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No. 10 **1959**

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1960

Annual Report
of the
National Institute of Genetics

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GENERAL STATEMENT

The year of 1959 was very important for our Institute because 10 years have passed since its establishment. On the 1st of June we have celebrated the 10th anniversary of its foundation. As the weather was fortunately fine, 281 friends of the institute who contributed directly or indirectory to its establishment and development attended the celebration, which closed on a cheerful and hopeful note. In commemoration of the Day, public lectures in cluding scientific movies were given in Tokyo and Misima.

The number of research reports assembled in this annual report is 145, which exceeds the figure of last year by 26.

In order to collect rice and related species, five members participating in the studies on the origin of cultivated rice traveled to China, Africa, India or Burma, where they collected many seeds and specimens of wild as well as cultivated rice. So far we could obtain 21 species. Five are still missing from our collection but we are confident that they will be added in near future.

Dr. T. Tateoka, Dr. S. Sakaguchi and Mr. S. Sakamoto are continuing their studies at the University of Montreal, Canada, Yale University and Minnesota University, U.S.A., respectively. Dr. Kondo came back after a 14 month study in the U.S.A.

Our institute had a great number of visitors including 28 from abroad.

X-ray laboratory, special silkworm house and a trasferable birdproof net-cage were newly built.

In addition, a neutron radiation apparatus, a ^{137}Cs γ-ray radiation apparatus, an inverted biological microscope, a micro-spectrophotometer and a low temperature centrifuge were obtained.

Dr. M. Kimura received the prize of the Japanese Genetic Society for his contributions to the knowledge of the genetic population load and its significance in evolution. Dr. H. Kihara was honored by a prize of Leopoldina, the Germany Academy of Biologists, on the occasion of the 100th Celebration of Charles Darwin's publication "On the origin of species by means of natural selection".

ABSTRACTS OF DIARY FOR 1959

Jan. 8. Committee meeting of Animal Genetics group.
Jan. 14. Meeting of Tobacco Research workers.
Jan. 15. Meeting of all Japan poultry Breeding Association.

Jan. 23. 72nd meeting of Misima Geneticists' Club.
March 11. 23rd Biological Symposium.
March 19. 24th Biological Symposium.
April 17. 73rd meeting of Mishima Geneticists' Club.
June 1. The 10th Commemoration day of National Institute of Genetics.
June 6. Memorial Lecture and movies (in Tokyo)
June 27. 75th meeting of Misima Geneticists' Club.
June 27. Meeting of all Japan Poultry Breeding Association.
June 29. 25th Biological Symposium.
July 17. 76th meeting of Misima Geneticists' Club.
July 22–25. 3rd series of Summer Seminar on Genetics.
Aug. 10. 26th Biological Symposium.
Aug. 24–26. Seminar on radio-isotopes.
Sep. 25. 77th meeting of Misima Geneticists' Club.
Sep. 26. 27th Biological Symposium.
Oct. 18. 28th Biological Symposium.
Oct. 23. 78th meeting of Misima Geneticists' Club.
Dec. 18. 79th meeting of Misima Geneticists' Club.

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

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Misima Branch of Hatano Tobacco Experiment Station

Masao TANAKA, Head Assistant 5

All Japan Poultry Breeding Association

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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 1959

Department of Morphological Genetics

Genetics of silkworm (TAZIMA and INAGAKI)
Studies on the food preference in silkworm (TAZIMA)
Radiation mutagenesis in silkworm (TAZIMA and ONIMARU)

Genetic effects of chronic γ-irradiation upon silkworm (TAZIMA and ONIMARU)
Theoretical studies of population genetics (KIMURA)

Department of Cytogenetics

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

Department of Physiological Genetics

Genetical studies on insecticide-resistance of Drosophila (OSHIMA)
Physiological studies on eye-pigment formation of Drosophila (TAIRA and OSHIMA)
Population genetics of deleterious genes in natural populations of Drosophia (OSHIMA)
Studies on the origin of wheat (KIHARA)
Studies on substitution of nucleus in wheat and related species (KIHARA)
Right- and left-handedness in plants (KIHARA and LILIENFELD)
Studies on stoneless pomegranates (KIHARA)

Department of Biochemical Genetics

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Embryological and biochemical studies of silkworm (TSUJITA 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)
Studies on the fine structure of gene (TSUJITA)
Genetics of virus (TSUJITA)

Department of Applied Genetics

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Quantitative genetics in Drosophila (YAMADA)
Theoretical studies of plant breeding (SAKAI)
Studies on competition in plants (SAKAI and IYAMA)
Population genetic studies of 'Red-Rice' growing among upland rice (IYAMA)
Genetic studies on 'cherry-red leaf' in tobacco plants (SAKAI and IYAMA)
Genetic studies on the migrating activity in Drosophila (SAKAI, IYAMA and NARISE)
Polyploidy and sterility in fruit plants (FURUSATO and MIYAZAWA)
Genetical studies on cytoplasmic inheritance in plant (SAKAI and IYAMA)
Genetic studies of some physiological and agronomic characters in rice (OKA)

Department of Induced Mutation

Studies on radiation-protection in animals (SUGAHARA)
Radiation genetics of mice (SUGAHARA and TSUTIKAWA)
Relation between the quality of radiations and mutations (MATSUMURA and KONDO)
Radiation genetics of wheat (MATSUMURA, FUJII and NEZU)
Radiation genetics and its practical application (MATSUMURA and FUJII)
Radiosensitivity in plants (FUJII)
Radiation dosimetry (KONDO)
Triploidy breeding of sugar beets (MATSUMURA)

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. Strains so far collected are more than 3,000, in which 20 different species are included.
b. Collection-tours were made by Dr. H. Kihara to Silkkim and Assam, India, by Mr. K. KATSUYA to Burma, and by Mr. K. FURUSATO to Africa, in this year.

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

a. Comparison of radio-sensitivity among *Oryza* species (T. FUJII)
b. Genome-analysis of *Oryza* species (M. NEZU)

c. Susceptibility of wild and cultivated rice strains to blast fungus (K. KATSUYA)
d. Surface structure of lemma and palea in *Oryza* species (H. KIHARA and T. KATAYAMA)
e. Investigation of photoperiodic responses in *Oryza* species (T. KATAYAMA)

Section 3. Population-genetics in wild and cultivated rice (K. I. SAKAI)
a. Estimation of genetic variability among and within populations of wild rice (K. I. SAKAI, S. IYAMA and T. NARISE)
b. Estimation of the percentage of out-crossing by a biometrical method (K. I. SAKAI, S. IYAMA and T. NARISE)
c. Competition between wild and cultivated rice strains (K. I. SAKAI and T. NARISE)
d. Variation studies of blast disease resistance in wild rice populations (T. NARISE and K. I. SAKAI)

Section 4. Genetic studies in wild and cultivated rice (H. I. OKA)
a. Statistical-systematic studies of *Oryza* species (H. MORISHIMA and H. I. OKA)
b. Survey of variations between *O. perennis* and *O. sativa* f. *spontanea* (H. MORISHIMA, W. T. CHANG and H. I. OKA)
c. Crossing-experiments for the sterility of hybrids between wild and cultivated rice strains (K. HINATA and H. I. OKA)
d. Investigation of floating habit in wild *Oryza* species (H. MORISHIMA K. HINATA and H. I. OKA)
e. Introgressive hybridization between wild and cultivated populations of rice (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. Comparative observation of chromosomes in haploid plants of *Oryza* species (C. H. HU)
c. Embryological studies in *Oryza* species (Y. DOIDA)

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

Section 1. Radiation mutagenesis in silkworm (Y. TAZIMA)
1. Nature of radiation induced mutations detected by specific loci method in silkworm (Y. TAZIMA and K. ONIMARU).
2. X-ray induced dominant lethals from irradiation of different stages of oögenesis in silkworm (Y. TAZIMA and K. ONIMARU).
3. Some observations relevant to the possible mechanism of radiation induced sterility in male silkworm (Y. TAZIMA).
4. Cytological studies of radiation-induced sterility in the male silkworm (T. SADO).

5. Recovery of irradiated spermatogonia of the silkworm and the stage limit for the production of functional sperms (T. SADO).

Section 2. Mutation in mammals (T. SUGAHARA).

1. Studies on mutation rate by chronic irradiation of mice (T. SUGAHARA, K. TUTIKAWA and Y. TAKEDA).
2. Dose distribution inside mice exposed to X-rays (S. KONDO)

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

1. Effects of X-rays on the rate of mitosis (T. H. YOSIDA and T. TAKAHASHI).
2. Relation between chromosome breakage at metaphase and fragment formation at anaphase after X-irradiation (T. H. YOSIDA and T. TAKAHASHI).
3. Nature of chromosome bridges after X-irradiation (T. H. YOSIDA and T. TAKAHASHI).
4. Relation between micronuclei and chromosome fragments after X-irradiation (T. H. YOSIDA and T. TAKAHASHI).

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

1. Studies on the mechanism inducing mutations after irradiation (S. NAKAI).
2. Effects of irradiated RNA components on mutation (T. IINO).
3. Relation of post-irradiation treatment to radiation-induced mutation frequency in yeast (S. NAWA and K. SAITO).

Section 5. Mutations in populations (C. OSHIMA).

1. 1. Heterozygous effects of induced lethals on pre-adult viability (C. OSHIMA and O. KITAGAWA).
2. Doubling dose of polygenic characters (Y. YAMADA and O. KITAGAWA)
3. The effect of X-ray irradiation on selection response (O. KITAGAWA)

FOREIGN VISITORS IN 1959

Feb. 11. Prof. F. J. RYAN (Columbia University, U.S.A.)
March 10. Associate Prof. B. S. STRAUSS (Syracuse University, U.S.A.)
March 24. Dr. J. J. MCKELVEY Jr. (The Rockefeller Foundation)
March 29. Dr. I. S. BALKE (Minister of Atomic Energy, West Germany)
Dr. H. SAVES
June 4. Dr. Chung Fu CHENG Dr. Keh Min LIN
Dr. Hsuin shwen CHANG Dr. Cheng I LIN
Dr. D. M. QUAN Dr. T. L. COPLEY
June 15. Dr. Miss D. PARKER (Librarian: the Rockefeller Foundation)
June 26. Mr. A. K. M. HUSSEIN (Pakistan)
June 29. Dr. H. H. SMITH (IAEA, Vienna)
Sep. 3. Prof. H. KAMENOTO, Prof. R. A. HAMILTON (University of Hawaii, U.S.A.)
Sep. 19. Prof. P. N. MEHRA (Botany Dept., Punjab University, India)
Dr. A. J. MACLEED (Federal Dept. of Veterinary Research vom. Nigeria)
Sep. 22. Mr. I. P. GUNAWARDENA (Dept., of Agriculture, Ceylon)
Dr. G. P. ARGIKAR (Bombay Agricultural Department, India)
Nov. 10. Dr. K. HOFMANN (Univ. Kicl. Germany)
Nov. 17. Prof. A. B. BURDICK (Purdue University, U.S.A.)
Dr. H. SLATIS (Argonne National Laboratory, U.S.A.)

RESEARCHES CARRIED OUT IN 1959

A. GENETICS, BIOCHEMISTRY AND CYTOLOGY OF INSECTS

1. *Studies on the E and U allelic genes in the silkworm, with special reference to the attachment form between the VIth and XIVth chromosome*

(By Mitsuo TSUJITA)

It is known that under natural condition one end of chromosome VIth and one end of chromosome XIVth are occasionally attached to one another. On the former chromosome the *E* complex locus (*E* region) and on the latter chromosome the *U* complex locus (*U* region) are located. These two complex loci effect somewhat similar phenotypes. It has been observed that in the spermatocytes of the hybrid between domesticated (Bombyx mori) and wild silkworm (Bombyx mandarina) a trivalent made up of two chromosomes of the former and one chromosome of the latter appears.

Table 1. Segregation from the Cross $Nl^2E^{Nc}/++\times+$.

	+	E^{Nc}	Nl^2	Nl^2E^{Nc}	Total
Total of 10 batches	1720	0	0	1573	3293

Table 2. Segregation from the cross $+\times Nl^2E^{Nc}/++$.

No.	+	Nl^2	E^{Nc}	Nl^2E^{Nc}	Total	C.O.V.
1	132	130	97	109	468	48.5
2	98	47	78	78	301	41.5
3	87	58	67	67	279	44.8
4	113	93	91	94	391	47.1
5	101	70	82	87	340	44.7
6	117	91	100	98	406	47.0
7	128	103	88	114	433	44.1
8	98	53	63	92	306	37.9
9	47	44	53	56	200	48.5
10	49	45	50	52	196	48.5
Total (mean)	970	734	769	847	3320	45.3

In the present experiment the following subjects were studied. i) Attachment form of the two chromosomes. ii) Eventual relationship between *E* and *U* regions. iii) Relationship between the attached chromosomes and the trivalent which is found in the hybrid between the domesticated and the wild silkworm.

From the cross between a female of the strain with attached chromosomes $E^{Nc}Nl^2$ and a normal male, only two types, Nl^2E^{Nc} and +, segregated, and from the reciprocal cross four types, Nl^2, $E^{Nc}Nl^2$, E^{Nc}, and + segregated. (Tables 1 and 2)

As shown in the above table, the apparent crossing-over value is 45.4. It seems, however, that this is not the real value for the following reason: In a small number of spermatocytes of the male from the cross $+ \times Nl^2E^{Nc}/++$, 27 chromosomes can be observed, one of them consisting of two attached chromosomes, but in most of them 28 chromosomes are counted, as shown in the following table.

Table 3. Chromosome numbers in the spermatocytes of the male from the cross $+ \times Nl^2E^{Nc}/++$.

No. individuals	No. chromosome							
	1st maturation division				2nd muturation division			
	26	27	28	29	26	27	28	29
1	1	2	23	1		1	15	
2		6	21			2	30	1
3		6	25		1	4	30	
4		7	30	1		2	25	
5	1	9	30	1		5	30	
Total	2	30	129	3	1	14	130	1
	164				146			

As shown in the above table, among 164 spermatocytes at the first maturation division, 30 had 27 chromosomes. On the contrary, among 146 spermatocytes at the second maturation division, 14 had 27 chromosomes. Thus, the number of the spermatocytes having the attached chromosomes is smaller in the former than in the latter division. It seems from this observation that the attachment of the VIth to the XIVth chromosome in the male germ cells is unstable. The number of male germ cells in which both chromosomes be- have as one up to the final maturation division amounts to about 10% of the second spermatocytes, while in 90% they maneuver independently, so far as our observation goes.

Thus, the crossing-over value shown in Table 2 cannot be taken as the linkage distance between E^{Nc} and Nl^2, because there are two modes of behavior of the VIth and XIVth chromosomes at spermatocyte division; in one of them the attached VIth and XIVth chromosomes behave as one chromosome, while in the other they do not attach and each behaves as an independent chromosome.

Further, the ratio of the two types, Nl^2 and E^{Nc}, to the total number of four types, +, Nl^2, E^{Nc}, and $E^{Nc}Nl^2$ from the cross $+\times E^{Nc}Nl^2/++$ should be different according to the form of the attachment, as shown in Fig. 1.

