation confounded with genetic, their variances may be used to estimate the relative magnitudes of the effects of genotype and environment. As an attempt to estimate the genetic contribution to the variation in the F_2 generation, analysis of variance was made for the data obtained from these mice (Table 3). In the present experiment, the magnitude of the heritability of the response of the leukocyte value to radiation was roughly estimated as 0.809 in the F_2 generation.

Table 3. Analysis of variance of response of leukocyte values to radiation in the F_2 generation.

Source of variation	d. f.	Sum of squares	Mean square	Expectation of mean square
Total	107	1377.76		
Between litters	10	606.20	60.62**	$\sigma_E^2 + 1/2\sigma_G^2 + \frac{n}{2}\sigma_G^2$
Within litters	97	771.56	7.95	$\sigma_E^2 + 1/2\sigma_G^2$

** Significant at the 1% level.

$\sigma_G^2 = 10.81$, $\sigma_E^2 = 2.55$, $\sigma_T^2 = 13.36$, $h^2 = \frac{\sigma_G^2}{\sigma_T^2} = 0.809$

I. RADIATION GENETICS IN PLANTS

69. *Radiosensitivity in Plants*

(By Tarô FUJII)

(A) Experiments with cultivated plants

Dry dormant seeds of 43 species or their commercial varieties were irradiated by γ-rays from radiocobalt and the radiosensitivity of these plants was determined by measuring the decrease in rate of germination and in number of growing plants. The germination rate decreased with the increase of dosage, but it varied widely, not only between different species but also between different commercial varieties. An irradiation effect was also noticeable in the delay of seedling growth proportionate to the dosage, as well as dwarfing, asymmetrical development and destruction of venation in young leaves. *Hordeum* was the most resistant and *Triticum* was the most sensitive among the Gramineae The Liliaceae were the most sensitive and next to them were the Umbelliferae. In some species of Cruciferae, Leguminosae and Compositae a few plants continued to grow even when irradiated at 100 kr. They were, therefore, more resistant to radiation than the Gramineae, Commelinaceae and Liliaceae. From our experiments, LD-50 for seed irradiation was about as follows:

Hordeum sativum	70 kr	*Glycine max*	40–70 kr
Triticum vulgare	20–40	*Vigna sinensis*	〃
Sorghum bicolor	40	*Pharbitis Nil*	70
Zea mays	〃	*Solanum gilo*	20–40
Raphanus sativus	70	*Calendula arvensis*	70
Arachis hypogaea	〃	*Cosmos bipinnatus*	70–100

The germination and growth up to the appearance of the cotyledons was not much decreased in several species, even at 100 kr, but most of the seedlings died immediately after germination and only a few plants could survive. Knowing this, the sensitivity of dormant seeds to irradiation can be determined by finding the percentage of seedlings which develop 2–3 leaves. Radiosensitivity was different even between commercial varieties within the same species. It seems that radiosensitivity of plants may be due to the genetic make-up of a race, and it may be modified by physiological or physical conditions as well.

(B) Radiosensitivity of mutants in Einkorn wheat

Five mutants of *Triticum monococcum flavescens* induced by X-rays were

irradiated again with X- and γ-rays, and the differences in radiosensitivity were examined with respect to the recessive genes induced by mutation. Three kinds of chlorophyll mutants and two morphological mutants were used in this study. Dry dormant seeds were irradiated by X-rays at 10, 20 kr and γ-rays at 20 kr, and the germination rate, plant height and fertility in the X_1 and frequency of chlorophyll mutation in the X_2 were examined (Table 1). The decreases in germination rate, survival rate and fertility of the X_1 mutants were not much different from those of the normals. But the chlorophyll mutation rate seemed to have become

Table 1.

Genetic effects of radiation in mutant strains of Einkorn wheat.

Strain	Dosage (kr)	Germination rate (%)	Plant height (cm)	X_1 fertility (%)	Frequency of chlorophyll mutation in X_2 (%)	% of non-germinating head-progenies
Normal	0	65.0	11.8	79.3	0.0	—
	X-10	70.0	12.0	79.5	4.6	8.5
	X-20	62.5	8.5	45.0	0.0	25.0
	γ-20	75.0	8.6	41.6	60.6	16.1
Chlorina	0	37.5	10.2	54.9	0.0	16.7
	X-10	50.0	9.0	53.7	3.3	26.8
	X-20	45.0	7.7	37.4	0.0	56.5
	γ-20	37.5	4.9	28.8	0.0	69.2
Basi-viridis II	0	30.0	9.6	51.5	0.0	—
	X-10	30.0	11.8	46.9	0.0	9.1
	X-20	50.0	8.4	48.4	0.0	47.8
	γ-20	26.7	9.2	23.9	0.0	25.0
Virido-albina	0	60.0	9.8			
	X-10	62.5	10.1			
	X-20	47.5	8.8			
	γ-20	47.5	6.4			
Early	0	55.0	12.2	76.1	0.0	0.0
	X-10	40.0	10.1	81.1	5.0	2.4
	X-20	20.5	4.3			
	γ-20	17.5	3.5	13.7	0.0	0.0
Slender	0	87.5	11.4	62.0	0.0	—
	X-10	75.0	9.8	54.4	0.0	37.7
	X-20	55.0	7.5	34.6	0.0	63.1
	γ-20	55.0	7.1	41.0	0.0	46.7

slightly lower than that of the normals, while the frequency of non-germinating head-progenies in the X_2 was higher in the mutants than in the normals. Thus we see that radiosensitivity is not different in the examined mutants in comparison with the normals. A decrease in viability in the X_2 of the mutants as shown in an increase of non-germinating head-progenies, may be due to an accumulation of genic mutations and radiation damage.

In this study, back mutation from recessive to dominant, such as from *chlorina* to normal, did not occur.

(C) Experiment with cultivated rice

A preliminary irradiation experiment was carried out in 1957 for the purpose of determining the lethal doses for rice. Dosages were 20, 40, 70 and 100 kr by γ-rays from radiocobalt, and they were applied to two commercial varieties, Omachi and Tetep. The germination rate in both varieties did not decrease greatly up to 40 kr, but it was very low at 70 kr. In 1958, three varieties, Tomoemasari (a typical Hokkaido variety), Mihonishiki (Chubu district) and Norin No. 18 (Kyushu district) were irradiated with 20, 40, 50, 60 and 70 kr. With regard to germination

Table 2.

Effects of radiations in three varieties of *Oryza sativa*.

Variety	Dosage (kr)	Germination rate (%)	Plant height (cm)	Survival rate (%)
Tomoemasari	0	79.5	15.03	77.0
	20	81.0	13.83	78.5
	40	67.5	12.06	65.0
	50	67.0	11.05	61.5
	60	1.0	—	0.0
	70	0.0	—	0.0
Mihonishiki	0	80.5	11.83	77.0
	20	82.0	10.50	77.5
	40	55.0	6.80	39.0
	50	18.5	6.13	13.5
	70	0.0	—	0.0
Norin No. 18	0	86.0	10.83	85.5
	20	81.0	10.51	81.0
	40	26.5	6.82	25.5
	50	13.0	5.20	15.0
	60	0.0	—	0.0
	70	0.0	—	0.0

rate, plant height and survival rate, Tomoemasari was most resistant, while Norin No. 18 was most sensitive (Table 2). Fertility in the X_1 generation was examined in Mihonishiki; it decreased with the increase of dosage, from 90.6% in the non-irradiated group to 55.3% at 20 kr, and to about 20% at 40 and 50 kr irradiation. A similar examination was carried out with several other varieties and a few wild rice species. From these results, it seems that the LD-50 of cultivated rice is about 30-40 kr, and 10-20 kr irradiation given to dormant seeds would be the proper doses for mutation work with rice.