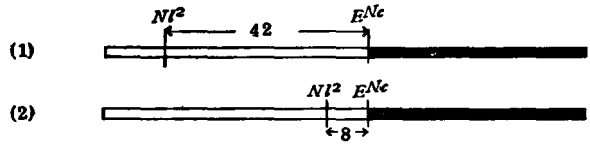


Fig. 1.

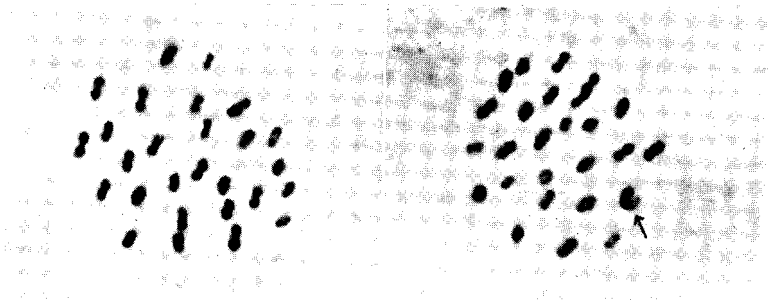
Based on these to forms of the attachment, crossing-over value was calculated.

In the case of the attachment form (1) $50\times0.9+4.2\times0.1=49.2$

In the case of the attachment form (2) $50\times0.9+8\times0.1=45.8$

The crossing-over value shown in Table 2 is 45.3. Judging from this value there is much stronger evidence for form (2) than for form (1).

Moreover, in most spermatocytes of the hybrid between the domesticated



Photograph 1. Chromosomes is the first maturation division of the hybrid between the domesticated (the strain having the attached chromosome) and the wild silkworm. 27 chromosomes can be observed. (×1600)

Photograph 2. The same material as in photograph 1. 26 chromosomes can be observed. An arrow indicates the attached chromosomes. (×1600)

(the strain having the attached chromosomes) and the wild silkworm, 27 chromosomes were observed, but in a small number of spermatocytes, 26 chromosomes, one of which consists of two attached chromosomes, are found (Photos 1 and 2).

This indicates that there is no relationship between the attached chromosomes and the trivalent appearing in the spermatocytes of the hybrid between the domesticated and the wild silkworm.

The fact that the VIth and XIVth chromosomes attach suggests an intimate relationship between them. Furthermore, as the present experimental results show, attachment form (2) is the more probable, placing the *U* and *E* complex loci relatively near to each other. Therefore, some important relationship between the two regions may be assumed.

2. *Genetic and biochemical studies on the new white-egg mutant in the silkworm*

(By Mitsuo TSUJITA)

The new mutant strain of the silkworm, w^{ox}, which exhibits a conspicuously transparent larval hypodermis and white eggs was obtained by X-ray treatment in 1954.

The gene w^{ox} belongs to the Xth linkage group, and crossing experiments show it to be allelic to w^{ol} on chromosome Xth.

Amounts of uric acid and isoxanthopterin produced in the hypodermis of larvae homozygous for w^{ox}, w^{3}, w^{ol} and of those of their hybrids were measured by a paperchromatographic method. In order to ascertain the amount of lost uric acid and isoxanthopterin in the procedure of measurement, definite amounts of the two substances were absorbed in a filter

Table 1. Isoxanthopterin (μg/g in dry matter weight, after paperchromatography)

Strain \ Exp. No.	I	II	III	Mean
+(Japanese 501 ×Chinese 501)	975	938	938	950.33
w^{ol}	188	150	163	167.00
w^{3}	375	375	370	373.33
w^{ox}	trace	trace	trace	trace
$w^{3}\times w^{ox}$	263	225	338	275.33
$w^{ol}\times w^{ox}$	75	113	113	100.33
$w^{1}\times w^{ox}$	1010	938	1013	987.00

paper. The amounts extracted from the spot appearing on the paper were measured. The remainder, initial amount−extracted amount, may be considered as lost in the procedures. The results obtained with the larval hypodermis were corrected accordingly.

The corrected amounts of uric acid and isoxanthopterin are given in the Table 1 and 2.

Table 2. Uric acid (mg/g. in dry matter weight, after paperchromatography)

Strain \ Exp. No.	I	II	III	Mean
(Japanese 501 ×Chinese 501)	88.50	86.94	85.50	86.98
w^{ol}	15.75	13.50	18.70	15.98
w^{3}	81.00	63.75	69.00	71.25
w^{ox}	trace	trace	trace	trace
$w^{3}\times w^{ox}$	21.75	26.25	22.50	23.50
$w^{ol}\times w^{ox}$	9.75	6.00	8.25	8.00
$w^{1}\times w^{ox}$	87.00	73.50	85.50	82.00

Tables 4 and 5 show that the amounts of isoxanthopterin and uric acid in the hypodermis tissue of larvae homozygous for w^{ox} are much smaller than in the larvae homozygous for w^{ol} or w^{3}. In the case of uric acid in comparison with isoxanthopterin, a large part is apt to be lost in the experimental procedure, so it is a matter of prime concern that the accurate amount of uric acid cannot be ascertained by paperchromatography. Therefore, in order to obtain more exact results, a method using uricase was applied, and the experimental data are given in Table 3.

Table 3. Uric acid (μg/mg. in dry matter weight, after uricase method).

Exp. No. \ Genotype	w^{ox}	*od*	w^{ol}	*oa*	+
1	0.25	2.5	24.3	25.6	108.2
2	0.23	2.6	23.1	26.1	106.5
Mean	0.24	2.6	23.7	25.9	107.4

The table shows that the amount of uric acid in the hypodermal tissue of the w^{ox} homogeneous larvae is only 1/100 of that found in the w^{ol} larvae and 1/10 of that found in the *od* larvae, which was hitherto assumed to have the smallest amount among the known mutant strains with transparent larval hypodermis.

Thus, it is characteristic of the w^{ox} mutant its amount of uric acid and isoxanthopterin are much smaller than in any other known mutant strain with transparent larval hypodermis.

It was found from in vitro experimental results that the activity of pterin dehydrogenase in the larval hypodermis of the mutant is somewhat stronger than in the normals. Therefore, it seems that there is nothing abnormal with the enzyme itself. In order to clarify the action of w^{ox}, a small amount of 2-amino-4 hydroxypteridine (AHP) was injected into the body-cavity of larvae homozygous for w^{ox} and the amount of isoxanthopterin produced in the hypodermis was periodically measured after 1, 2, 3, 4, 14, 20, and 24 hrs. The experimental results are shown in the following table:

Table 4. Change of the amount of isoxanthopterin in the hypodermal tissue after injection of AHP into the larval body cavity.

Genotype / Duration after injection	w^{ox}	w^{ol}
Control	19*	40*
1 hr.	28	49
2 hrs.	29	73
3	30	69
4	31	69
14	16	65
20		64
24	18	45

* Relative amounts

As the table shows, the amount of isoxanthopterin in the hypodermis of the larvae homozygous for w^{ox} increases during the first 4 hours after the injection of AHP, and then decreases rapidly, showing the initial amount 14 hours after the injection. On the contrary, the amount of isoxanthopterin in the hypodermis of the larvae homozygous for w^{ol} increases in the course of the first 3 hours after the injection and then decreases very slowly. The normal amount of isoxanthopterin appears 24 hours after the injection. Thus, it takes more time for w^{ol} than for w^{ox} larvae to regain the initial amount.

It may be assumed that isoxanthopterin and uric acid newly produced in the hypodermal tissue penetrates into the body cavity passing through cell membranes and that the permeability of the substances is controlled by w^{ox} or w^{ol}.

3. *X-Ray induced Nl-type mutants of the silkworm*

(By Mitsuo TSUJITA)

Female moths homozygous for *HKp* whose pupae were treated with 8000γ X-rays were mated with normal male moths. Many *Nl*-type mutants (non-lunar silkworm larvae) were obtained from these hybrids. The results of the experiments which were carried out during the years 1954–1958 are summarized in the following table.

Table 1. *Nl*-type mutants obtained from 1954–1958.

Year	Rearing season	No. of examined batches	Exceptional types				Total	*HKp*/++	Total
			Double crescent	Nl^1 type	Nl^2 type	Other types			
1954	Summer	167	13	2	3	15	33	7346	7379
1955	Spring	230	16	20	3	26	65	29568	29633
1955	Summer	31	2	1	0	1	4	204	208
1956	Summer	150	20	23	3	35	81	25657	25738
1958	Summer	100	15	10	2	58	85	15682	15767

Since examination for exceptional types was done in the early larval stage, it seems likely that a part of the *Nl*-type mutants were not detected. Therefore, the total number of the *Nl*-type mutants may be actually larger than the number shown in the above table.

There are two kinds of *Nl*-type mutants. Larvae with the genotype $Nl^1/+$ show almost the same characteristics as those with the genotype *Nl*/+ in that they both lack the lunar patterns on the 5th segment and the star-shaped markings on the 8th segment, and in the presence of modifiers, rudimentary patterns appear on both segments. However, larvea with the genotype $Nl^2/+$ are different from those with the genotype *Nl*/+ or $Nl^1/+$; they completely lack lunar markings, but in certain genetic background, star-shaped markings appear. Furthermore, they lack the caudal horn on the 11th segment.

Two Nl^1 type mutants obtained in the spring of 1955 were crossed with the *odk* strain and crossing-over values were calculated. The results were 4.6 and 5.2, respectively, while the crossing-over value between Nl^2 and *odk* was 7.2.

Three Nl^1 type mutants and 2 Nl^2 type mutants, which were obtained in the experiment of 1958, were examined. Two types segregated from the cross between the Nl^1 mutants and the *oa* strain, namely, Nl^1 larvae with transparent hypodermis and normal larvae with non-transparent hypodermis. And from the cross between the Nl^2 mutants and the *oa*

strain, segregation of two types, Nl^2 larvae with transparent hypodermis and normal larvae with non-transparent hypodermis, was observed.

The crossing-over value between the new Nl^1 and *odk* was 3.2 and the linkage distance between the new Nl^2 and *odk* was 6.3, as shown in the following tables.

Table 2. Segregation from the cross $odk \times odk/Nl^1$.

Phenotypes / Batches	$+^{Nl^2}$		Nl^1		Total	C.O.V.
	$+^{odk}$	*odk*	$+^{odk}$	*odk*		
10 batches	37	1122	1014	37	2210	3.4

Table 3. Segregation from the cross $odk \times odk/Nl^2$.

Phenotypes / Batches	$+^{Nl^2}$		Nl^2		Total	C.O.V.
	$+^{odk}$	*odk*	$+^{odk}$	*odk*		
10 batches	109	1433	1294	73	2909	6.3

From the crosses $+/Nl^1 \times Di/Di$ and $+/Nl^2 \times Di/Di$, respectively, Nl^1 and Nl^2 larvae and normals segregated. These larvae exhibited slightly "dirty" larval marking characteristic of *Di*.

From the experimental results above-mentioned it might be inferred that in all of these mutants a deficiency was involved in the portion extending from the *U* region to the marker *odk* of the 14th chromosome, and that the extent of the deficiency is not always the same.

A spontaneous *Nl*-type mutation occurs very rarely. However, it may be said from the present experiment that the frequency of the mutation increases strikingly under irradiation with X-rays.

4. *Genetic maps of the eighth linkage group of the silkworm*

(By Yataro TAZIMA, Tamiji OZAWA, Eiichi INAGAKI and Takao KOBAYASHI)

In his classical paper on amylase activity of silkworm larvae, MATSUMURA (1934) reported that there are two different genes, *ae* and *be*, which control the activity of enzymes in the digestive juice and in the body fluid respectively, and that they belong to the same linkage group. According to him, recombination value between these two genes is 1.1%. This group was temporarily called the eighth group, according to the order of discovery, though it was not certain whether the group is actually a new one or not. Since then nobody has tested the linkage relation of *ae* and *be* with other

morphological genes, until a joint survey of silkworm linkage groups was undertaken on an extensive scale in 1955.

As a member of the committee, the senior author was assigned to carry out the survey of this group. He confirmed its independence from the already established 14 groups.

In 1956 one of the authors, Ozawa, found that the *st* gene (stony, a larval character) belonged to this linkage group among a number of other genes tested with *ae*. Recombination values of *st*−*ae* and *st*−*be* were 5.7% and 11.4% respectively, which indicated the order of these three genes to be *st*−*ae*−*be*, though the values themselves were not reliable enough.

In order to determine the exact loci of these genes, we undertook three point tests. The Indian polyvoltine race, "Pure Mysore", which was the only triple dominant strain among the many tested, was outcrossed to the *st ae be* stock. F_1 males were backcrossed to triple recessive females. However, it was found that the activity of the $+^{be}$ gene of "Pure Mysore" was too weak to confirm the amylase action in heterozygotes. Therefore, $+^{st}ae+^{be}/st+^{ae}be$ individuals were built up by using other strains, Hinode (+*ae*+), Taishohaku (++*be*) and Stony (*st ae be*). These triple heterozygotes were backcrossed to *st ae be*. Recombination values obtained were as follows:

st−*ae*, 2.8%; *ae*−*be*, 1.4%; *st*−*be*, 4.2%.

From those data, the linkage map of the eighth group may be represented as:

st(0.0)−*ae*(2.8)−*be*(4.2).

5. *Structure and function of pleiotropic genes of the silkworm III. Genetical study of four mutant strains induced independently from the E^{Bl} gene*

(By Yataro TAZIMA and Eiichi INAGAKI)

The pleiotropic effect of genes of the *E* pseudo-allelic group suggests that the *E*-region might be a complex of several closely linked component subunits having different functions. The most reliable method of examining this supposition is to test recombination between those subunits. However, no marker genes contiguous to the *E*-region have been found, which makes the recombination test inapplicable. Therefore, approaches were made by inducing mutations at the supposedly specific sites which might correspond to components of a compound character.

Up to the present, we have obtained a large number of presumed mutants for each component. In this report reference will be made only

to the information concerning four mutant strains obtained from the complex gene E^{El}. The characteristics of those strains are shown in Table 1.

Table 1. Characteristics of four mutant strains as represented by the obliterating effect on components of compound characters.

Strain	Representative phenotype	Obliterating effect in terms of penetrance			
		4th segment		5th segment	
		Markings	Legs	Markings	Legs
El_{122}	*El*-1	0.14	0.92	0.00	0.29
El_{22}	*El*-4	0.51	0.93	0.00	0.29
El_{33}	*El*-6	1.00	1.00	0.60	0.09*
El_{131}	*El*-6	1.00	1.00	0.41	1.00**

1.00: 100% disappearance in individuals with extra markings or legs.
* legs of 5th segment considerably degenerated.
** legs of 5th segment completely degenerated.

Even though the penetrance varies in the same mutant strain according to the component trait, specificity of each strain is clear so far as the site and magnitude of change are concerned. Representative phenotypes of each mutant strain are given in the second column of the table. These mutant strains are maintained by backcrossing to the normal and yields several types of pheno-variants in every generation. For example, when type *El*-1 of the El_{122} strain is crossed to the normal, pheno-variants *El*-1 (26.3%), *El*-2 (13.3%), *El*-4 (5.9%) and quasi-P (1.0%) appear (for type classification, refer to Ann. Rept. Nat. Inst. Genetics No. 9, p. 11). This fact suggests that all these types might be modified expressions of one mutant gene, presumably derived from E^{El}.

Each type was then backcrossed to the normal to confirm the differences in phenotypic expression. The results are given in Table 2.

Even in the same strain considerable differences in expression were observed according to the type of parents used for the cross. For instance, within the El_{122} strain, disappearance of additional crescent markings on the 4th segment was more extreme in the effspring of quasi-*P* than in those of *El*-1 or *El*-2. The obliterating offect on extra-abdominal legs on the 4th and 5th segments was more extreme in the offspring of *El*-2 than in those of *El*-1. The similar drift in phenotypic expression was also observed between the different phenotypes of El_{22} strain. It may be inferred, therefore, that the cause of phenotypic modification within the same strain is due to modifiers.

Thus, two characeristics are distinguished, one is proper to the strain and the other is variable within the same strain. The former seems to be controlled by a mutant gene specific to each strain while the latter is under the control of non-specific strain modifiers.

Table 2. The results of testcrosses of different phenotypes of mutant strains to normals.

Strain	Type of parent used for cross	Breeding season	Obliterating effect in terms of penetrance			
			4th segment		5th segment	
			Markings	Legs	Markings	Legs
El_{122}	*El*-1	59-1	0.14	0.92	0.00	0.29
El_{122}	*El*-2	59-1	0.49	1.00	0.00	0.39
El_{122}	quasi-P*	59-3	0.59	0.99	0.00	0.17**
El_{22}	*El****	59-3	0.22	0.85	0.00	0.11
El_{22}	*El*-4	59-1	0.51	0.93	0.00	0.11

* Phenotypically almost similar to *P* (Normal) except for the traces of extra legs; considered to be a modified form of El_{122}.
** Lower penetrance might be attributed to a difference in environmental conditions.
*** Abbreviation of "E^{El}".

Table 3. Modifications of phenotypic expression in the offspring of different phenotypes crossed to normals

Strain and type of parent	Incubation temperature	Obliterating effect in terms of penetrance			
		4th segment		5th segment	
		Markings	Legs	Markings	Legs
El_{122}	30°C	0.12	1.00	0.00	0.33
El-1	20°C	0.15	0.92	0.00	0.03
El_{122}	30°C	0.16	1.00	0.00	0.15
El-2	20°C	0.16	0.97	0.00	0.05
El_{122}	30°C	0.71	1.00	0.00	0.12
El-4	20°C	0.70	0.99	0.00	0.00
El_{22}	30°C	0.41	0.98	0.00	0.00
El-1	20°C	0.38	0.86	0.00	0.00
El_{22}	30°C	0.61	0.99	0.00	0.01
El-4	20°C	0.52	0.82	0.00	0.00
El_{33}	30°C	1.00	1.00	0.03	0.75
El-6	20°C	1.00	1.00	0.86	0.65
El_{131}	30°C	1.00	1.00	0.07	1.00
El-6	20°C	1.00	1.00	0.77	1.00

Since it had been known that incubation temperature affects the expressivity of some gene controlled characters, the effect of incubation temperature upon the expression of the mutant traits was tested by subjecting the eggs to high or low temperatures. The results are shown in Table 3.

The response pattern to extreme temperatures differed according to the mutant strain but was rather similar among the different types of the same strain. This result supports the above interpretation that the different types in the same strain represent modified phenotypic expressions of the same gene.

From these observations, it may be assumed that the four mutant strains used in the present experiments might have been the products of mutation at different sites. If this is true the E^{El} gene may be understood as being composed at least of four sites which can mutate independently.

6. *Genetic variations in DDT-resistance of laboratory strains and natural populations of Drosophila melanogaster.*

(By Chozo OSHIMA)

The level of DDT-resistance of twenty laboratory strains of *D. melanogaster*

Table 1. Mortality of laboratory strains observed 24 hours after exposure to 4% DDT test paper and their LD50s and slops.

Laboratory strain	Exposure time (Hours)		LD50	Slope
	8 Mortality (%)	16 Mortality (%)		
1 Osyoro	32.3	89.7	9.4	7.19
2 Otaru	20.8	73.0	11.4	6.53
3 Sapporo	33.9	84.2	9.6	6.29
4 Toyama	34.1	49.8	16.2	1.96
5 Katsunuma	24.5	58.8	13.2	4.29
6 Asitakayama	15.5	78.0	11.4	7.81
7 Hatijyozima	0.8	42.3	17.2	5.19
8 Anjyo	18.9	89.9	10.2	8.88
9 Hikone	1.0	19.6	26.8	2.33
10 Kyoto	16.9	68.0	12.2	6.39
11 Hirakata	8.7	55.1	15.0	5.80
12 Miyazu	17.4	46.4	17.6	3.63
13 Tateba-sima	14.5	73.9	12.0	7.43
14 Kama-sima	4.6	33.1	21.8	3.56
15 Iguro-sima	18.1	45.8	18.8	3.46
16 Koti	12.4	69.2	12.6	7.10
17 Uwajima	24.4	45.1	19.2	2.59
18 Hikosan	43.1	99.1	8.4	7.00
19 Kirisima	6.9	74.7	12.4	8.48
20 Ao-sima	6.1	58.9	14.4	6.60
Overall average	17.7	62.7	14.49	5.63

collected from different localities in Iapan and maintained by mass-mating for several years was tested by exposure to 4% DDT test paper prepared by the WHO in Geneva. The level of DDT-resistance of F_3 flies originated from many female flies captured in nine natural populations was tested also by the same method.

The LD50 and slope of the dosage-mortality regression line of these strains were obtained from mortality records 24 hours after exposure to two time-determined dosages. These results are shown in Table 1 and 2.

Table 2. Mortality of natural populations observed 24 hours after exposure to 4% DDT test paper and their LD50s and slopes.

Population	Exposure time (Hours)		LD50	Slope
	8 Mortality (%)	16 Mortality (%)		
Suyama-1	38.4	75.7	9.6	4.66
Suyama-2	54.8	91.9	7.7	4.63
Suyama-3	43.2	81.0	8.8	4.72
Juriki-1	11.3	67.4	13.0	7.01
Juriki-2	16.9	73.3	11.8	7.05
Kofu	17.9	56.7	14.0	4.85
Katunuma-1	9.6	38.9	20.4	3.66
Katunuma-2	3.8	28.1	24.0	3.04
Katunuma-3	11.4	47.4	16.6	4.50
Overall average	23.0	62.3	13.99	4.91

The difference between the two overall average LD50s of laboratory strains and F_3 flies of natural polulations was not as large as might have been expected considering the anticipated effect of extended laboratory culturing without selection in the former.

A positive correlation between slope and population size was expected on the basis of the original populations of some strains. This was verified in natural populations which were collected by the authors in October, 1959.

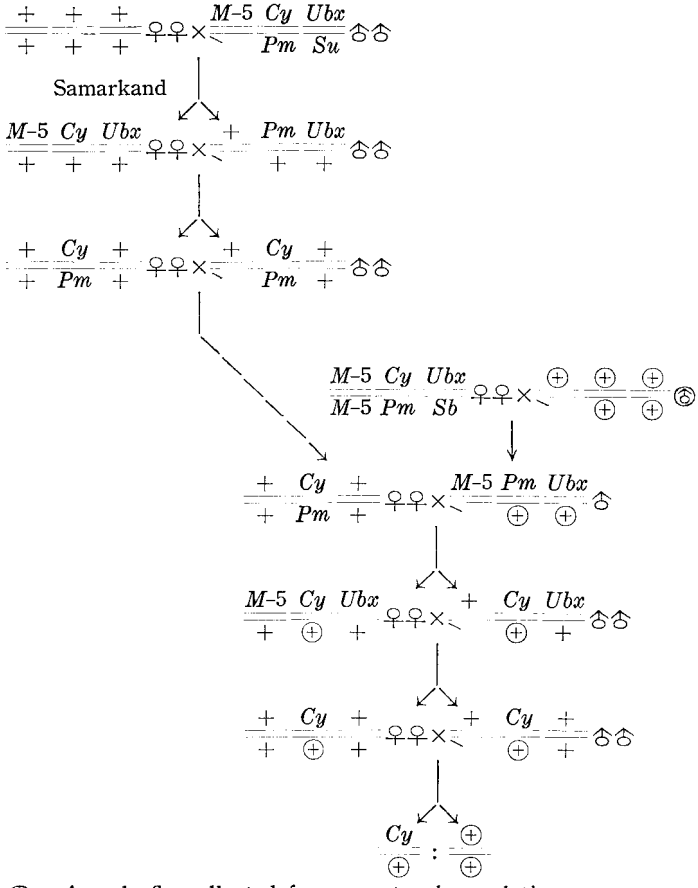
There was a significant negative correlation ($r=-0.63$) between slopes and LD50s. Strains having flat slopes were, in general, more resistant than those with steep slopes.

7. *Frequencies of deleterious chromosomes in natural populations of Drosophila melanogaster.*

(By Chozo OSHIMA and Osamu KITAGAWA)

Many flies were collected from natural polulations at three places in Suyama and two places in Juriki in Shizuoka Prefecture and one place in Kofu and three places in Katunuma in Yamanashi Prefecture in October 1959. The second chromosomes of male flies were isolated individually by the CMI method.

The mating scheme of the CMI method.



Ⓜ: A male fly collected from a natural population.

A single male fly of the flies collected from natural populations was crossed

to female flies having inversions and dominant marker genes on each chromosomes. The *M*-5, *Pm* and *Ubx* heterozygous male fly was crossed to female flies having *Cy*, *Pm* marker genes and inversions on the second chromosomes and homozygous background. In the following generations, female and male flies heterozygous for the *Cy* chromosome and for one of the chromosomes obtained from natural populations were mated. If the wild-type second chromosome had deleterious genes, the proportion of wild type flies in the next generation would be decreased below 0.33. When a number of chromosomes were tested by the above method, a considerable number of lethal, semi-lethal and deleterious second chromosomes were detected. The frequencies of lethal and semi-lethal chromosomes found in each natural population are shown in Table 1.

When the data are compared with that reported by several other investigators, we find that the frequencies of lethal and semi-lethal chromosomes in Japanese natural populations are somewhat than those in American and Israel populations and almost equal to those in Korean populations. The natural populations of Suyama and Juriki were small. The natural populations of Kofu and Katsumuma were large and coexisting with other species, *D. lutea*, *D. hydei* and *D. immigrans*. The causes for lower frequencies than expected in Suyama and Juriki populations are presumed to be associated with conditions with make most of the lethal

Table 1. Frequencies of lethal and semi-lethal second chromosomes in natural populations.

Population	No. of chrom. tested	No. of lethal chromosomes	%	No. of semi-lethal chrom.	%
Suyama-1	43	4		1	
Suyama-2	19	2		3	
Suyama-3	22	3		2	
Total	84	9	10.71	6	7.14
Juriki-1	43	5		1	
Juriki-2	27	2		1	
Total	70	7	10.00	2	2.86
Kofu	47	5		12	
Katunuma-1	32	5		6	
Katunuma-2	40	6		6	
Katunuma-3	38	5		3	
Total	157	21	13.38	27	17.20

Table 2. Frequencies of deleterious and normal second chromosomes in natural populations collected at three localities.

Population	Percentage of wild type flies					No. of chromosomes
	0	0-16.7	16.8-25.0	25.1-33.3	33.4-41.6	
Suyama	9	6	11	43	9	78
Juriki	7	2	9	30	8	56
Kofu & Katunuma	21	27	25	68	7	148
Total	37	35	45	141	24	282
%	13.12	12.41	15.96	50.00	8.51	

and semi-lethal genes partially dominant or produce local inbreeding in small populations. The viability of each homozygous second chromosome originated from natural populations was estimated by the ratio of *Cy* and wild type flies in the later generations. The classification of deleterious and normal chromosomes is determined as follows; no wild type flies:lethal, 0.0-0.167 wild type flies:semi-lethal, 0.167-0.250:deleterious, 0.250-0.416:normal and more than 0.416:super vital second chromosome. The results shown in Table 2 were obtained. The difference in number of the tested chromosomes shown in Tables 1 and 2 was caused by the loss of some homozygous strains in the later generations and the addition of a few lethal and semi-lethal chromosomes.

8. *Effects of X-irradiation on biosynthesis of nucleic acid in Drosophila.*

(By Toshifumi TAIRA and Toshiteru MORITA)

We have shown that the biosynthesis of nucleic acids in *Drosophila* is markedly inhibited by X-irradiation. The nucleotide, precursors of nucleic acids, also are much influenced by irradiation. In the experiment in which this was shown, 2rd instar larvae and early pupae (24 hrs. after pupation) were subjected to 3,000 r irradiation. Twenty-four hrs. after irradiation, each material was immediately homogenized with cold 10% aqueous perchloric acid. After centrifugation, the supernatants were neutralized with 5 *N* KOH in an ice-bath. The supernatants were used as the acid-soluble nucleotide fraction and were charged on columns of Dowex-1-X 8-formate resin. The eluting solutions ranged from 0.125*N* HCOOH to 4*N* HCOOH-0.8*M* $HCOONH_4$. Fractions of 5 *ml* were collected by the automatic fraction-collector. The absorbance of each fraction was read in the Beckman DU

spectrophotometer at 260 $m\mu$. Paper chromatographical analysis, ratios of E_{260}/E_{280}, and quantity of inorganic phosphorus (measured in each main fraction by means of a modified Fiske-Subba Row method) were used also for the identification of nucleotides. The results are shown in Table 1.

Table 1. Effects of X-irradiation on nucleotide level in *Drosophila* (E_{260}/g in wet weight)

Nucleotides	CMP+DPN	AMP	TPN	GMP	ADP	GDP	ATP
Control:							
Larvae	6.6	1.6	10.0	8.4	12.6	—	1.1
Pupae	5.5	1.3	0.5	4.2	4.0	3.9	1.6
Irradiated:							
Larvae	8.1	10.4	13.0	1.2	—	—	—
Pupae	5.2	3.7	9.7	10.6	10.2	—	—

From these results, the following conclusions can be drawn: (1) Nucleotides of di- and tri-phosphate increase considerable in amount during metamorphosis. (2) Nucleotide levels are strikingly influenced by irradiation; nucleotide phosphorylations from monophosphate to triphosphate are strongly inhibited.

9. *Distribution of the "Sex-Ratio" agents in the tissues of adults of Drosophila Willistoni.*

(By Bungo SAKAGUCHI and Donald F. POULSON*)

It has been demonstrated by Malogolowkin and Poulson (1957, 1959) that the abnormal "sex-ratio" condition in *D. willistoni* is transmitted through egg cytoplasm and that the agent in such eggs is capable of infectious transfer into normal females converting them to the "sex-ratio" condition.

The "sex-ratio" agent has been experimentally examined from the view point of its distribution among the various organs and tissues of the adult fly.