70. *Differences in Radio-sensitivity and -mutability between Cultivated and Wild Strains of Japanese Morning Glory*

(By H. KIHARA and S. SAKAMOTO)

In order to compare the differences in radio-sensitivity and -mutability between cultivated and wild strains of Japanese morning glory (*Pharbitis nil* Chois.), the following four strains were used in this experiment. (A) wild strain "850"—collected in Nepal with standard leaf and blue flower, (B) wild strain "Tendan"—collected in North China with standard leaf and blue flower, (C) cultivated strain "Violet"—with dragon-fly leaf and reddish purple flower, (D) cultivated strain "P7"—with standard leaf and blue flower. Dormant seeds were irradiated with γ-rays from a ^{60}Co source. The seed-coats were cut open and the seeds were sown.

Table 1. Percentage of germinating and surviving plants in 3 strains.

Strain	(B)			(C)			(D)		
Dosage (kr)	No. of sowing	Germination	Survival	No. of sowing	Germination	Survival	No. of sowing	Germination	Survival
Cont.	46	100	100	49	100	95.5	49	100	100
10	50	100	100	50	96.0	97.9	50	100	100
20	50	98.0	95.9	50	92.0	100	50	98.0	95.9
30	48	97.9	100	50	92.0	95.7	50	100	96.0
40	50	98.0	89.8	50	100	82.0	50	100	60.0
50	50	96.0	79.2	50	96.0	66.7	50	100	28.0
60	50	100	44.0	50	96.0	43.8	50	100	26.0
70	50	100	48.0	50	98.0	38.8	50	100	28.0
80	50	96.0	27.1	50	94.0	4.3	50	100	4.0
90	50	100	22.0	50	100	0	50	98.0	0
100	50	96.0	8.3	50	98.0	0	50	98.0	0

(I) Difference in radiosensitivity

Table 1 shows the percentage of germinating and surviving plants in 3 strains, (B), (C) and (D), after irradiation at dosages of 10 to 100 kr. Germination and survival were observed 5 and 45 days after sowing, respectively. The germination of the 3 strains was very good and uniform at any dosage. The number of surviving plants was high under 40 kr but at dosages over 50 kr it decreased rapidly with the increasing dosage. All plants of the cultivated strains (C) and (D) were dead at 90 kr or more. The wild strain (B) showed a higher survival rate than the cultivated strains at all dosages. In this experiment, radiosensitivity of the cultivated strains was higher than in the wild ones. Several abnormalities, such as irregular leaf, shrunken leaf, variegated leaf, blocked hypocotyl elongation, and abnormal anthocyanin formation in hypocotyl and cotyledons were observed.

(II) Difference in radiomutability

In this experiment dormant seeds of (A), (B) and (D) strains were treated with 25 kr. Radiomutability was measured by the frequency of induced mutations. 330 X_2-plants derived from 22 X_1-plants of (A) were observed but no visible mutation could be found. Among 105 X_2-plants

Table 2. Pollen- and seed-fertilities of delicate leaf mutants on the "P7" strain. (No. of flowers in parentheses)

Culture number	Pollen-fertility (%)	Seed-fertility (%)* selfed	backcrossed**	open pollination
36-1***	6.06	0 (Many)	–	ca. 0.80 (ca. 40)
-5	12.05	0 (17)	5.50	8.17 (65)
-31	41.03	0 (7)	0 (1)	0 (28)
37-6	21.93	2.67 (61)	4.67 (18)	5.00 (230)
-7	27.07	0 (79)	11.16 (9)	10.33 (69)
-10	73.85	0.83 (37)	11.83 (7)	5.17 (152)
-21	25.72	0 (19)	2.83 (12)	0.83 (38)
Control	90.51	81.00 (43)	–	71.33 (57)

* Seed-fertility=(No. of seeds / No. of ovules)×100
(No. of ovules=6×No. of flowers)

** Backcrossed to control.

*** Did not form elongated branches.

obtained from 36 X_1-plants of (B) only one mutant with deep purple flowers was observed but there was no difference from the control plants in the other characters. Out of 912 X_2-plants obtained from 43 X_1-plants

of (D), 9 mutants were found. One was a maple leaf (Tatsutaba) mutant with high seed-fertility which could be easily backcrossed to the control. The other 8 individuals were delicate leaf (Sasaba) mutants. Their growth was slow and dwarflike but later they formed elongated branches. Their flowers with slightly separated petals did not fully open and the development of the stamens was abnormal. Table 2 shows pollen- and seed-fertilities of the delicate leaf mutants. They were both very low. In this experiment the frequency of induced mutations in the cultivated strains was higher than in the wild ones.

71. *Relation of Ploidy to Radiation Effect*

(By Mitsuya NEZU)

The effect of radiation is influenced by various factors. Polyploidy is

Table 1. Frequency of chromosome aberrations at MI in PMC's induced by γ-rays in wheats.

Material	Dosage (kr)	Total no. of cells observed	Total number observed						Average no. of breaks per cells
			fragments	univalents	multivalents ④	⑥	⑧	⑩	
T. aegilopoides	Cont.	4							0.00
	20	4			2	1			1.75
T. monococcum	Cont.	6							0.00
	20	30			10				0.66
T. dicoccum	Cont.	7	1						0.14
	20	19	7	1	12	3			2.15
	30	9		4	14				3.55
T. durum	Cont.	7							0.00
	20	27		2	12	1			1.07
	30	27		3	22	2			1.96
	40	27		2	30	2			2.51
T. Spelta	Cont.	17							0.00
	20	26	2	6	27	3			2.73
	30	18	1	6	28	8		2	5.38
	40	9		6	11	3	5	1	6.88
T. vulgare	Cont.	6		2	1				0.66
	20	27	1	2	24	5	1	1	2.77
	30	16		11	17	6	2		4.43
	40	14		3	15	4	4	2	5.07

one of them. The author has examined in this connection meiotic irregularities and seed fertility in three groups—di-, tetra- and hexaploid —of wheat. As material 6 species were used, namely *Triticum aegilopoides*, *T. monococcum*, *T. dicoccum*, *T. durum*, *T. Spelta* and *T. vulgare*. Dormant seeds were exposed to γ-rays of 20, 30 or 40 kr for 18 hours.

The percentage of PMC's with meiotic irregularities, mostly reciprocal translocations, increased with increasing ploidy. In 4x species the great majority (83.4%) of PMC's showed aberrations at 30 kr and in 6x species 88.7% cells showed similar irregularities at 20 kr, while in 2x species only 38.3% had aberrations at 20 kr. At higher dosages (30–40 kr) all cells were abnormal. A similar increase of multivalents (⑥, ⑧, ⑩) was observed, especially in 6x species (Table 1). As to other abnormalities, asynapsis and deficiency were observed, and rarely heteroploidy. The number of breaks per cell increased almost linearly with the dosage within each group. Comparison between the three groups—2x, 4x and 6x—showed an increase in breakages in a ratio of 1 : 2 : 4. Seed fertility decreased with increasing dosage. The 2x species were most susceptible in this respect, next to them the 6x, followed by the 4x. The fertility of the 6x species decreased suddenly at dosages between 20 and 30 kr. The higher the ploidy, the more aberrations arise. The highest number of multivalents was found in the 6x plants.