The "sex-ratio" strain SRB-3 was used in this experiment. This strain was maintained for about twenty generation by back crossing to the original "sex-ratio" strain with wild type males of Barbados-3 (B-3) strain. To test the possibility of transfer of the "sex-ratio" agent from SRB-3 into normal B-3 abult females, blood, ovaries, wing muscles, adipose tissues and gut were carefully taken out of two or three week old

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SRB-3 females. The blood was directly sucked into a micropipette from abdomens of the donor flies and immediatley injected into the abdomens of young virgin B-3 females. In the case of ovaries, muscles, gut, and fat body, the tissues were taken out of about fifty to one hundred donor flies and repeatedly washed with Ringer solution.

These organs and tissues were then homogenized separately in a small volume of Ringer solution using a small glass homogenizer. Small quantities of each homogenate were injected into the abdomens of the B-3 host flies. The injected females were mated to males of their own strain. Both collection of eggs and examination of progenies were carried out in accordance with the procedure previously reported by Malogolowkin, *et al* (1959).

In the generation from B-3 females injected with blood or homogenates of adipose, musclar, or ovarian tissue from the SRB-3 females, the progeny in the early period of egg production consisted of approximately normal proportions of females and males. However, as the injected flies aged the proportions of females from every mother increased and finally, except in some cases, they produced mainly daughters. The results of second and third generations showed almost complete unisexual bloods, again with a few exceptions. In most of the cases of blood injection, the incubation period from the time of injection to the beginning of the effect of the "sex-ratio" agent was very short, only a few days, as compared to about twenty or twenty five days after injection of homogenates of the other organs and tissues. It seems from these results that the "sex-ratio" agent is widely distributed in the bodies of SRB-3 flies, viz. blood, fat body, flight muscles, and ovary, and that there is a high concentration of "sex-ratio" agent in the blood compared to other organs and tissues. In the case of injection of gut there were no unisexual bloods among the progenies. However, it is not clear at present whether the "sex-ratio" agent is present. in the gut tissues, because the survival rate of injected flies was very low, only 2 out of 12 individuals.

A series of controls in B-3 flies were injected with blood or homogenates of various organs and tissues of other B-3 flies was carried out. In these experiment one individual from the series of 12 ovarian injected and one of the 8 muscle injected hosts produced the "sex-ratio" condition. This "sex-ratio" condition was maintained in further generations. It seems that the "sex-ratio" agent may be maintained in a latent phase in individuals of the B-3 strain.

That the occasional males which survive in "sex-ratio" strains also carry the "sex-ratio" agent was demonstrated by injection of blood into normal B-3 females. In each case injected females soon produced only daughters and the condition was transmitted in further generations.

10. *Mass migrating activity in inbred lines derived from four wild populations of Drosophila melanogaster.*

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

Sixty five inbred lines derived from four wild populations of *Drosophila melanogaster*, eleven strains from Tateba-sima (TB), fourteen from Katsu-

Table 1. Analysis of variance of mass migrating activity of inbred lines of four wild populations of *D. melanogaster*.

Source of variation	KN and TB		TS and IG	
	d.f.	mean square	d.f.	mean square
Population	1	9156.8639**	1	7910.0822**
Lines w/n population	23	1960.9507**	38	1063.7599**
Density	5	6676.1363**	3	7612.5266**
Density × population	5	1769.4330**	3	312.8952
Density × line w/n population	115	305.7090	114	413.6496*
Error	232	427.0929	640	215.8361

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

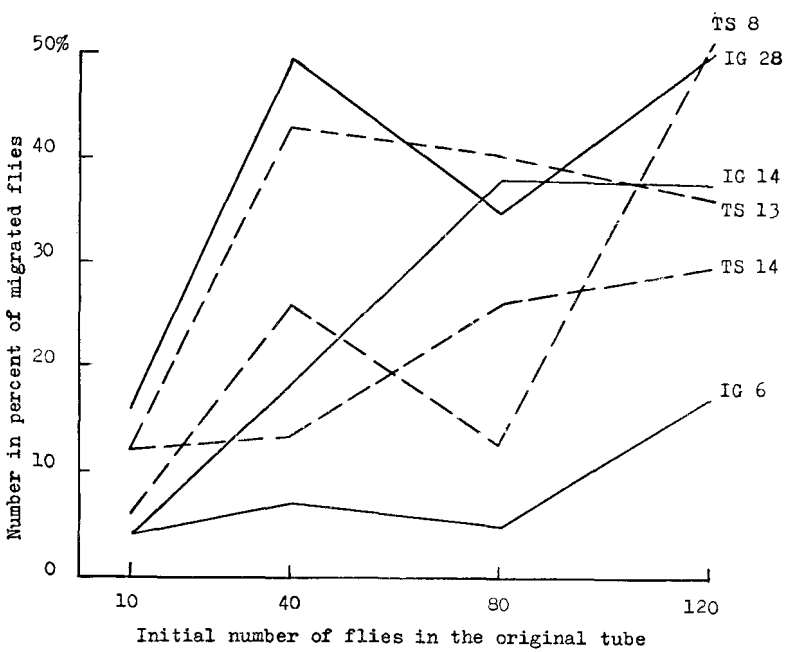


Fig. 1.

numa (KN), sixteen from Iguro-sima (IG) and twenty four from Te-sima (TS), were used in this investigation. After the completion of twenty inbred generation in TB and KN and thirty in IG and TS, the mass migrating activity presumably caused by the pressure of population density was investigated for each of the inbred strains. The details of the method of investigation were given in the 1957 issue (No. 7, pp. 72–73.) of this Annual Report. Counting of the number of flies migrated to the connected tubes from the original one was made six hours after connection. The analysis of data was made for KN and TB populations and for IG and TS populations separately. Table 1 shows the analysis of variance of the mass migrating activity of the inbred lines from the four wild populations of *D. melanogaster*. Migrating activity of a few strains of the IG and TS populations is shown graphically in Fig. 1.

From Table 1 and Fig. 1 it can be concluded that the migrating activity varies genetically not only among populations but also among inbred lines within populations.

11. *Genetical aspects of two colour mutants of rats caught in the suburbs of Misima*

(By Tosihide H. YOSIDA)

Two coat colour mutants of the rat (*Rattus norvegicus*) were caught in the suburbs of Misima. One of them, a female rat, was characterized by a reddish yellow coat and pink eye. This rat was crossed with a CASTLE black-strain rat with non- agouti coat (*aa*). All F_1 hybrids showed agouti coat (*A/a*) and black eye. The F_2 progeny fell into four classes, namely agouti and black eye (101 rats), non-agouti and black eye (35 rats), reddish yellow and pink eye (49 rats) and light reddish yellow and pink eye (13 rats), according to the dihybrid Mendelian ratio. The expected ratio of segregation in the above cases was 111.5:37.1:37.1:12.4. The above result indicates that two independent genes, *A* for agouti coat and *p* for pink eye, are involved in this mutant. The expression of *A* is conditional on the presence of *p*. *A* and *p* together effect reddish yellow coat and pink eye. When allele *a* occurs together woth *p* the coat is light reddish yellow. A pink eyed yellow mutant has been reported already by CASTLE (1915). The mutant found by the present author may be the same as that obtained by CASTLE.

The other mutant rat (含) was characterized by brownish gray coat and ruby eye. This mutant is similar to the Albany-strain which has light brownish gray coat and ruby eye. To find out whether the coat clour gene concerned is the same as that of the Albany-strain, the two were

crossed. All F_1 hybrids showed non-agouti coat and black eye, but the F_2 segregated into three types, namely non-agouti (29 rats), brownish gray (19 rats) and light brownish gray (11 rats). From the above investigation it seems that the wild mutant and the Albany-rat have different coat colour gene or genes, but have in common the agouti allele *a*.

By successive inbreeding of rats with new mutant characters, several inbred strains, such as NIG-I, NIG-II, NIG-III, ..., are being established in the author's laboratory.

12. *Study on the new mutant "falter" found in the house mouse*

(By Tosihide H. YOSIDA)

"Falter" (*fa*) is a recessive mutant wich appeared spontaneously in 1958 in a sib-mated stock (DBA/Ma-c^e) bred in the author's laboratory. In homozygous condition, falter can be recognized by an abnormal side to side, swaying walk. The mutant was found in mice of about 10 days age. The abnormality became more pronounced with advancing development, and all the mice died at about 20 days.

By the mating of two heterozygotes (*fa*/+), 81 offspring were obtained. Among them 59 were normal, and 22 were abnormal, which indicates that the falter character is due to a recessive mutation. The expected segregation ratio is 60.75 and 20.25 ($\chi^2=0.202$).

Table 1. F_2 segregation with respect to genes *p* and *fa*

Types	*P Fa*	*P fa*	*p Fa*	*p fa*	Total
Observ. No.	23	7	16	2	48
Expect. No.	27	9	9	3	48
	$\chi^2=6.82$		$p=0.1$-0.05		

Characters similar to falter have been reported by FALCONER, 1951, (reeler *rl*), by DICKIE *et al.*, 1949, (ducky *du*), and by HOECKER *et al.*, 1954, (agitans *ag*). These mutants allow development for a considerably longer period of time and some animals grow to maturity. Reeler (*rl*) is very similar to falter and was proved to belong to the 3rd linkage group. In order to investigate whether *fa* is in the same group or not, *fa*/+ mice (♂) were mated to NH-strain mice (♀) which are characterized by having genes *p* and *s*. Gene *p* (pink eye) is in the 1st linkage group and gene *s* (recessive spotting) is in the 3rd linkage group. The F_2 progeny from the above mating segregated into four categories with respect to *p* and *fa* (Table 1). From the segregation ratios it seems that *fa* is independent

of the 1st linkage group.

Table 2. F_2 sagregation with respect to genes *s* and *fa*

Types	*S Fa*	*S fa*	*s Fa*	*s fa*	Total
Observ. No.	30	5	9	4	48
Expect. No.	27	9	9	3	48

$\chi^2=2.44$ $p=0.95\text{-}0.50$

In relation to genes *s* and *fa*, the F_2 offspring segregated into four categories as shown in Table 2. The segregation ratios indicate that *fa* is independent of *s*, i.e. does not belong to the 3rd linkage group.

13. *Peripheral blood analyses of various strains of mice and rats*

(By Tadao HAMADA, Tosihide TABATA, Yoshinori KURITA and Tosihide H. YOSIDA)

The present paper deals with the normal peripheral blood analyses of various strains of mature mice and rats.

The methods used for the examination were as follows: 1) The blood was withdrawn, always before meals between 9.00 a.m. and 10.00 a.m.,

Table 1. Peripheral blood analyses of various strains of mice

Strain of mice	R.B.C. ($\times 10^6$)	W.B.C. ($\times 10^4$)	Neutrophils (%)
A/Ms	1,148.0	1.06	43.1
AKR/Jax	976.0	1.08	29.0
BALB/cJax	1.008.0	0.94	21.8
C57BR/Jax	1,025.0	1.83	15.4
C57/BL/6Ms	888.0	1.52	21.4
C57L/Ms	892.0	2.01	33.2
C58/Ms	1,085.0	1.79	32.6
CBA/Ms	934.0	1.17	38.0
CFW/Ms	893.0	2.65	35.3
C3H/Ms	887.0	1.46	40.3
DBA/MaMs	1,200.0	1.26	47.1
dba/Ms	993.0	2.86	47.9
dd/Ms	885.0	2.59	28.1
DM/Ms	870.0	1.13	30.8
D103/Ms	963.0	1.76	35.6
NH/Ms	1,109.0	0.80	18.3
S4/Ms	1,078.0	2.11	18.8
SM/Ms	1,001.0	2.17	34.6
SPS/Ms	865.0	1.33	32.8
Swiss Albino	964.0	1.19	42.8

5 mice were observed in each strain.

Table 2. Peripheral blood analyses of various strains of rat

Strain of rats	R.B.C. ($\times 10^4$)	W.B.C. ($\times 10^4$)	Neutrophils (%)
WKA	812	1.27	15
W	777	1.01	23
Wayne	827	1.25	14
Albany	858	1.64	15
Fischer	821	0.92	16
Buffalo	887	1.45	20
Long-Evans	759	0.71	16
N	788	1.07	24
SH	917	0.92	19
NIG	855	1.13	9.0
CW-I	890	1.36	13
CW-II	880	1.06	15
AN	806	1.97	10
WT	875	1.43	26

4-5 rats were observed in each strain.

from the right lateral vein in the middle part of the tail. 2) Hemocytometers for human hematology were used. Blood counts for each animal were repeated three to five times to minimize errors. 3) Smear preparations of the peripheral blood were made by the usual technique for leukocyte counts. GIEMSA's staining technique was used. The morphological estimation of white blood cells was always followed by the usual hematologic technique. The total number of cells observed per strain were about 10,000. The results of observations in mice and rats are shown in Tables 1 and 2, respectively.

As shown in these tables, no marked differences were found between the blood pictures of various strains. Noticeable is the fact that mice such as A/HeMs, C3H/Ms, dba/Ms, etc., which show high incidence of mammary tumor, have a comparably higher percentage of leukocytes, while C57BL/6HeMs, BALB/cJax etc., strains with low tumor incidence, have a higher percentage of lymphocytes.

14. *Reciprocal transplantation of skin between rhino and non-rhino mice*

(By Shigekatsu TSUZI and Tosihide H. YOSIDA)

Rhino mice used in the present study were obtained in December, 1958, from the Jackson Memorial Laboratory. They are characterized by loss

of hair and development of skin folds (HOWARD 1940). Experiments with skin transplantations between rhino and non-rhino mice have been carried out by DAVID (1934) and FRASER (1946). According to DAVID (1934), hairless skin grafts behaved autonomously on a normal host, while FRASER (1946) demonstrated in young mice that the rhino skin on normal hosts showed depilation in the center; on the borders, however, a zone of hair persisted.

The results obtained by the present authors from reciprocal transplantations of skin between adult rhino and non-rhino mice are as follows. Rhino skin grafts on non-rhino hosts had on the surface the appearance of reddish and smooth sloughs about one week after operation. Later, the grafted skin became thicker and the characteristic skin folds appeared. After a month, the grafts showed the characters of hyperkeratosis. It is noteworthy that thin hairs appeared on the adult rhino skin which was grafted on non-rhino hosts 10 to 20 days after operation, but were shed completely about one month later.

In order to investigate why thin hairs developed on rhino skin grafts, the following experiments were performed: (1) Rhino skins were grafted on other rhino hosts. (2) Rhino skins were retransplanted to the same place of the same host in various orientations. The results obtained from these experiments were similar to those obtained from those mentioned above. Thus, it seems that the temporary growth of thin hairs on the transplanted rhino skin was due to a mechanical stimulation by the operation.

Non-rhino skin grafts on rhino hosts showed typical hair growth about one month after operation.

15. *Study on phosphatase activity in rhino mouse skin*

(By Kazuo MORIWAKI)

A mouse homozygous for the mutant gene rhino (hr^{rh}) begins to lose its hair at the age of about 14 days, while no such change occurs in a heterozygote. From experiments with reciprocal ectodermal transplants performed at an early stage, FRASER (1946) stated that after birth the hair-loss character developed autonomously, freely from other control mechanisms in the body (e.g. hormonal control). As a biochemical property changed with the appearance of the autonomous character, the author referred the change to acid phosphatase activity of the skin. It has been suggested in this connection that in canaries the acid phosphatase has a relation to feather formation through the fat metabolism of the skin (KOBAYASHI and MARUYAMA, 1957). Also, in the mouse skin, the depilation seems to be accompanied by an accumulation of subcutaneous fat and activation of acid

phosphatase. In mice younger than 2 weeks, both skins of rhino and non-rhino animals showed normal hair growth and similar phosphatase

Table 1. Comparison of acid phosphatase activity at various ages in the skin of rhino homo- and heterozygotes.

Age / Exp. no.	Extinction of dephosphorylated PNP (-log T at 400 mμ/7.5 mg wet tissue)				
	8 days	18 days		40 days	
	hr^{rh}/hr^{rh}, $hr^{rh}/+$	hr^{rh}/hr^{rh}	$hr^{rh}/+$	hr^{rh}/hr^{rh}	$hr^{rh}/+$
1	1.96	1.47	1.19	1.57	0.73
2	2.04	1.43	1.15	1.82	0.84
3	2.30	1.45	1.19	1.90	0.62
4	2.23			2.00	0.73
5	1.55			2.21	0.73
6	2.12				
7	1.96				
Average	2.02	1.45	1.18	1.90	0.73

activity. After 2 weeks, according to the progress of depilation, the enzyme activity in the skin of the homozygotes was shifted in comparison with the heterozygotes (Table 1), and accumulation of subcutaneous fat was observed in the former (TSUJI, unpublished).

A problem for further study involves which process induces the rhino behavior, depilation, subcutaneous fat accumulation, or activation of skin acid phosphatase.

In this experiment, para-nitrophenolphosphate (PNP) was employed as substrate of the enzyme, and the amount of dephosphorylated PNP after incubation for 20 minutes at 37°C and PH 4.2 was estimated at 400 mμ.

16. *Effect of energy-rich phosphate esters on the swelling of mouse diaphragm*

(By Kazuo MORIWAKI)

KREBS et al. (1949) found that the water content of a kidney slice is augmented by metabolic inhibitors such as KCN or 2,4-dinitrophenol, which indicates that energy is necessary for keeping the water content in the tissues at a constant level. The present report deals with the correlation between the swelling of mouse diaphragm and the decrease of the energy-rich phosphate esters.

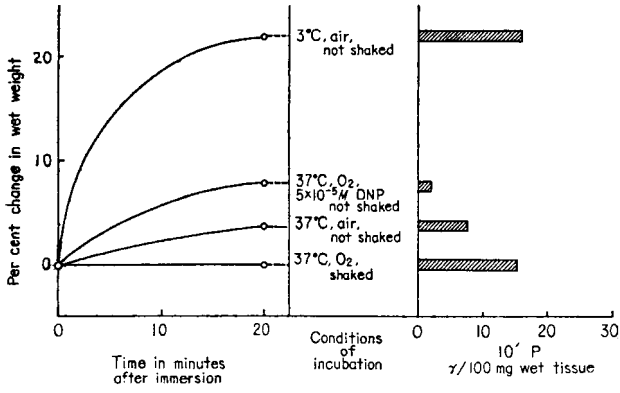


Fig. 1. Swelling and 10′P change in mouse diaphragm immediately after removal under various conditions of incubation.

Immediately after excision, the mouse diaphragm contains the largest amount of 10′P. The 10′P from such diaphragms was dissolved in 10% cold trichloroacetic acid solution, precipitated by Ca-reagent and hydrolysed for 10 minutes in 1 N HCl at 100°C.

The swelling of the excised diaphragm during 20 minutes of incubation at 3°C in the KREBS-RINGER-phosphate solution was prevented by shaking the tissue in the gas phase of oxygen or under air conditions at 37°C in WARBURG vessels, whereby, to a considerable extent, the 10′P content was maintained. Under the same conditions, marked swelling of the diaphragm was induced in the presence of 5×10^{-5} M 2,4-dinitrophenol, whereby 10′P content was reduced (Fig. 1).

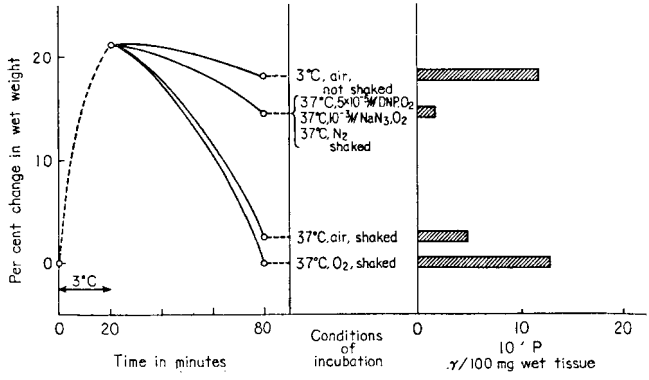


Fig. 2. 10′P content and recovery of the swollen diaphragm of mouse under various conditions of incubation.

The tissue swollen by incubation in the ordinary medium for 20 minutes at 3°C returned to the initial state when incubations for 60 minutes in the presence of 100% O_2 or 20% (air) and shaking were applied, whereby the amount of 10'P reached a comparatively high level. The swelling described above failed to disappear when the tissue was treated with 5×10^{-5} M 2,4-dinitrophenol or 10^{-3} M sodium azide or under anaerobic conditions, whereby little of 10'P was found. At 3°C, the diaphragm was swollen for 20 minutes under air conditions, and the swelling continued for 60 minutes, although a considerable amount of 10'P was found (Fig. 2).

In view of those findings, it seems likely that the extrusion of the water penetraing into the tissue is conditioned by an endergonic mechanism dependent upon the temperature.

17. *Interaction of Aegilops cytoplasm and various wheat nuclear complements*

(By Hitoshi KIHARA)

KIHARA (1958) obtained a male-sterile strain of *Triticum vulgare* by substituting its nucleus into cytoplasm of *Aegilops caudata*.

In order to investigate the fertility effect of interaction between "caudata" cytoplasm and various nuclear complements of *Triticum*, *T. vulgare*, *T. spelta*, *T. compactum*, *T. macha* and *T. durum* were crossed twice to the male-sterile strain (SB_8) of *T. vulgare*. Pollen and seed-fertility of hybrids in F_1 and B_1 generations of these crosses are summarized in Table 1.

F_1 and B_1 generations of $SB_8\times T.$ *vulgare* (=SB_9) and $SB_8\times T.$ *spelta* were both male sterile but female fertile, giving fairly good seed-set by artificial pollination. It can be concluded from this result that these two species have the same genotype with respect to the male sterility manifested in "caudata" cytoplasm.

F_1s of $SB_8\times T.$ *compactum* were male-sterile but female-functional. In the B_1 generation, male sterility segregation was observed. In some plants male fertility was restored to a normal level. B_1 plants were too few to predict the segregation ratio of male fertile vs. male sterile plants. Apparently, *T. compactum* carries a gene or genes responsible for restoration of male fertility. According to its effect the gene(s) must be recessive.

Crosses between SB_8 and *T. macha* produced only lethal F_1s, indicating that *T. vulgare* and *T. macha* carry complementary dominant lethal genes as noticed by other workers.

F_1s of $SB_8\times T.$ *durum* were male-sterile but female-fertile. A further cross of this hybrid to *T. durum* produced plants (probably 2n=28) whose male and female gametes were almost completely functionless. The result

indicates that nuclear complement of *T. durum* cannot allow for a normal development of male and female gametes when placed in "caudata" cytoplasm. This female sterility controlled by the nuclear complement of *T. durum* seems to be recessive.

Table 1. Average pollen and seed fertilities of hybrids obtained by crossing a male-sterile strain of *Triticum vulgare* with five *Triticum* species.

Strain	Pollen fertility (%)	Seed setting (%)	
		Self	Cross
SB_8: *Ae. caudata*×(*T. vulgare*)^6	—	0.0	23.2
SB_8×*T. vulgare*=SB_9	0.3	0.0	41.2
SB_8×(*T. vulgare*)^2=SB_{10}	0.2	0.0	56.3
SB_8×*T. spelta*	0.0	0.0	27.9
SB_8×(*T. spelta*)^2	0.0	1.3	39.1
SB_8×*T. compactum*	—	4.7	33.4
SB_8×(*T. compactum*)^2	39.9	51.6	53.6
SB_8×*T. macha**	—	—	—
SB_8×*T. durum*	4.9	2.0	39.2
SB_8×(*T. durum*)^2**	—	0.8	4.1

* All F_1s were lethal.

** Pistilloidy of anthers was observed.

18. *Genome analysis in the genus Oryza*[1]

(By Hitoshi KIHARA and Mitsuya NEZU)

We have collected a majority of the wild species of rice under a grant from the Rockefeller Foundation. At the start of our work on genome analysis, *O. sativa* was crossed with 6 diploid and 3 tetraploid species, all belonging to the Section Sativa ROSCHEVICZ, and seed fertility and chromosome pairing in F_1 hybrids were examined.

The F_1 hybrids between *O. sativa* and *O. sativa* var. *spontanea* or *O. perennis* showed 12 bivalents at meiosis but they were semi-sterile. The F_1 hybrids of the crosses *O. sativa*×*O. glaberrima* and *O. sativa*×*O. breviligulata* failed to produce seeds, though chromosome pairing was very regular. In the meiosis of F_1 hybrids, *O. sativa*×*O. officinalis* and *O. sativa*×*O. australiensis*, very few bivalents were formed and no seeds were set. From these findings it can be concluded that 4 diploid species, *O. sativa* var.

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

Table 1. Chromosome pairing and fertility observed in the F_1 hybrids between 8 species of *Oryza*.

Combination	No. of cells observed	Bivalents (Mode)	Trivalents	Univalents	Fertility (%)	
					Seed	Pollen
O. sativa×*O. sativa* var. *spontanea* (108×W0139)	30	12 (12)	0	0	37.0	—
〃 (414×W0122)	35	12 (12)	0	0	63.9	—
O. sativa×*O. perennis* (414×W0032)	20	12 (12)	0	0	54.5	—
〃 (414×W0149)	55	12 (12)	0	0	57.4	—
O. sativa×*O. glaberrima* (414×W0040)	45	12 (12)	0	0	0.0	—
O. sativa×*O. breviligulata* (647×W0042)	25	12 (12)	0	0	0.0	—
O. glaberrima×*O. glaberrima* (W0025×W0492)	45	12 (12)	0	0	96.4	—
O. sativa×*O. australiensis* (647×W0008)	62	0–1 (0)	0	22–24	0.0	0
O. sativa×*O. officinalis* (563×W0006)	65	0–1 (0)	0	22–24	0.0	0
O. sativa×*O. minuta* (4*x*) (414×W0051)	55	0–9 (6)	0–1	18–36	0.0	0–10

spontanea, *O. perennis*, *O. glaberrima* and *O. breviligulata*, have homologous genomes while the other 2 diploid species, *O. officinalis* and *O. australiensis*, have different genomes from that of *O. sativa*.

So far as the crosses between *O. sativa* and the tetraploid species are concerned, we could study only the F_1 hybrids of a cross between *O. sativa* and *O. minuta*. They had at MI 18 to 36 univalents and 0 to 9 bivalents with the mode at 6. Other workers have reported different results from the same cross. MORINAGA (1940) observed in most cases 36_I, though he found occasionally 1 to 3 bivalents, while OKURA (1937) observed $12_{II}+12_I$ in the majority of PMC's. NANDI (1936) studied hybrids between *O. officinalis* and *O. minuta* and found that *O. officinalis* has the same genome as one of the genomes of *O. minuta*.

Thus, it seems that *O. minuta* has one modified *sativa* and one *officinalis* genome. This hypothesis will be tested by studying colchicine produced amphidiploid between *O. sativa* and *O. officinalis*.

In addition, we are making diallel crosses between 18 species belonging to all four sections, namely, Sativa, Granulata, Coarctata and Rhynchoryza (T. KATAYAMA & M. NEZU). On the basis of the F_1 hybrids from these crosses, we will analyse the genome constitutions of all four sections of the genus *Oryza*.

19. *Application of SUMP method in taxonomic studies in Oryza*[1)]

(By Hitoshi KIHARA and Tadao KATAYAMA)

The surface structure of the lemma is an important taxonomic character in *Oryza*, as was recognized by ROSCHEVICZ. In order to examine the details, we studied the lemma of 18 *Oryza* species by the convenient SUMP (Suzuki's Universal Microprinting) method with the following results:

(1) Section Sativa ROSCHEV.

The cells are square-shaped or rectangular. Their arrangement is regular. Each cell bears a round tubercle and has a smooth or rough surface. The tubercle gives the impression of a bursting membrane. Hairs occur in most of the species.

(2) Section Granulata ROSCHEV.

The cells are rectangular and have irregular contours. Each cell has a tubercle composed of several tuberclets.

(3) Section Coarctata ROSCHEV.

In general, the cells are slender and without tubercles. They are arranged in long stripes with deep grooves between them. Species belonging

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

to this section show a great variation in structure. *O. coarctata* has stomata on the lemma.

(4) Section Rhynchoryza ROSCHEV.

The cells are polygonal, varying in size and are arranged like the scales of a tortoise shell. They have in the middle a globe-shaped tubercle, from which furrows radiate.

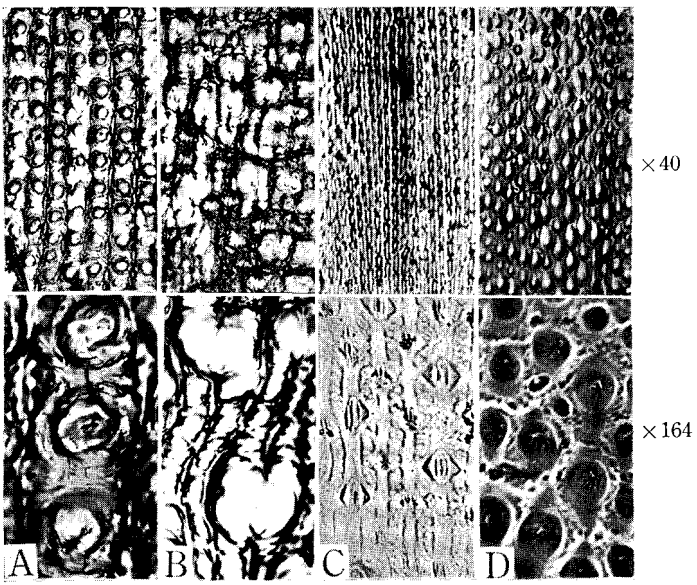


Fig. 1. Surface structures of the lemma in genus *Oryza*.
A: *O. sativa*, B: *O. granulata*, C: *O. coarctata*, D: *O. subulata*

20. *Studies on germination in Oryza*[1]

(By Tadao KATAYAMA)

I. Germination behavior

The germination behavior, i.e., germination percentage and rate, were studied using 18 species of genus *Oryza* collected abroad. Forty to 200 grains of each strain were freed from the glumes and sown in a petri-dish kept at 30°C. Experiments were made 8 times between April 10 and June 28.