72. *Genetic Effects of β- and γ-radiation on Einkorn Wheat*

(By Seiji MATSUMURA)

To compare the genetic effects of β-radiation with those of γ-radiation, seeds of *Triticum monococcum flavescens* were soaked in ^{32}P and ^{131}I solutions for 2 days just before sowing. Radioactive solutions of pH 6 to 7 contained 0.05~0.4 mc/gm of ^{32}P and 0.2~0.8 mc/gm of ^{131}I. Also γ-irradiation with ^{60}Co was applied at the dosages 2.5, 5, 10 and 20 kr immediately after soaking the seeds in water for 2 days. The growth of seedlings, single-spike fertility and chromosome aberrations of treated plants (X_1) and the chlorophyll mutations in the X_2 were compared for β- and γ-irradiations. The data are shown in Table 1. The seedlings were measured 18 days after sowing. The relation between the inhibition of seedling growth and dosage of β- and γ-radiations coincides roughly with that between the decrease of fertility and dosage. γ-rays at 2.5 kr slightly inhibited the growth of seedlings and reduced the fertility and the effects correspond roughly to those of β-radiation from 0.2 mc/gm ^{32}P solution and 0.8 mc/gm ^{131}I solution. γ-irradiation at 10 kr was markedly

Table 1. Genetic effects of β- and γ-radiations in Einkorn wheat.

Dosage		Germination rate (%)	Length of seedlings* (cm)	Fertility in X_1 (%)	Chromosome aberrations in PMC (%)	Chlorophyll mutations in X_2 (%)
Control		38.0	13.46	92.09	0.00	0.00
γ-ray	2.5kr	42.0	10.12	83.17	2.44	14.00
	5.0 〃	56.0	9.10	64.63	24.53	17.46
	10.0 〃	16.0	2.71	47.36	90.91	28.57
	20.0 〃	0.0	–	–	–	–
^{32}P	0.05mc/gm	52.0	14.33	92.25	6.89	5.19
	0.1 〃	54.0	13.13	89.02	1.35	5.33
	0.2 〃	76.0	12.70	82.25	7.69	11.11
	0.4 〃	48.0	7.97	71.18	7.94	18.87
^{131}I	0.2 mc/gm	68.0	12.46	95.00	2.13	0.00
	0.8 〃	44.0	11.63	88.38	0.00	6.45

* The seedlings were measured 18 days after sowing.

effective. There was no germination at 20 kr with γ-irradiation.

In the treated plants (X_1), white and yellow stripes were often found, especially after ^{32}P. The frequency of ears with chromosome aberrations in X_1-plants was strikingly high after γ-irradiation. The majority of induced chromosome aberrations were : ④+5_{II}, often ④+④+3_{II} or ⑥+4_{II} and seldom ⑧+3_{II} or asynapic 14_I.

The frequency of head progenies with chlorophyll mutations in the X_2-generation increased with the increase of radiation dosage. The effects of β-radiation from 0.4 mc/gm ^{32}P solution correspond roughly to those of 5.0 kr γ-radiation. The majority of chlorophyll mutations after β-irradiation were *albina*, *xantha* and *viridis*, while with γ-irradiation *albo-viridis*, *virido-albina* and *basi-viridis* were most often observed.

In conclusion, the effects of β-radiation from 0.2 mc/gm ^{32}P solution and 0.8 mc/gm ^{131}I solution, correspond to those of 2.5 kr γ-radiation. If we assume that the effects of β-radiation are confined to the embryo, by calculation we find that the 0.2 mc/gm ^{32}P solution equals about 2.4 krad. This will account for the present data.

73. *Genetic Effects of Thermal Neutrons on Wheat*

(By Seiji MATSUMURA)

The thermal neutron irradiations discussed here were conducted in the

thermal column of the Japan Atomic Energy Research Institute's Nuclear Reactor, JRR-1. Hole No. 7 (North 6″ ϕ Thermal Column Access Port) was selected to keep gamma contamination as small as possible. The thermal neutron flux was calculated to be 2.6×10^{8} n/cm²·sec when the reactor was operated at 25 kilowatts.

In a preliminary experiment, dormant seeds of *Triticum monococcum flavescens* were kept in this hole for 1~5 weeks (I~V: actually 131.5~1068.7 kWh: $4.9\sim40.0\times10^{12}$ n/cm²). The data are shown in Table 1. The higher the dosage of thermal neutrons, the more delayed were the germination and growth of seedlings. The seeds were almost uniformly injured. There was no germination after a 4~5 week treatment (IV~V: $31.4\sim40.0\times10^{12}$ n/cm²) and the seedlings resulting after shorter exposure died in an early stage. In the root tips just after germination the frequency of chromosomal aberrations increased with the increase in dosage, and in IV and V more than 95% of the dividing cells had chromosome aberrations. In PMC's a ring of 4 chromosomes was observed. In the X_2, chlorophyll mutations such as *albina*, *xantha*, *viridis*, etc., were found. The frequency of chromosome aberrations and chlorophyll mutations increased in proportion to the dosage.

In the next experiment the reactor was operated at 40 kW and seeds of *T. monococcum* (2x), *T. durum* (4x), and *T. vulgare* (6x) were subjected to thermal neutrons at 5 different distances in reactor hole No. 7 for 2 weeks (actually 990.8 kWh). The thermal neutron flux ranged from 0.49 to 4.2×10^{8} n/cm²·sec (total flux: $4.4\sim37.5\times10^{12}$ n/cm²). After exposure to

Table 1. Genetic effects of thermal neutrons on Einkorn wheat.

	Kilowatt hours	Thermal neutron flux ($\times10^{12}$ n/cm²)	Germination rate* (%)	Length of seedlings (cm)	(Index)	Abnormal cells in root tips (%)	Chromosome aberrations in PMC's (%)	Chlorophyll mutations in X_2 (%)
Control	–	–	65.0	9.41	(100)	0.24	0.00	0.00
I	131.5	4.9	65.0	7.76	(82.47)	22.04	3.13	3.77
II	363.2	13.6	79.0	7.08	(75.24)	28.30	18.52	10.53
III	491.5	18.4	87.5	4.86	(51.65)	43.67	15.79	8.00
IV	838.0	31.4	20.0	0.50	(5.31)	96.43	–	–
V	1068.7	40.0	40.0	0.47	(4.99)	96.61	–	–

* The seedlings were measured 14 days after sowing.

37.5×10^{12} n/cm², *T. monococcum* did not germinate, while the seeds of *T. durum* and *T. vulgare* germinated but the seedlings died in an early stage without further growth. At 20.6×10^{12} n/cm² about 2/3 of the seeds of *T.*

monococcum germinated, but the seedlings soon died, while in *T. durum* and *T. vulgare* slow growth of the seedlings was observed. Thus we see that *T. monococcum* is most sensitive to thermal neutrons and *T. durum* is unexpectedly most resistant. There is no significant difference between *T. durum* and *T. vulgare*.