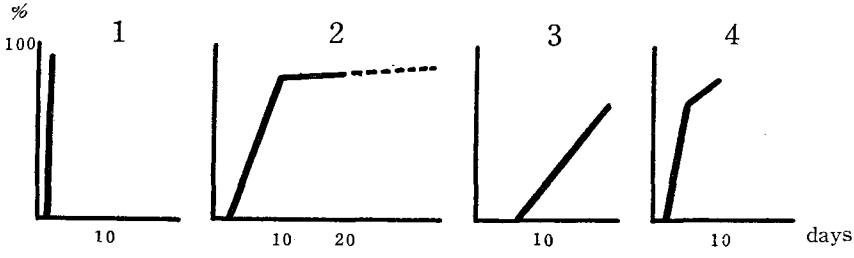
The species can be classified into four groups with respect to their germination behavior according to the data obtained. First group: Germina-

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

tion occurred within 1–2 days after sowing: Afterwards no germination occurred. *O. sativa* and *O. glaberrima* belong to this group. Second group: Germination started within 2–3 days after sowing and afterwards its percentage gradually increased. Even 270 days after sowing some seeds still germinated. *O. sativa* var. *spontanea* and *O. perennis* belong to this group. Third group: Germination started within 5–6 days after sowing. During the following 3 weeks, the germination rate increased, reaching the maximum at about 70%. *O. granulata* belonged to this group. Fourth group: Germination started within 2–3 days after sowing. The germination percentage gradually increased and reached the maximum 10 days after sowing. *O. alta*, *O. australiensis*, *O. brachyantha*, *O. breviligulata*, *O. eichingeri*, *O. latifolia*, *O. minuta*, *O. officinalis*, *O. ridleyi* and *O. subulata* belong to this group.

II. Germination test with plant hormones

Forty seeds of each of 13 *Oryza* species were soaked 12 hours in water at 30°C and treated with Indole acetic acid (IAA) and gibberelline (GIB) in a concentration of 1, 10 or 100 ppm for 12 or 24 hours in order to examine the physiological effects of plant hormones on the germination behavior of *Oryza* species.



Vertical axis; per cent germination.
Horizontal axis; number of days after sowing.
Fig. 1. Germination behavior in the genus *Oryza*.

Effects of the treatments varied with the species. Germination of the cultivated rice was little influenced by hormone treatment. Germination rate of wild species treated for 12 hours showed maximum acceleration by 100 ppm GIB. Only a few species (*O. australiensis*, *O. granulata*, *O. latifolia*), responded to IAA of the same concentration. When applied for 24 hours, IAA and GIB showed almost the same effect. The effect was stronger than that of the 12 hour treatment in all species except *O. perennis* which responded in the same way to both 12 and 24 hours treatments.

No effect on breaking dormancy of wild rice was detected.

21. *Studies on the flowering time of genus Oryza*[1)]

(By Tadao KATAYAMA)

A number of strains of cultivated and wild rice collected abroad belonging to 18 species of the genus *Oryza*, were grown in the greenhouse. Their flowering and its duration were investigated. Observations were made 21 times between September 12 and November 18 for several strains of each species. The flowering time means the time of opening of the lemma and palea of the spikelet that flowers first on a panicle. The duration of flowering time indicates the interval between opening and closing of a flower.

Results of the observations on the flowering time are given in Fig. 1. *O. sativa* var. *spontanea* and *O. perennis* were not much different from cultivated rice which flowers in the morning. All the other wild species opened earlier in the morning than the cultivated rice except *O. australiensis*

Fig. 1. The flowering behavior of *Oryza* species.

Species	Flowering time (4–17 o'clock) 4 5 6 7 8 9 10 11 12 13 14 15 16 17	Flowering response to climatic conditions	Flowering duration
O. sativa		L	M
O. s. v. *spontanea*		L	M
O. perennis		L	M
O. glaberrima		L	M
O. breviligulata		L	L
O. stapfii		T	L
O. officinalis		T	M
O. australiensis		T	L
O. minuta		T	L
O. malabarensis		C	L
O. latifolia		C	L
O. alta		C	M
O. eichingeri		L	L
O. granulata		C	S
O. brachyantha		C	S
O. coarctata		T	S
O. ridleyi		T	L
O. subulata		T	L

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

and *O. coarctata*, both of which opened in the afternoon.

Temperature, relative humidity and the time of sunrise were correlated with the flowering time of each species. The species studied can be classified into three groups with respect to their flowering response to climatic conditions. In the first group, indicated with "C" in Fig. 1, the flowering time was very stable regardless of the climatic conditions. In the second group, indicated with "L" in Fig. 1, the flowering time varied mainly with the time of sunrise, *i.e.*, it became gradually later from September to November in accordance with the calendar. Very few wild species belong to this group. In the third group, indicated with "T", the flowering time was mainly determined by temperature.

As the relative humidity during the experiment was kept higher than 70%, the critical humidity at which flowering does not occur could not be determined.

The species studied were classified into three groups based on flowering duration; (1) species indicated by M showed approximately the same duration of flowering as that of *O. sativa*, (2) species indicated by L showed relatively longer duration than *O. sativa*, and (3) species indicated by S showed a shorter duration than *O. sativa*. As shown in Fig. 1, species distantly related to cultivated rice showed in general not the same but either longer or shorter duration.

In *O. ridleyi* the male-sterile spikelet at the top of each rachis always opened earlier than the male-fertile spikelets.

22. *Photoperiodic responses of Oryza species II*[1]

(By Tadao KATAYAMA)

(1) The materials used in this experiment include two cultivated species, *O. sativa* and *O. glaberrima*, and wild growing *O. sativa* var. *spontanea* and *O. perennis*. Several strains which have the "critical day-length" of about 13 hours were selected for this experiment. They were grown in an experimental short-day field and in pots.

Studies in this experiment, were the relationships between the photoperiodic reaction and age of plants on one hand and between the former and number of short-day treatments on the other hand.

Up to the critical short-day length, the number of short-day treatments necessary to induce flowering increases proportionally with the length of illumination (Table 1). With the same short-day treatments flowering was accelerated in relation to the increased number of treatments. Photoperiodic sensitivity increases with the age of seedlings, reaching to the most sensitive

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

Table 1. Photoperiodic sensitivity of some rice strains in the vicinity of critical day-length.

Strains	Age in days	12:35*					12:50					13:05				
		6**	9	15	21	27	6	9	15	21	27	6	9	15	21	27
Kyoto Asahi I	50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
〃 〃 II	35	−	−	+	+	+	−	−	−	+	+	−	−	−	+	+
108	50	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
124	50	−	+	+	+	+	−	−	+	+	+	−	−	−	+	+
230	50	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
W0026	50	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−
W0039	50	−	−	+	+	+	−	−	−	−	+	−	−	−	−	−
W0106	50	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+
W0107	50	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−
W0120	50	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
W0149	50	−	−	−	+	+	−	−	−	+	+	−	−	−	−	−

* Day-length of short-day treatment
** Number of treatments
\+ Effective
− Ineffective

age when the plants can respond to the "critical day-length". The number of treatments required for induction of flowering was smaller in plants collected from higher latitudes than in those collected from lower latitudes. Furthermore, it seems that this number increases in the order, *O. sativa*, *O. glaberrima*, *O. sativa* var. *spontanea* and *O. perennis*.

(2) Variation of photoperiodic sensitivity and critical day-length were studied in each species. The variations were found to decrease in the order, *O. sativa*, *O. glaberrima*, *O. sativa* var. *spontanea* and *O. perennis*. As a whole, cultivated rice showed a greater variation than wild rice.

(3) The author previously reported (Katayama *et al.* 1959) a positive correlation between the critical day-length and the latitude of the habitat of the wild species. The same experiment was carried out with 383 strains of cultivated rice, collected from 10 countries in various latitudes. The data indicate that there is a similar correlation between the critical day-length and latitude of the habitat. However, the correlation was less pronounced in the cultivated rice than in the wild rice.

23. *Monosomic analysis of lethal factors in common wheat*

(By Koichiro TSUNEWAKI)

Identification of chromosomes carrying complementary lethal factors widely distributed in common wheat varieties is the subject of this investigation.

The F_1 hybrids from crosses between Prelude and Kharkov or Jones Fife were all semi-lethal, bearing about 10 seeds per plant. In the F_2 generation the cross Prelude×Kharkov produced a ratio of 41 lethals to 30 normals and the cross Prelude×Jones Fife, 57 lethals to 43 normals. Both closely fit a 9:7 ratio indicating that lethality is caused by two dominant complementary genes. Kharkov and Jones Fife apparently carry the same gene because the cross (Kharkov×Jones Fife) F_1×Prelude produced 252 lethal plants and a single normal plant. The normal plant might have been produced by out-crossing.

In order to determine the location of the complementary lethal genes, the monosomic series of Prelude was crossed with Kharkov and the Kharkov monosomic series with Prelude. Monosomic series of Prelude and Kharkov were derived by McGinnis and Jenkins, respectively, from the Chinese Spring series. Both series, particularly that of Prelude, are in an early stage of development. Because the monosomic plants used for crosses were obtained by self-pollination, it is expected that genes on a disomic chromosome can be heterozygous or homozygous for either dominant or recessive factors. However, genes on the monosomic chromosome must be derived from Prelude in the Prelude monosomics and from Kharkov in the Kharkov monosomics.

In the non-critical monosomic lines, one can expect an F_1 ratio of either 1:0, 1:1 or 0:1 for the semi-lethal vs. normal plants depending upon the genotype of the ♀ parent. On the other hand, only a 1:3 ratio is expected for the critical monosomic line, because all disomic F_1's must be semi-lethal and the monosomics, normal. Based on these considerations, the actual F_1 ratio obtained for each monosomic line was compared with the nearest ratio among the three expected for the non-critical line and the 1:3 ratio expected for the critical line.

All F_1 lines from crosses between Prelude monosomics and Kharkov, except those derived from mono-V, fitted one of the three ratios possible for a non-critical line. The ratios obtained from lines of mono-V fitted the critical 1:3 ratio only, indicating that chromosome V of Prelude carries one of the complementary genes. In the crosses between the Kharkov monosomics and Prelude, all F_1 lines except those derived from mono-XIII satisfied one of the three ratios expected for a non-critical line but disagreed

with ratio for the critical line. On the other hand, the F_1 ratio obtained in the progenies of mono-XIII failed to fit any ratio for a non-critical line but satisfied the ratio expected for the critical line. These results indicate that chromosome XIII of Kharkov carries the other gene for lethality.

The Prelude gene on chromosome V will be designated as Le_1 and the Kharkov and Jones Fife gene on chromosome XIII as Le_2. The Le_1 gene is identical with Caldwell and Compton's *Le* gene. It is yet uncertain whether their Le_2 gene is the same as the gene of Kharkov and Jones Fife.

24. *Developmental studies in the genus Oryza I. The process of embryo sac formation.*[1]

(By Yukio DOIDA)

From the view point of comparative morphology, the process of embryo sac formation was studied in some species of the genus *Oryza*. The materials used are listed in Table 1.

Table 1. Materials and Origin

Species	Strain No.	Origin
O. sativa	Kyoto Asahi	Nat. Inst. Genet.
O. glaberrima	W0026	Central Rice Res. Inst., Cuttack, India. (C.R.R.I.)
O. alta	W0017	C.R.R.I.
O. breviligulata	W0009	C.R.R.I.
O. ridleyi	W0001	Bangkok

O. sativa L.

"Kyoto Asahi", a rice of Japonica type, was used. Its embryo sac formation was found to resemble the so-called "*Polygonum* type" which is the commonest type in the angiosperm. An archesporial cell (=embryo sac mother cell) undergoes normal meiosis resulting in a tetred. Three cells at the micropylar end degenerate. The innermost cell undergoes three successive mitoses, and eight nuclei are formed. The three nuclei at the micropylar end form the egg apparatus and the three at the charazal end form the antipodal cells, the remaining two constituting the polar nucleus. Thereafter, the three antipodal cells become multinuclear or polyploid.

O. glaberrima Steud.

The process of embryo sac formation is almost the same as in common

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

rice. The three antipodal cells hypertrophy and become multi-nuclear or polyploid.

O. alta Swallen

The process of embryo sac formation is almost the same as in common rice. The antipodal cells become multi-nuclear or polyploid. Twenty-four bivalents were observed at MI in PMCs.

O. breviligulata A. Cheval et Roehr.

The process is the same as mentioned above.

O. ridleyi Hook.

The three antipodal cells show a secondary development and the antipodal apparatus consists of several cells, sometimes ten or more. Thus, the process in *O. ridleyi* is somewhat different from that described above for the other 4 species.

In the 5 examined species, the megasporangium consists of a nucellus and two integuments which envelop the mature embryo sac. The ovule is anatropous. The nucellus is well developed at the chalazal end.

25. *The karyotype and intra-genome pairing of chromosomes in haploid plants of Oryza glaberrima*

(By Chao-Hwa HU and Yô TAKENAKA)

Oryza glaberrima Steud., cultivated in the West Africa, is considered to be a distinct species since it differs in several characters from the common cultivated rice, *O. sativa*, and the F_1 hybrid with *O. sativa* is highly sterile. Haploid plants of *O. glaberrima* were obtained from a twin seedling. The chromosomes in root tip cells and PMCs were observed, and the results were compared with those previously obtained in haploid plants of *O. sativa*.

In both *O. glaberrima* and *O. sativa* nine types of chromosomes could be identified on the basis of size, position of constriction, and presence or absence of satellites and secondary constrictions. No karyotypic difference could be found between the two species. Also in the frequencies of bi- and tri-valent chromosomes and of secondary associations in PMCs, the two species showed no significant difference. It is known that the diploid F_1 plants of these two species show no disturbance in chromosomes pairing. It was concluded, accordingly, that these cultivated species of *Oryza* might have cytologically the same chromosomes.

26. *Systematic studies in the genus Oryza by statistical methods*[1)]

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

For evaluating interspecific relationships in the genus *Oryza*, the writers have been engaged in statistical-taxonomical studies of the strains kept in the collection of the Institute. The results of a preliminary investigation were reported last year (Annual Report No.9: 35–36); this paper presents the results of further studies by two methods, factor analysis and Sokal's weighted variable group method, of between-species correlation matrices. Between-species correlations based on measurements of 42 characters, which were assumed to be a quantitative index of the degree of resemblance between species, were calculated among 16 species.

Of the 42 characters, 22 were recorded qualitatively using code numbers, and the rest were measured in standard deviation units. The correlation matrix obtained from the data was analysed first by the *averoid* method of factor analysis, extracting the first to fourth factors. Then, the 16 species were scattered in the space defined by the factor axes. The scatter diagrams showed that certain species are gathered in clusters.

Secondly, the same correlation matrix was analysed by Sokal's method to illustrate interspecific relationships in a tree-like diagram (Fig. 1). The results obtained by both methods were comparable and were in general in accord with the classification of the genus by Roschevicz (1931). However, Roschevicz's Section *Sativae* can be sub-divided into two distinct

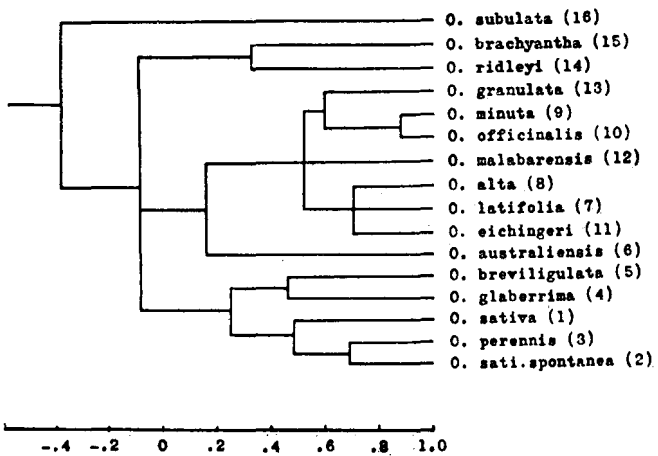


Fig. 1. Tree-like diagram showing interspecific relationships obtained by Sokal's weighted variable group method.

1) This work was supported by Grant RF57080 from the Rockefeller Foundation.

groups of species, one represented by *O. sativa*, *O. perennis*, *O. sativa* f. *spontanea*, *O. glaberrima* and *O. breviligulata* (called the "Sativa Group"), and the other by *O. officinalis* and the other six related species (called the "Officinalis Group"). Section *Coarctatae* (*O. ridleyi* and *O. brachyantha*), as well as Section *Rhynchoryza* (*O. subulata*), are found to form distant groups. It is suggested from the results that the main lines of evolutionary progress in the genus *Oryza* might be directed to the "Sativa" and "Officinalis" groups, leaving other species outside the main lines.

(Published in Evolution 14:153-165, 1960.)

27. *Variation studies in wild rice species, Oryza pernnis and O. sativa f. spontanea, by multivariate analysis*[1]

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

The two wild rice species, *O. perennis* and *O. sativa* f. *spontanea*, can easily be crossed with each other and with the cultivated rice, *O. sativa*, to produce fertile hybrids. They have been, however, considered to be distinct species. The major difference so far recognized between them is that the former is perennial and has rhizomes, while the latter is annual. In order to look into the pattern of variations in these wild rice species, a number of strains belonging to either of the two, collected from India, Thailand and Malaya, were grown in Misima, and records were taken for various characters. In all characters, the strains showed a continuous range of variation.

The multi-character variation in this group was then investigated by the "component analysis" method. The original data were taken for 60 strains, among which seemingly *perennis*, *intermediate* and *spontanea* types were included. Correlation coefficients among five characters, i.e., anther length (A), length/width ratio of grains (G), ligule length (L), panicle length (P), and branch number per panicle (B), were caluculated from the data. Then, two principal components were extracted from the resultant correlation matrix. The two component axes gave the following combinations of characters.

$$X_1 = 1.00\,A + 0.94\,G + 0.26\,L + 0.44\,P - 0.52\,B\,.$$

$$X_2 = 0.12\,A - 0.28\,G + 1.00\,L + 0.74\,P + 0.86\,B\,.$$

The 60 strains investigated were then scattered on a plane defined by the X_1 and X_2 axes (Fig. 1). The scatter diagram showed no particular cluster of strains.

We cannot know, however, from the results of this computation what

[1] This work was supported by Grant RF57080 from the Rockefeller Foundation.

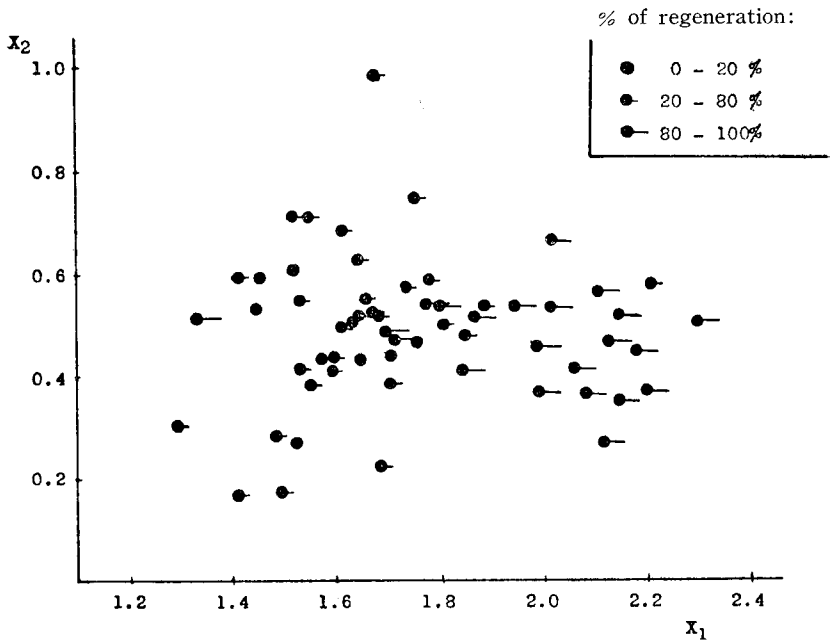


Fig. 1. Strains of *Oryza perennis* and *O. sativa* f. *spontanea* scattered in the plane defined by the first and second component axes.

the component axes mean. We computed two discriminant functions in an attempt to find their meanings, one (Y_1) to maximize the difference between seemingly *perennis* and *spontanea* types, and the other (Y_2) to maximize the difference between wild and cultivated strains. They were found to be

$$Y_1=1.00\,A+0.64\,G-0.02\,P+0.46\,L\,,$$

and

$$Y_2=1.00\,S+0.38\,D-0.20\,W-0.13\,N\,,$$

where A, G, P and L are the notations used in X_1 and X_2, and S, D, W and N represent measurements for degree of shedding, degree of seed dormancy, grain weight, and grain number per panicle, respectively. Having then examined correlations between the 'scores given by X_1, X_2, Y_1 and Y_2, we found high correlation coefficients between X_1 and Y_1 ($r=0.906$), as well as between X_2 and Y_2 ($r=0.519$). It may then be assumed that the first component X_1 shows differentiation in the direction of *perennis* vs. *spontanea* types, and the second component X_2 shows that in the direction of cultivated types.

A further attempt was made to investigate the pattern of variation in this group. Using data for 12 populations belonging to either of the two

species grown in Taichung, Taiwan, a discriminant function to maximize the variance among populations relative to that within populations was constructed. It was

$$Z=-0.09\,Aw+1.00\,L+0.64\,P-0.67\,W\,.\quad (Aw\text{: Awn length})$$

The scores given by this formula seemed to discriminate clearly the *perennis* and *spontanea* types. Frequency distributions of the score, in each population and among populations, were then examined. Among populations, there appeared a continuous array of intergardes from the *perennis* to the *spontanea* types. The variation within populations, which might be indicative of heterogeneity in the population, was narrow in range in *spontanea* types, and was wider in *intermediate* and *perennis* types.

The percentage of regeneration after cutting at maturity was examined, further, with the view to measure the perennial vs. annual habit. Populations of the *perennis* type showed higher percentage than those of the *spontanea* type, but the two species could not be clearly discriminated by the percentage, as shown in Fig. 1. From these variation studies, it was concluded that in so far as Asian materials are concerned, there is no clear distinction between *O. perennis* and *O. sativa* f. *spontanea*, while the two species may be considered to represent both extremities of a continuous variation in the group to which they belong.

28. *Hybrid sterility relations among cultivated and wild strains of rice*[1]

(By Kokichi HINATA and Hiko-Ichi OKA)

In his previous paper on phylogenetic differentiation of Asian cultivated rice varieties, the junior author (OKA 1953) reported on hybrid sterility relationships among the varieties. Hybrid sterility was scored by means of "reaction types" indicated by a combination of plus and minus signs; a plus (or minus) sign respresents the pollen fertility of the F_1 of a given strain crossed with a certain test strain being higher (or lower) than 87.5 %. Thus, the percentages of good pollen, though measured in each cross combination, could not be fully utilized to deduce a final conclusion by this method. In order to overcome this defect, we have attempted in this study to use "component analysis" and other mathematical techniques for treating the data.

A number of wild rice strains, collected from various localities of India and belonging to *Oryza perennis* or *O. sativa* f. *spontanea*, were used as the materials for crosses. They were each crossed with five test strains of *O. sativa*, 108, 414, 521, 563 and 647. The first two, 108 and 414, be-

[1] This work was supported by Grant RF57080 from the Rockefeller Foundation.

long to the so-called "Continental" or "Indica", 521 and 563 to the "Temperate-Insular" or "Japonica" and the last one, 647, to the "Tropical-Insular" type. These are the same strains used by the junior author (OKA 1953) before; therefore, his old data for many cultivated strains could be analysed together with the data for the wild strains.

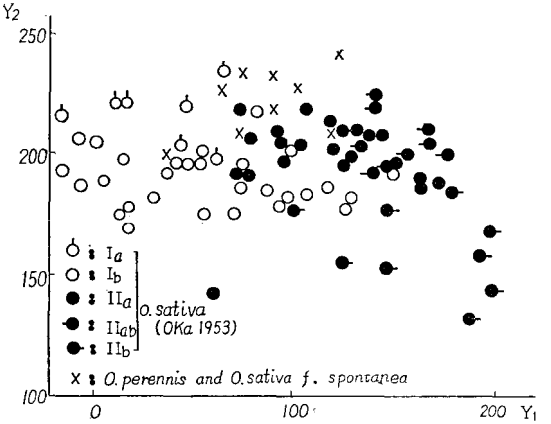


Fig. 1. Distribution of wild and cultivated strains in the plane defined by the first and second component axes extracted from the data for hybrid sterility.

In the original data, each strain to be examined was represented by a set of five good-pollen-percentages (A, B, C, D, E), one each from the five test strains. Taking those values as variates of the strain, the first and second components were extracted from the data for cultivated strains. The two components gave weights (relative magnitudes of direction cosines) to the five variates as follows:

$$Y_1 = -0.97\,A - 0.56\,B + 1.00\,C + 0.93\,D + 0.86\,E\ ,$$

$$Y_2 = \ \ 0.57\,A + 0.77\,B + 0.02\,C + 0.16\,D + 1.00\,E\ .$$

Secondly, from the same data, a discriminant function was computed to combine the five variates so as to maximize the difference between the "Continental" and the "Insular" types, into which the strains had been divided. The discriminant formula was found to be

$$Y_3 = -1.00\,A - 0.18\,B + 0.12\,C + 0.58\,D + 0.62\,E\ .$$

Comparing the Y_1 and Y_3 formulas, we find that they have the same arrangement of signs for the five variates.

Further the scores given by another discriminant function, computed by Oka (1956) to classify the "Continental" and the "Insular" types by combining measurements for various characters, showed a high correlation

coefficient ($r=0.65$) with the scores given by Y_1. It may then be assumed that the first component, Y_1, represents the differentiation of these two phylogenetic groups in *O. sativa*.

Then, we examined the scatter diagram in Fig. 1, which shows the distribution of the strains used in the plane defined by the two component axes. The "reaction-types" classified by the old method are also shown in the figure. It is recognized in the figure that the old and new methods have given comparable results, though the continuity of variation could be illustrated only by the new method. Further, an interesting fact found in Fig. 1 is that most wild strains appear to be intermediate between the "Continental" and "Insular" types in the score given by Y_1, while they have larger scores for Y_2 than the cultivated ones and are located in the upper part of the diagram. It may then be assumed that the second component, Y_2, represents the direction of variation from wild to cultivated types.

In the original data, a greater part of the wild strains tested showed high fertility with all of the five test strains, though the latter show sterility in their mutual F_1s. It may then be said that the differentiation among wild strains toward the "Continental" and "Insular" or the "Indica" and "Japonica" types is not much advanced. However, in the whole group including both wild and cultivated forms, the major direction of differentiation might be toward the "Continental" and "Insular" types. After subtracting this component, the remaining part of the variation given by the second component, Y_2, might represent the differentiation of wild and cultivated types. Thus, the scatter diagram given by the two component axes may be regarded as a pictorial representation of the development of cultivated types from wild ones.

29. *Variation in hybrid sterility with certain test strains within populations of wild rice* [1]

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

Populations of wild rice (*O. sativa* f. *spontanea and O. perennis*), collected from various spots in India, were raised in the experimental field in Taichung, Taiwan, and a number of plants taken at random were crossed with two test strains of cultivated rice (*O. sativa*). Thus, two kinds of F_1 and self-pollinated seeds were obtained from each plant. Peiku (or 108, Indica) and Taichung No. 65 (or 504, Japonica), whose F_1 shows semi-

[1] This work was supported by Grant RF57080 from the Rockefeller Foundation.

sterility, pollen fertility being about 45 %, were chosen as test strains.

Variations in pollen fertility found among the F_1s and self-pollinated lines thus obtained are given in Table 1. As the data in the table show, in the case of a population of *O. sativa* f. *spontanea*, W203, all self-pollinated lines as well as those crossed with 108 showed high fertility, and semi-sterility was found only in a few lines crossed with 504. As reported in another paper (HINATA and OKA), the F_1s between this species and various cultivated rice varieties are generally fertile, though between the cultivated ones, those distantly related show semi-sterility in their F_1s. The above-mentioned fact suggests, however, that the populations of *O. sativa* f. *spontanea* might contain plants which produce semi-sterile F_1s with a certain cultivated type.

In the case of the W208 population of *O. perennis*, semi-sterility occurred not only in the F_1s but also in self-pollinated plants. The mechanism of sterility in self-pollinated plants and in the original population is a problem to be studied.

Table 1. Variations in pollen fertility among self-pollinated lines of wild rice and their F_1s with two test strains of *O. sativa*.

Population		Percent of good pollen													No. of lines
		100	95	90	85	80	75	70	65	60	55	50	45	40	
W203	Self	10	8												18
(*spontanea*)	108 x	4	4												8
	504 x		8	1			2								11
W208	Self		2	5	3		1								11
(*perennis*)	108 x	1		1		2		2	1						7
	504 x		3	2	2		1				1			1	10

30. *An investigation of floating habit in wild rice species* [1]

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

In tropical Asian countries, delta areas of rivers are flooded in the rainy season and the depth of water reaches several meters. In such areas, floating rice is the only food crop which can be grown. The floating habit of rice is potential; when deep water conditions are given, rapid elongation of internodes, branching of culms, and development of adventitious roots from water-submerged nodes take place. However, if grown in an ordinary paddy field, the floating varieties show the same growing habit as the non-floating ones.

[1] This work was supported by Grant RF57080 from the Rockefeller Foundation

Oryza perennis and *O. sativa* f. *spontanea* are wild species closely related to *O. sativa*. The former usually grows in deep-water swamps and seems to be of floating nature. In order to examine if they have floating habit or not, 10 strains, belonging to *O. perennis* and *O. sativa* f. *spontanea* and 9 cultivated strains, collected from various localities in India and Thailand, were tested in a pond as follows: Seedlings were raised in a nursery bed for two months and were transplanted at the bottom of a pond 72 cm deep. Starting from the tenth day after transplanting, water was added to the pond increasing its depth by 4 cm a day. The plants then remained in the pond for two months. Records were taken regarding (1) number of internodes longer than 3 cm, (2) total length of elongated internodes, (3) number of branches per tiller, (4) number of nodes with adventitious roots, (5) presence or absence of rhizomes, and (6) tiller number. It was assumed that floating ability would be measured by characters 1 to 4.