74. *Genetical Analysis of a Few Chlorophyll Mutants in Einkorn Wheat Induced by Radiation*

(By Tarô FUJII)

(A) Crossing experiments between *basi-viridis* II and other mutant strains

Crosses between *basi-viridis* II and *basi-viridis* I or other mutant strains were carried out in 1957–'58, and double recessive segregants were obtained from each cross. *Basi-viridis* II and *basi-viridis* I recovered a normal chlorophyll content when they were placed in the phytotron, but in the seedling stage the chlorophyll content was only about 50% in comparison with that of the normals. The chlorophyll content of the double recessives derived from the cross *basi-viridis* I×II was further decreased and the plants had the appearance of *virido-albina* mutants. When they were placed in the phytotron, their leaves gradually recovered a normal green color, as did both parents. On the other hand, early, slender and irregular-ear mutants were crossed to *basi-viridis* II and double recessive plants were also obtained from these crosses. As to chlorophyll, all double recessive segregants in the seedling stage were similar to the *basi-viridis* II parent.

From these results and similar symptoms exhibited by double recessive plants obtained from crosses between *virido-albina* and the chlorophyll mutants (Ann. Rep. No. 8), it seems that the chlorophyll content of the double recessives in the seedling stage is decreased more than in the parents. On the contrary, the chlorophyll content and recovery in double recessive plants obtained from crosses between chlorophyll mutants and mutants of other kinds were quite similar to those of the parental chlorophyll mutant, and they resembled morphologically the other parental mutant. This may be due to the fact that in the crosses between chlorophyll mutants the genes involved have an additive action causing a decrease of chlorophyll content, while the genes for morphological traits have no effect on chlorophyll formation.

(B) Doubly recessive plants from crosses between *chlorina* and *striata*

All F_1 plants from a cross between *chlorina* and *striata* were of normal

green color and showed at meiosis the chromosome conjugation $1_{IV}+5_{II}$ while in both parents seven normal bivalents were formed. Normal, *chlorina*, *striata*, and doubly recessive plants segregated in the F_2 generation. The segregation ratio was also observed in the F_3 generation, but the linkage relationship was not clear because the germination rate of *chlorina* was low, and that of *striata* was still worse. In the F_3 *striata* (or *chlorina-striata*) plants were segregated only from the F_2 plants with a tetravalent (Table). Moreover, F_1 plants from crosses between *striata* and the mutants *basi-viridis* I or II, slender and early also showed the conjugation $1_{IV}+5_{II}$. From these facts, we judge that the *striata* gene must be located on one of the chromosomes involved in the reciprocal translocation.

Character in F_2	Chromosome conjugation in F_2	Segregation rate in F_2			
		normal	*chlorina*	*striata*	*chlorina-striata*
Normal	7_{II}	98			
〃	〃	141	46		
Chlorina	〃		147		
Normal	$1_{IV}+5_{II}$	207		34	
〃	〃	176	67	21	8
Chlorina	〃		201		35

75. *Radiation-induced Mutation of the Self-incompatibility Gene in Brassica*

(By S. S. RAJAN)

The present note is a preliminary report of attempts to induce mutations of the self-incompatibility gene in some species of *Brassica*. Mature pollen grains and flower buds at various stages of meiosis were subjected to X- and gamma-radiations with doses ranging from 200 to 3,000 r units. The pollens treated was used in self-pollinations. The gamma-ray treatments were confined to pollen grains only. Even the highest dose did not affect the normal functioning of the pollen grains as checked by test crosses with cross-compatible genotypes. It was noticed that neither of the radiations had any mutational effect when applied to mature pollen grains. There was, however, considerable stimulation in the development of fruits, mostly with very few seeds. True seeds with 100% viability were found after self-pollination with pollen from flowers opening on the fourth day after irradiation, and later. There was a steady decline in

the seed set as buds treated at younger stages came to flower and were used in pollination. This is probably due to the high sensitivity of the earlier stages of meiosis to the radiation. Buds that were at early prophase to anaphase I stages during irradiation produced no fertile pollen. Considering each seeds set as due to the mutation of a single self-incompatibility gene, the mutation and the dosage showed a linear relationship, being maximum (average of 5 seeds out of 10 ovules per fruit) at 500 r units and minimum (1 seed) at 220 r units, (control 0 seeds per fruit). If these are true mutations, the fact that they could be induced at the tetrad stage lends further support to the hypothesis that the pollen reaction in these materials is under sporophytic control. Alternative explanations for the seed setting might be (i) pseudo-fertility, (ii) mutation of modifiers, specific or nonspecific in reaction, (iii) physiological weakening or destruction of the incompatibility reaction without genetic consequences. These have to be examined by results from test crosses with genotypes homozygous for the *S* alleles. This work is in progress.

J. GENETICS AND CYTOLOGY OF TUMORS

76. *An Attempt to Explain the Origin of Tumor Cells with V- or J-elements*

(By Tosihide H. YOSIDA)

Tumor stem cells in almost all transplantable rat tumors contain prominently large V- or J-shaped elements. According to MAKINO and SASAKI (1958), the original stock of the Yoshida sarcoma of the rat contained a large V-shaped element in the tumor stem-cells, but three tumor sublines which were characterized by containing two, three and four large V-shaped elements have been obtained from the original stock tumor. Recently a new subline which contains two large V- and one large J-shaped element was derived from the Yoshida sarcoma (YOSIDA 1958, 1959).

On the other hand, some primary rat hepatomas which developed after administration of an azo dye were observed by YOSIDA 1957, and YOSIDA and ISHIHARA 1958. According to their reports, some of the hepatomas primarily developed lacked such large V- and J-shaped elements as observed in the transplantable rat tumors, while other hepatomas were characterized by containing a large V- or J-shaped element. The frequency of the occurrence of the cells containing these elements varied with the tumor.

Based on the above investigations, a scheme representing the development of tumor cells containing large V- or J-shaped elements is given in Fig. 1. Developmental events occurring in the hepatomas and the Yoshida sarcoma are represented on the left side and the explanations for these occurrences are given on the right side of the diagram. The process going on in the cells is tentatively termed "primary somatic mutation". The original hepatoma cells which do not contain a large V- or J-shaped element may have undergone this change. In the course of successive cell generations a secondary somatic mutation may occur. Hepatomas which are characterized by tumor cells containing large V-shaped elements may belong to this category. If the tumor cells thus developed are serially transplanted into other rats, the selection process which takes place may determine which of the cell types will persist in the population. In this way, a stem line of the transplantable tumor could be

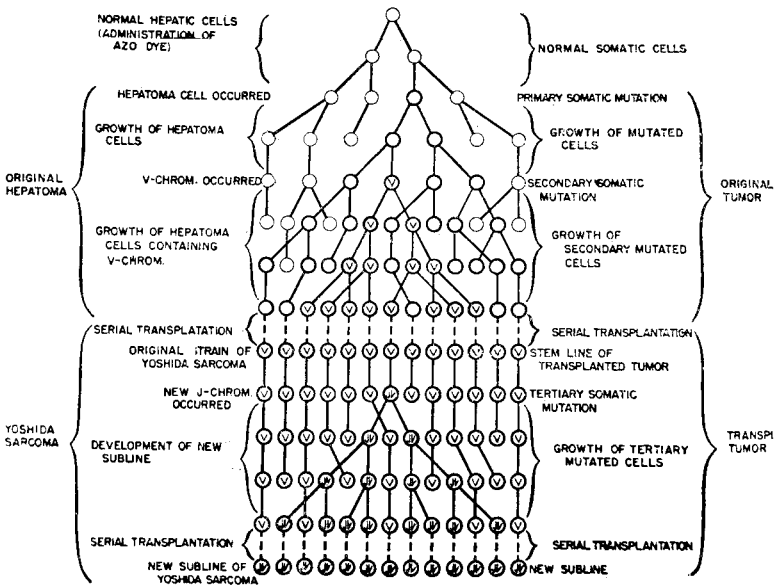


Fig. 1. A diagram representing the author's concept of the development of tumor cells containing the characteristic V- or J-shaped element in the course of cell generations. Thin circles denote normal somatic cells. Heavy circles show the tumor cells. V and J inside the circles denote the characteristic V- and J-shape chromosomes of tumor cells.

established. The stemline karyotype may readily be altered by ensuing somatic mutations. The new subline of the Yoshida sarcoma which con-

tains a large J-element is a good example of a tertiary somatic mutation.

This study was reported on August 23, 1958 at *X* International Congress of Genetics, Canada.

77. *Chromosome Breaks in Yoshida Sarcoma Cells Induced by X-irradiation**

(By Toshihide TABATA, Hiroyuki HIRUMI and Tosihide H. YOSIDA)

The tumor stem cells of the Yoshida sarcoma used in this experiment are characterized by 40 chromosomes, among them two large V-shaped elements. Rats carrying the Yoshida sarcoma were irradiated by X-rays under the following conditions: Deep therapy installation 160 kVp; filter of 1.0 mm Al; five different dosages, namely 10,000 r (25.9 minutes), 5,000 r (17.9 minutes), 2,000 r (5.1 minutes), 1,000 r (2.5 minutes) and 500 r (1.7 minutes). The chromosomes of the tumor cells were prepared by the acetic orcein squash technique immediately after irradiation. The results of our observations are as follows:

(1) Remarkable chromosome breaks were induced by X-ray irradiation with high doses such as 10,000 r, 5,000 r, 2,000 r and 1,000 r, but the cells were unsuitable for cytological analysis because the chromosome breaks were too numerous to study. The X-ray dose most suitable for chromosome observation was 500 r.

(2) Idiograms of thirty four tumor stem cells containing chromosomal breaks were analysed. The chromosomes of the Yoshida sarcoma comprise three types, namely J-, V- and rod-shaped chromosomes. As a standard for measurement of each chromosome the mean value of the largest V-shaped chromosome measured from thirty-four stem cells was used. Chromosomes of stem cells were classified into nine different groups, 0.2, 0.3, 0.4,—1.0 according to their α-ratio (mean length of chromosome/standard length) (Table 1). As shown in the table, the frequencies of breaks in the rod chromosomes were higher than in the other two groups. The χ^2 test shows that there is a significant difference between the frequency of breaks in the rod-chromosomes and in the V-shaped ones.

(3) The locations of breaks are represented by a calculated value of f/a (a=length from the kinetochore to the tip of the chromosome; f= length from the kinetochore to the location of the break). Most chromosome breaks were in the middle of the chromosome arms.

It may be concluded that the rod-chromosomes, especially those having

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

an α ratio of 0.8, are most sensitive to X-ray irradiation, and that the radiosensitive region is close to the middle of the chromosome arms.

Table 1. Frequencies of breaks in J-, V- and rod-shaped chromosomes

α-ratio	J-shaped chrom.				V-shaped chrom.				Rod-shaped chrom.			
	nJ	αnJ	fJ	fJ/αnJ	nV	αnV	fV	fV/αnV	nR	αnR	fR	fR/αnR
0.1	0	0	0	0	0	0	0	0	0	0	0	0
0.2	6	1.2	0	0	4	0.8	0	0	4	0.8	0	0
0.3	10	3.0	0	0	10	3.0	0	0	4	1.2	2	1.7
0.4	4	1.6	3	1.8	2	0.8	0	0	6	2.4	11	4.6
0.5	4	2.0	8	4.0	0	0	0	0	4	2.0	11	5.5
0.6	2	1.2	7	5.8	0	0	0	0	2	1.2	8	6.7
0.7	4	2.8	12	4.2	0	0	0	0	2	1.4	16	11.4
0.8	2	1.6	6	3.7	2	1.6	4	2.5	2	1.6	25	15.6
0.9	2	1.8	8	4.4	0	0	0	0	0	0	0	0
1.0	2	2.0	11	5.5	2	2.0	9	4.5	0	0	0	0
Total	36	17.2	55		20	8.2	13		24	10.6	73	

$\chi^2=37.67$ $P<0.001$

α-ratio: Mean length of each chromosome/mean length of the largest V-shaped chromosome; nJ, nV and nR: Number of daughter chromatids in J-, V- and rod-shaped chromosomes observed in this experiment; fJ, fV and fR: Frequencies of breaks in each chromosome type.

K. GROWTH, DIFFERENTIATION AND REGENERATION

78. *Growth-Promoting Effect of Kinetin on Embryos of Triturus pyrrhogaster* Boie.

(By Yoshito OGAWA)

Kinetin (6-Furfuryl-aminopurine) was isolated first by MILLER et al. (1956) from DNA obtained from herring sperms, and was found to be a substance stimulating cell division in excised tobacco pith tissues. Its effect on cell division has been reported in onion root tips (GUTTMANN, 1957), ascites tumor cells of rats (OGAWA, et al., 1957), jejunum mucosa of mice (CUDKOWICZ, 1957) and recently, *Paramecium caudatum* (GUTTMANN, 1958).

This paper deals with the growth-promoting effect of Kinetin on the early development of embryos of *Triturus pyrrhogaster* Boie. Embryos

at Stage 20 were raised in Kinetin solution (10.0‰; 1.0‰; and 0.1‰) at 20°C., and their growth with respect to organ formation was investigated.

It was found that Kinetin produced a significant effect in promoting early embryo development, while keeping the natural balance in organ formation. The most effective dose of Kinetin was 1 mg. per 1.000 cc. This effect of Kinetin was first observed seven days after treatment (significant at the 1% level), and lasted for at least one week, as shown in Fig. 1. The difference between Kinetin-treated and non-treated embryos on the seventh day was found to be four developmental stages, which corresponds to 120 hours at 20°C.

It is interesting to note that the time at which Kinetin becomes effective (the seventh day in this experiment) was always Stage 30, irrespective of the dosage and the developmental stage at which treatment was started.

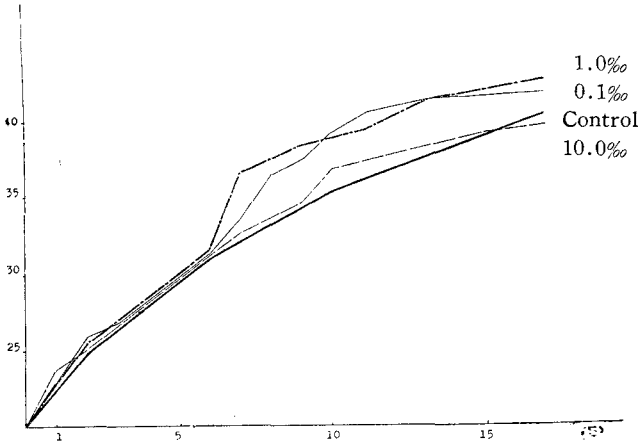


Fig. 1. Effect of Kinetin on the growth of embryos of *Triturus pyrrhogaster*. Abscissa: Days after treatment. Ordinate: Developmental stages.

79. *Mitosis-Promoting Activity of a Pregnancy Serum*

(By Yoshito OGAWA)

The presence of mitosis-promoting substances has been recognized in early developmental stages of animal embryos (GUSTAFSON, 1953; ANDRES. 1955; OGAWA, 1958).

The object of this note is to confirm the existence of a mitosis-stimulating substance in human pregnancy serum. The effect of pregnancy

serum on cell division was investigated using Yoshida sarcoma cells transplanted into the abdomens of Wistar rats. Normal female serum of the same age was prepared for a control.

The pregnancy serum (ten months) was injected (2.5 cc. per 100 g. body weight) into the abdomens of rats (two months old) 48 hours after the transplantation of the sarcoma, and the daily change in the frequency of metaphasic cells was observed. The results are given in Fig. 1.

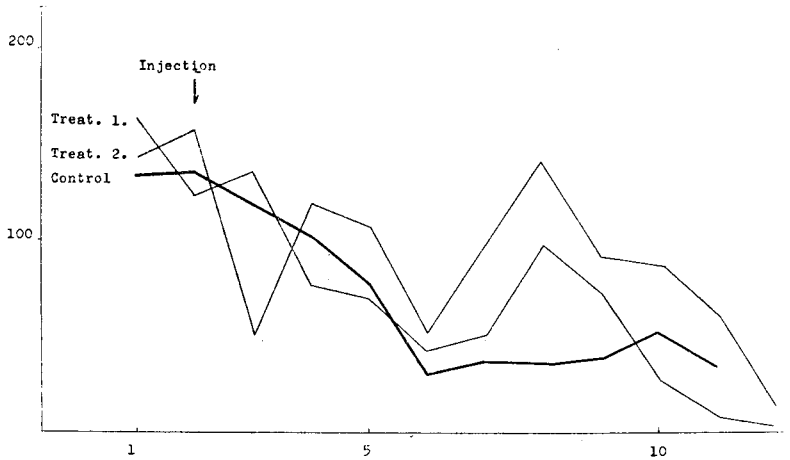


Fig. 1. Daily change of frequency of metaphasic cells after injecting a pregnancy serum into the abdomens of rats, 10,000 cells being observed per day.
Abscissa: Days after transplantation. Ordinate: Number of metaphasic cells observed.

A significant increase in the frequency of moitotic figures was found in the tumor cells six or seven days after the injection of the pregancy serum (both significant at 5% level). Thus the activity of this serum in promoting cell division was confirmed. This stimulus might be due to the action of some diffusible substance produced in the fetal tissue. The writer has formerly pointed out that such a diffusible cell division-promoting substance is produced in the tissues of a regenerating liver after partial hepatectomy.

In spite of their mitotic stimulants, somatic cells in pregnant rats were able to keep a normal functional balance. This might not be due to the action of any anti-mitotic substance, since the pregnancy serum showed no anti-mitotic activity in the above experiment. Whether the somatic cells reach a stage of division or not depends upon their physiological condition, as many workers have pointed out. Chemicals promoting cell

division, Kinetin and Na-glucuronate (OGAWA, 1958), show their activity only in cells already preconditioned to mitosis, such as in regenerating liver tissue after partial hepatectomy, fetal tissues or sarcomas.

80. *Synthesis of Contractile Proteins in the Early Development of the Embryo of Triturus pyrrhogaster.*

(By Yoshito OGAWA)

The determination of the presence of actin and myosin in skeletal muscles of the chick 72 and 96 hours after incubation, respectively, was reported by the present writer last year. In order to test whether the appearance of actin in the developing skeletal muscle before the formation of myosin is limited to chick embryos or is a general phenomenon in animals, the formation of skeletal muscle protein in the embryonic stages of *Triturus pyrrhogaster* Boie was investigated using a serological technique. The embryo of *Triturus pyrrhogaster* is suitable material for research on the biochemical mechanism of muscle protein formation, since developmental progress is relatively slow.

G-actin and myosin were isolated from the skeletal muscle tissue of good-sized specimens of *Triturus pyrrhogaster* by the method of Szent-Gyorgyi. Both proteins, at a concentration of 25 mg. per m*l*., were injected intravenously into rabbits at three-day intervals, until they reached a total of 100 mg. The rabbits were bled by heart puncture on the fifth day after the last injection, and the sera obtained were raised to their serological specificity by a resorption test with saline extracts of liver, spleen and skin tissue of *Triturus pyrrhogaster*. Titration with a homologous antigen was then carried out before the precipitin reaction with a saline extract of the embryo. The titer of both sera was 1 : 512. Possible failure due to an excess of antigen was prevented by using a constant volume of serum and progressively decreasing the amount of antigen.

It was found that actin first become detectable in the early embryo at stage 19 (about 132 hours at 20°C) and myosin at stage 24 (about 176 hours at 20°C), as shown in Table 1.

Therefore, it is probably generally true that in the skeletal muscle actin appears before the formation of myosin in the early stages of development in animals. On the other hand, in the heart muscle of early chick embryos, myosin appears before actin (EBERT, 1953; OGAWA, 1958). With a view to this finding, the morphological and physiological differences of heart and skeletal muscle should be examined.

The correlation between the formation of actin and myosin in early developmental stages is now being studied.

Table 1. Determination of developing skeletal muscle proteins in early embryos of *Triturus pyrrhogaster*, Boie

Developmental stages	No. of Embryos Extracted	No. of Reaction Series	Lowest antigen concentration giving positive results*	
			Actin	Myosin
18	12	2	–	–
19	12	2	1 : 20	–
20	12	2	1 : 40	–
23	12	2	< 1 : 160	–
24	12	2		1 : 160
25	12	2		< 1 : 160
26	10	2		< 1 : 160
Control (Non-fertilized)	24	2	–	–

* Antigen concentrations used ranged from 1 : 10 to 1 : 160 in embryo weight.

81. *Effect of Na-glucuronate on Liver Regeneration after Partial Hepatectomy*

(By Yoshito OGAWA)

The writer found a promoting effect of Na-glucuronate on animal cell division (Exp. Cell Res. 15 : 415, 1958). The present report is the result of a study to observe how the Na-glucuronate affects the regeneration of liver tissue after partial hepatectomy. The relation between tissue weight and mitotic activity in the regenerating liver after this operation was investigated using the Wistar strain of rats (two months old). The frequency of metaphasic cells was determined 48 hours after the operation in 2,000 cells per liver lobe.

The mitotic activity of regenerating liver cells after partial hepatectomy depends upon the extent of surgical infringement. When more than 50% (by weight) of the whole liver was removed by operation, the remaining tissue greatly increased in weight after 48 hours, but no mitotic cells were seen. On the other hand, if the amount of liver removed was less than 50%, the increase in weight of the remaining part was relatively small but the mitotic activity was quite high. Only in the latter cases was the postoperative prognosis good (OGAWA, Y, et al.: Med. and Biol. 45 : 12, 1957).

When Na-glucuronate was injected (the most effective dose is 750 mg per 100 g body weight), the increase in the weight of the remaining liver tissue was relatively small but a remarkable increase in the mitotic index was found in the regenerating tissue, even if more than 70% (by weight) of the whole liver was removed by the operation (Table 1).

It is concluded that Na-glucuronate may help the recovery of liver function and allow a good postoperative prognosis after extensive hepatectomy which without Na-glucuronate would normally be impossible.

Table 1. Regeneration of liver tissue after partial hepatectomy

	No.	Sex.	Body weight (g)	Weight removed tissue in %	Increase in weight of regenerating liver tissue (%)*	Mitotic index
Treated	31	♂	147.1	37.6	13.9	72.5
	32	♀	159.0	45.9	25.0	60.0
	33	♀	106.5	52.0	33.4	35.0
	34	♀	125.0	62.1	45.4	18.5
	35	♂	120.2	67.8	94.5	21.5
	36	♂	98.0	71.7	84.6	16.7
	37	♀	132.5	79.0	146.2	0.0
	38	♀	169.0	88.6	211.6	0.0
Control	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

* Percent of the remaining liver tissue weight.

L. TECHNICAL NOTE

82. *Simultaneous Measurement of Thermal Neutron Fluxes and γ-Ray Dose in Thermal Column by Silver-Activated Phosphate Glass*

(By Sohei KONDO)

Thermal neutron irradiation of biological materials is usually carried out in thermal columns of reactors which are always more or less contaminated with γ rays. Therefore, in such experiments it is absolutely necessary to know the γ ray dose in the presence of an intense thermal neutron flux. The ionization chamber method so far used for this dosimetry is very delicate and difficult. The method proposed here is a very simple and reasonably accurate way to measure simultaneously the γ ray dose and the thermal neutrons. It was discovered by SCHULMAN et al.[1] that silver-activated phosphate glass exposed to X-rays acquires the ability to fluoresce when excited by ultra-violet rays and that the amount of fluorescence is linearly proportional to the exposure dose. We have found that the response of this glass to thermal neutrons increases linearly with increasing dose and that the fluorescence is almost all caused by β rays emitted from activated silver nuclei.

Experimental: The method for measuring the induced fluorescence is essentially that of SCHULMAN and ETZEL[2] except for a modification of the holder to fit the glass plates used in this experiment. Glass plates of $AgPO_3$ added to a base phosphate glass ($Al(PO_3)_3$ 50%, $Ba(PO_3)_2$, 25%, KPO_3 25%) in ratios of 8:100, 10:100 and 12:100 by weight, were provided through the courtesy of Mr. G. E. BLAIR of the Bausch and Lomb Optical Co., Rochester, N. Y. Piles of 4 glass plates of each composition were exposed for 24 hrs to thermal neutrons rather highly contaminated with γ rays at the bottom of the water tank above the thermal column of the Oak Ridge Graphite Reactor.[3] The ratio of the fluorescent output, $I(x)$, of a glass plate exposed in the thermal column to that, $I_0(x)$, of the same glass plate exposed to ^{60}Co γ rays of 259 r is plotted in Fig. 1 against the weight percentage of $AgPO_3$, x. The linear relationship between $I(x)/I_0(x)$ and x, and the application to thermal column dosimetry are explained in the following paragraph.

Theory: The fluorescent output per rad of silver-activated phosphate glass exposed to thermal neutrons is equal to that of γ rayed glass. Thus taking wD_n as the energy imparted by thermal neutrons to a glass plate of weight w and wD_γ as the energy released to it by γ rays in the

thermal column, we may assume the following equation for fluorescent output $I(x)$:

$$I(x)=f(x)w(D_\gamma+D_n), \tag{1}$$

where f is a proportionality constant which parametrically depends on the concentration x of $AgPO_3$ in the glass.[1] From Equation (1) we have the equation for fluorescent output $I_0(x)$ of the glass plate calibrated by ^{60}Co γ rays of absorbed dose wD_0

$$I_0(x)=f(x)wD_0. \tag{2}$$

From Equations (1) and (2) we obtain

$$I(x)/I_0(x)=D_\gamma/D_0+D_n/D_0. \tag{3}$$

The energy D_n can be given by

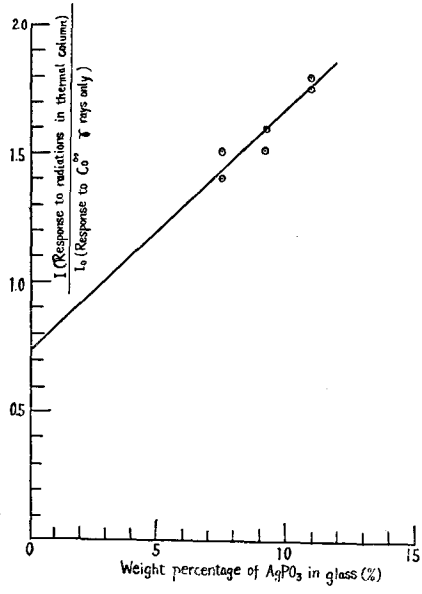
$$D_n=\Phi\sum_{i,j}n_i\sigma_{ij}E_{ij}=\Phi[A(1-x)+Bx] \tag{4}$$

where Φ is the number of thermal neutrons per cm^2, n_i the number of i nuclei per gram, σ_{ij} the cross section for any interaction (j) involving nucleus j, E_{ij} the total energy imparted as a result of this interaction to secondary radiations, and x the fraction by weight of $AgPO_3$ in the glass.

Combining (4) with (3), we find

$$I(x)/I_0(x)=ax+b. \tag{4}$$

This explains the linear relationship shown in Fig. 1. For the calibra-



tion exposure dose γ_0 r of ^{60}Co γ rays, we have the following numerical equations:

$$\Phi = 2.41 \cdot 10^8 \gamma_0 a \ (n_{th} \cdot cm^{-2}) \ ; \ \gamma = \gamma_0 (b - 6.05 \cdot 10^{-3} a) \ (r) \qquad (5)$$

Thus, from the gradient a, intercept b and calibration dose γ_0 r, we can easily obtain the thermal neutron flux Φ and the gamma contamination in the thermal column, γ.

From (5) and Fig. 1 we find

$$\Phi/t = 6.8 \cdot 10^6 \ n_{th} \cdot cm^{-2} \cdot sec^{-1} \ ; \quad \gamma/t = 7.4 \ r/hr. \qquad (6)$$

Compared with the value $\Phi/t = 7.1 \cdot 10^6 \ n_{th} \cdot cm^{-2} \cdot sec^{-1}$ obtained from the exposed gold foils and $\gamma/t = 8.1$ r/hr obtained by the ionization chamber method[3], the agreement is good. The accuracy of this method can easily be improved by using more samples with wider differences in their silver concentration.

1) J. H. Schulman et al., *J. Appl. Phys.* **22**, 1479 (1951)
2) J. H. Schulman, H. W. Etzel, *Science* **118**, 184 (1953)
3) R. O. Bagley, et al., *EPS-X-269* (unpublished) ORNL (1956)

83. *The Mechanism of Serological Reactions Examined by using Steroids*

(By Yoshito OGAWA)

A serological reaction obtained with cholesterol, the Wassermann Reaction and the cephalin cholesterol flocculation test, is a useful clinical tool, although its mechanism is not well known.

The present study was made in order to determine the role of cholesterol in the cephalin cholesterol flocculation test using cholesterol analogues. Cholesterol analogues used in this experiment were cholesteryl-acetate, cholestin-5, 7-ketocholesteryl-acetate, 3·3-dichrol-cholesterol, and cholesten-5·diol-3·4.

In the case of cholesteryl-acetate, cholesten-5, 7-keto-cholesteryl-acetate, and 3·3-dichrol-cholesterol, negative results were obtained with all of the sera tested, but when cholesten-5·diol-3·4 was employed, reactions were positive with all of the sera except that of Kala-Azar, as shown in Table 1.

It may be argued that a chemical constitution of the steroid, the OH-radical of C_3, is an important factor in determining the results of this reaction. It was recognized that the protein which reacts with cholesterol remained in the serum of hepatoma patients after the resorption of the protein which reacted with cholesten-5·diol-3·4. Furthermore, examina-

tion of the serum of the same patients with cholesten-5·diol-3·4 after the exclusion of the protein which reacts with cholesterol, gave positive results.

These results may indicate that the protein which reacts with the OH-radical of C_3 is different from that which reacts with the OH-radical of C_4 of the steroids. The results of a serological reaction using steroids thus depend on the position of the hydroxyl radical of 1:2-cyclopentano-perhydrophenanthrein.

Table 1. Results of cephalin cholesterol floculation test using cholesterol analogues

(at 24 hrs.)

No.	Sex	Age	Disease	Cholesterol (original method)	Cholesterol analogues I	II	III	IV	V
14	♂	46	Hepatoma	+++	−	−	−	−	+++
3	♀	40	Necropneumonia	+++	−	−	−	−	+++
4	♀	36	Gastroscirrhus	++	−	−	−	−	+++
12	♂	32	Pulmonary tuberculosis	++	−	−	−	−	+++
9	♂	29	Pulmonary tuberculosis	++	−	−	−	−	+++
5	♀	38	Cancer of the rectum	+	−	−	−	−	+++
7	♂	29	Pyothorax (tuberculosis)	+	−	−	−	−	+++
15	♂	36	Cystic kidney	±	−	−	−	−	+++
18	♂	58	Cholecystitis	±	−	−	−	−	+++
8	♂	20	Pulmonary tuberculosis	±	−	−	−	··	+++
1	♂	44	Pulmonary tuberculosis	−	−	−	−	−	+++
2	♀	27	Pulmonary tuberculosis	−	−	−	−	−	+++
10	♂	34	Pyothorax	−	−	−	−	−	+++
16	♂	34	Purulent peritonitis	−	−	−	−	−	+++
6	♀	57	Cholecystitis	−	−	−	−	−	+++
11	♂	19	Trauma of urethra	−	−	−	−	−	+++
17	♂	20	Fracture of femur	−	−	−	−	−	+++
19	♂	41	Kala-Azar	+++	−	−	−	−	−
101	♂	22	Normal	−	−	−	−	−	+++
102	♂	20	Normal	−	−	−	−	−	+++
103	♀	35	Normal	−	−	−	−	−	+++
104	♀	19	Normal	−	−	−	−	−	+++
105	♀	42	Normal	−	−	−	−	−	+++
Control				−	−	−	−	−	−

I. Cholesteryl-acetate. II. Cholesten-5 III. 7-Keto-cholesteryl-acetate.
IV. 3·3-Dichrol-cholesterol. V. Cholesten-5·diol-3·4.

84. *A Simple Method for the Determination of Chromosome Number in Rice**

(By Mitsuya NEZU)

When the usual squash method is applied to the root tips of rice, good staining is very difficult. The writer obtained good results when the root tips were fixed in glacial acetic acid and then squashed. For staining, FEULGEN's reaction was used.

Using this method, the writer determined the somatic chromosome numbers of 2 varieties of Japanese cultivated rice and 10 strains of 8 wild species. Among them the chromosome numbers of the following 3 species were not reported before: *Oryza ridleyi* (W0001) 2n=48, *O. australiensis* (W0008) 2n=24, *O. alta*? (W0017) 2n=48.

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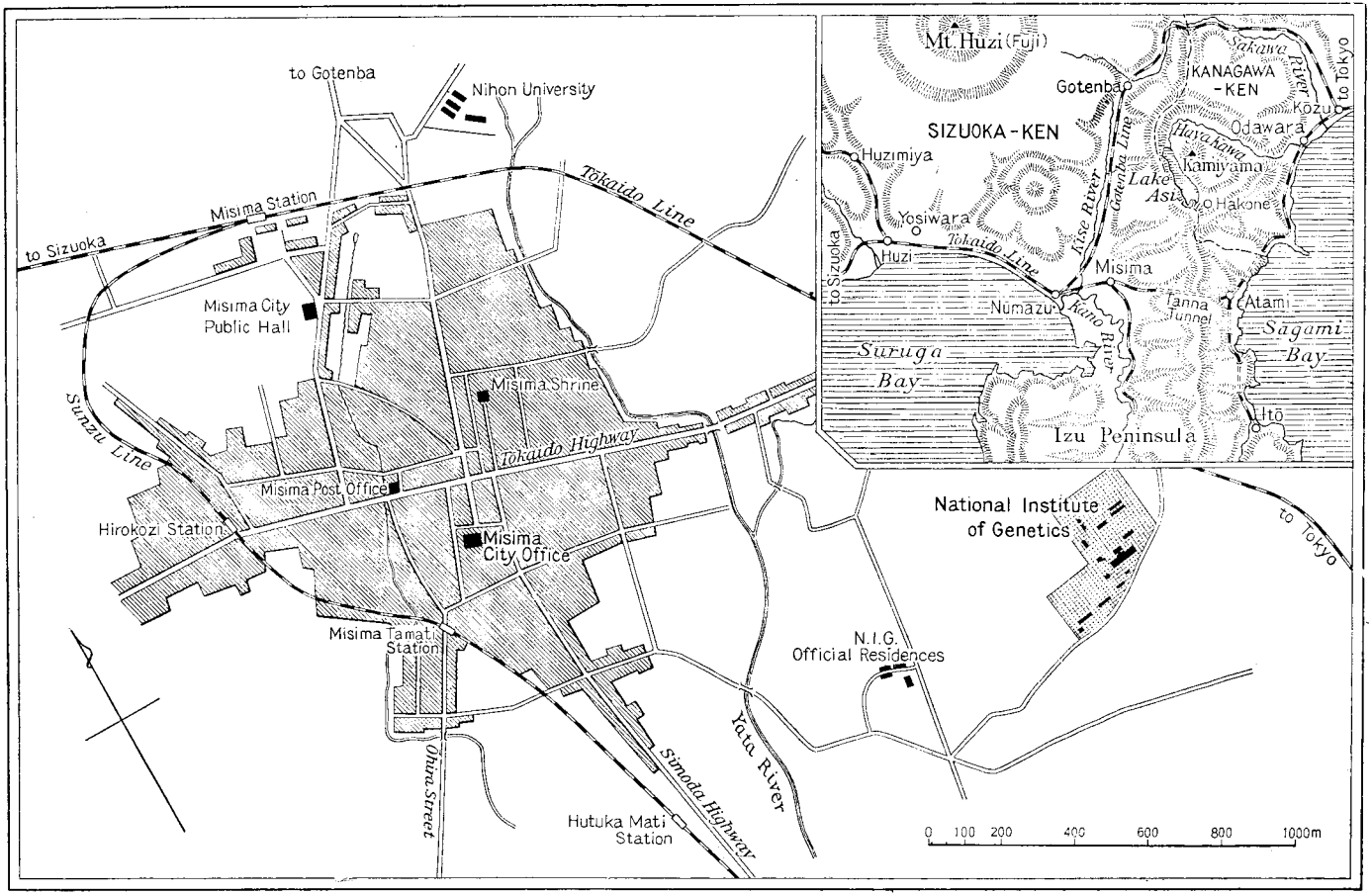
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