The results of this experiment showed that all strains seemingly belonging to *O. perennis* had floating ability, and those belonging to *O. sativa* f. *spontanea* had it also to some extent. Among cultivated strains, however, those with and without floating habit could be distinguished relatively clearly. Rhizomes were found in two strains of *O. perennis*.

It was then suggested that *O. perennis* as well as *O. sativa* f. *spontanea* might generally have floating ability, though its degree may vary among populations in accordance with the depth of water in their habitat. As reported in another paper (MORISHIMA and OKA), these two wild species show a continuous array of intergrades and cannot be distinctly classified. It is possible that the differentiation of *perennis* and *spontanea* types results from adaptation to different depths of water.

31. *Variation in seed dormancy of wild rice species*[1]

(By Hiroko MORISHIMA and Hiko-Ichi OKA)

It seems that dormancy of seeds is an important character distinguishing the wild from the cultivated types of rice. Twenty eight strains of *Oryza perennis* and *O. sativa* f. *spontanea*, collected from various localities in India, and 92 cultivated strains (*O. sativa*) from various Asian countries, were tested for seed dormancy. Seeds harvested about 20 days after heading were kept in a desiccator, and germination tests with hulled and unhulled grains of each strain were made three times, 50, 90 and 180 days after heading. The germinating capacity and the average number of days required for germination were thus obtained for each sample. In order

[1] This work was supported by Grant RF57080 from the Rockefeller Foundation.

to evaluate the germinating activity of a sample as a whole, however, the two measurements were combined as follows:

"Germinating activity" = (% of germination in 10 days) $\times \frac{11-\text{No. of days}}{10}$.

The number of days required for the "germinating activity" to reach 40 % was then calculated for each strain as the measure for the degree of dormancy. Frequency distributions of the number of days thus obtained with hulled seeds are given in Table 1.

Table 1. Variation in number of days required for "germinating activity" reaching 40 %. (Tests with hulled seeds)

Type	No. of days								No. of strains
	40	60	80	100	120	140	160	180 or more	
Prennis							2	2	4
Intermediate	5	1		1				3	10
Spontanea			2	1		2		6	11
Cultivated (*O. sativa*)	86	3	1		1	1			92

As shown in the table, wild strains generally had stronger dormancy than the cultivated ones. It seems, however, that some strains of intermediate type between *perennis* and *spontanea* had a relatively weak dormancy like that of cultivated strains. If the hulls were not removed, the seeds of wild strains showed a low germinating activity even though six months had elapsed after maturity.

32. *Variation in photoperiodic response among wild rice population of Thailand* [1]

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

In the Annual Report for 1958 (No. 9: 47–48), the writers have reported variations in photoperiodic response among Indian populations of *Oryza perennis* and *O. sativa* f. *spontanea*. In 1959, similar investigations were made using materials collected from Thailand. The results are given in Table 1. The data show that, in the same manner as in the Indian materials, strains seemingly belonging to *O. perennis* are highly sensitive to photoperiod, and those belonging to *O. sativa* f. *spontanea* have a relatively low sensitivity. As discussed in another paper (MORISHIMA and OKA), however, these two species show a continuous array of intergrades. Some

[1] This work was supported by Grant RF57080 from the Rockefeller Foundation.

Table 1. Variation in photoperiodic response in wild rice populations collected from Thailand.

Species	Population	Index of sensitivity (TDM)			Critical day-length		
		Mean	Earliest	Latest	Mean	Earliest	Latest
O.sativa f. *spontanea*	W101*	86°			13h28′		
	W168	45°	14°	71°	13h21′	13h49′	13h01′
	W170	37°	55°	17°	13h13′	13h57′	13h30′
	W173	45°			13h27′		
"Intermediate"	W163	90°			12h42′		
	W164	90°			12h44′		
	W171	86°	73°	90°	12h38′	12h53′	12h25′
	W179	61°	11°	72°	12h57′	13h20′	12h44′
O.perennis	W169	90°			12h32′		
	W172	90°			12h30′		
*O.sativa*** (Cultivated)	108	3°	0°	11°			
	414	46°	49°	41°	14h04′	14h07′	14h02′
	504	11°	3°	19°			
	521	41°	40°	36°	14h23′	14h26′	14h21′
	647	15°	23°	6°			

* From India.

** The difference between the earliest and the latest plants in cultivated strains shows the magnitude of sampling error.

populations of *intermediate* type showed a high sensitivity like that of *O. perennis*. Regarding variation whthin populations, the data of Table 1 suggest that it is wider in *O. sativa* f. *spontanea* than in *O. perennis*.

33. *The impact of cultivation on populations of wild rice, Oryza sativa f. spontanea* [1]

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

In the Raipur District, Madhya Pradesh, India, *Oryza sativa* f. *spontanea* is a common grass, which is also found in paddy farms as a weed. The senior writer visited this area in November, 1957, and collected seeds of this species from populations growing in a grass land apart from paddy

[1] This work was supported by Grant RF57080 from the Rockefeller Foundation.

farms (I), in a patch between paddy farms (II), and in a paddy field (III), and seeds of a cultivated variety (*O. sativa*) from the paddy field in which Population III was found (IV). The plants from those seed samples were grown and investigated in Taichung, Taiwan, in a plant-to-row experiment. First, it was found that the four populations showed a continuous range of variation in heading date, panicle length, grain weight and other characters. A discriminant function was computed to maximize the difference between Populations I and IV by combining those characters. Its score also showed a continuous variation. It was then assumed that Populations II and III might be due to introgressive hybridization between wild and cultivated plants.

Secondly, the percentages of out-crossing, estimated by a biometrical method from variances of grain size and heading date, were found to be 25% – 30% in Population I, less than 10% in Population IV, and 10% – 20% in Populations II and III. As to the degree of seed dormancy, it was also found that Populations II and III could be regarded to be intermediate between I and IV. In parallel to this, the disturbance of habitat by man seemed to increase from I to IV. It was then hypothetically assumed that a population produced by natural hybridization between wild and cultivated plants might acquire characters of the cultivated type in accordance with the "pressure of cultivation" under which the population grows.

(Published in Phyton 13: 105–117, 1959)

34. *Distribution of the glutinous gene in wild and cultivated populations of rice in Thailand* [1]

(By Hiko-Ichi OKA)

In the Northern and North-Eastern regions of Thailand, glutinous rice is grown in almost all paddy farms as the principal food crop. The writer travelled through those "glutinous area" from November to December, 1958, and collected seeds from a number of wild and cultivated populations. Seven samples of cultivated glutinous rice, each consisting of 40 to 80 plants, were found to be a mixture of non-glutinous and heterozygous plants, and the frequencies of the glutinous gene varied from 97% to 62%. The percentage of out-crossing was estimated from the frequency of non-glutinous seeds in glutinous homozygous plants and from the frequency of the glutinous gene in the population. It varied from 0% to 3.6%.

[1] This work was supported by Grant RF57080 from the Rockefeller Foundation.

Secondly, 14 wild populations of *Oryza perennis* or *O. sativa* f. *spontanea*, surrounded by glutinous cultivated rice, were investigated in the same manner. Six of them were purely non-glutinous, other six contained the glutinous gene with a low frequency, but the remaining two showed high frequencies of the glutinous gene, i.e., 28% and 26%; for the former, the percentage of out-crossing was estimated to be 44%. This indicates that under certain conditions, wild rice populations can absorb genes from adjacent cultivated plants.

Plants from the seeds of those cultivated and wild populations were investigated in Taichung, Taiwan, in a plant-to-row experiment. The genetic components of variances of seed length, estimated for the wild population with 44% out-crossing, are shown in Table 1.

Table 1. Genetic variances of seed length in classes with different combinations of the glutinous gene.

Class	σ_p^2,	σ_0^2	No. of lines	Mean(mm)
++	.0567	.0680	31	8.43
+*gl*	.0499	.0906	41	8.30
glgl	.0488	.0059	8	8.47

The data in Table 1 show that in inter-plant variance (σ_p^2, among lines in the experiment), the three classes++, +*gl*, and *glgl* did not differ so much from one another, while the *glgl* class had an apparently smaller offspring variance (σ_0^2, within line) than the other two classes. This may indicate the effect of self-pollination.

35. *Population-genetic studies of wild rice of Taiwan* [1]

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

The senior writer once assumed that the wild rice found in Tao-Yuan Prefecture, Taiwan, might be *O. sativa* f. *spontanea* (Annual Report No.6: 46–47), but its rhizomes indicate that it is rather *O. perennis*. Three small populations, called A, B and C by the writers, can be found at present. Seeds were taken from a number of plants of the three populations, and the offspring were investigated in the experimental field in Taichung. First, the results proved that the majority of plants bore the characters of cultivated rice, such as non-shedding, heavy seed weight, etc., though the plants in the natural habitat appear to be completely of wild type.

Secondly, with regard to seed length and seed width, the genetic vari-

[1] This work was supported by Grant RF57080 from the Rockefeller Foundation.

ances were estimated among plants of the natural population (σ_p^2), among line means of the offspring ($\sigma_{p'}^2$) and within lines (σ_o^2). Also the covariance between parental plants and offspring line means ($\sigma_{pp'}$) was estimated. The frequency of heterozygotes for genes controlling those characters ($2y$), relative to the percentage of out-crossing (λ), was computed by using the formulas given below. The results showed that within the range $\lambda=0.20-0.70$, the frequencies of heterozygotes ($2y$) were much higher than those expected under the assumption that the population was not affected by selection, $2y=\lambda/1+\lambda$ (assuming that gene frequency $x=\frac{1}{2}$). In other words, the populations might consist mainly of heterozygous plants owing to their selective advantage. In view of this fact, it was suggested that the populations of wild rice can absorb and preserve a large amount of genetic material from cultivated plants and release it if sexually propagated.

Formulas used for computation:

$$\sigma_p^2 = \Sigma(1-2y)d^2+\Sigma 2y(1-2y)h^2 ,$$

$$\sigma_{p'}^2 = \left(1-\frac{\lambda}{2}\right)^2\Sigma(1-2y)d^2+(1-\lambda)^2\Sigma\frac{y}{2}(1-2y)h^2 ,$$

$$\sigma_{pp'} = \left(1-\frac{\lambda}{2}\right)\Sigma(1-2y)d^2+(1-\lambda)\Sigma y(1-2y)h^2 ,$$

$$\sigma_o^2 = \Sigma\left[y+\frac{\lambda}{2}\left(1-\frac{\lambda}{2}\right)(1-2y)\right]d^2+\Sigma\left[\frac{y}{2}+\frac{\lambda}{2}\left(1-\frac{\lambda}{2}\right)(1-2y)\right]h^2 .$$

36. *Variation in yielding capacity among isogenic lines of rice*

(By Hiko-Ichi OKA)

A variety of crop plants represents an assembly of genotypes which respond to outer conditions in a certain manner. When a few particular genes of the genotype are replaced by others, the genotype may in some cases be disharmonized as the whole, resulting in a break-down of the reaction-norm. In other cases, however, certain characters may be changed without causing disintegration of the reaction-norm determined by the genetic back-ground. For instance, early varieties of rice generally have lower yielding capacity than late ones. Taking a variety with high yielding capacity, if a few genes controlling the duration of growing period only could be replaced by proper ones introduced from an early variety, the potential yielding capacity of the genotype may be utilized to the maximum.

From the above-mentioned view-point, the writer has been engaged, with collaboration of Mr. K. H. TSAI of the Taiwan Provincial Agricultural College, in the breeding of isogenic lines of Taichung no. 65, a representative "Horai" variety grown in Taiwan. The results for glutinous

isogenic lines were reported last year (Annual Report No. 9: 34-35); in this paper, data for early lines are presented.

A strain of Taichung no. 65 was crossed with two early varieties, and then back-crosses were repeated seven times using the former as the recurrent parent. As the donor parent for earliness, "Tatong-tsailai" from Northern China, and "Bozu no. 5" from Hokkaido, Japan, were used. The B_7F_3 lines thus obtained were tested together with the recurrent parent. Fifty two lines from "Tatong-tsailai" (Class A), and 5 lines from "Bozu no. 5" (Class B) were 15 to 19 days earlier than the control (Taichung no. 65), and the other 25 lines from "Bozu no. 5" (Class C) were 3 to 7 days earlier, though they showed the same growing habit as Taichung no. 65. Variation in rough rice yield per plant of those lines, tested in the second crop season of 1959, is given in Table 1. The data in the table show that a part of the early isogenic lines may have the same yielding capacity as Taichung no. 65.

Table 1. Variation in yield among early isogenic lines of Taichung no. 65.

Class	Yield per plant, gm								No. of lines	Heading date
	16	18	20	22	24	26	28	30		
Control	1	2	3	11	3				20	Oct. 8-12
B_7F_3 A	11	14	16	9	2				52	Sep.21-25
〃 B		3	2						5	Sep.21-25
〃 C		1	5	7	6	1	3	2	25	Oct. 3- 7

37. *Genetic variability in established varieties of rice*[1]

(By Kan-Ichi SAKAI)

The genetic variability in this experiment is measured in terms of heritability, i.e. amount of genetic variance as a percent of total variance. Three rice varieties of hybrid origin in Ceylon were investigated. The heritability values estimated for seed size characters, yield capacity, tiller number and plant height are listed in Table 1.

It is found in Table 1 that of these three varieties, H 106 is the most while H 105 is the least heterogeneous. A number of lines were selected from different yield levels in each variety, and they were planted in the following season. The correlation between yields of the two season is computed (Table 2).

1) This study was conducted in the Division of Botany, Department of Agriculture, Ceylon.

Table 1. Heritability values of different quantitative characters in three rice varieties.

	H 105	H 106	H 501
Seed length	21.85	82.51	37.95
Seed width	11.51	24.95	28.55
Yield	11.58	38.17	20.19
Tiller number	0.84	19.72	0.00
Plant height	30.64	66.61	47.06

Table 2. The total, between-classes, and within-classes correlation coefficients between yields of two consecutive seasons.

	Correlation coefficients		
	H 105	H 106	H 501
Total	0.7811	0.6005	0.5585
Between-classes	0.9951	0.9319	0.6890
Within-classes	0.3480	−0.0140	0.2759

Table 2 shows that in general, the yields of the same lines in two consecutive seasons are positively correlated. The correlation is very high if different classes are compared, but very low if comparison is made within classes. On the basis of these findings, it is concluded that:

1) Established varieties of rice, a plant which is believed to be an autogamous plant, are not always genetically pure. They are more likely mixtures of different lines, at least with respect to polygenic characters.

2) In view of this, an established variety of rice may be capable of altering its performance during its maintenance and propagation.

3) A suggestion is made that seed of high genetic purity could be produced by improving the scheme of seed farm operation by introducing simplified performance tests accompanied by elimination of inferior lines. Heritability tests should also be included in the scheme.

Details of the present study will be published at another opportunity.

38. *Variability of seed size characters in wild rice*[1]

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

Variability of seed size characters was investigated for several wild rice

[1] The main part of this study was conducted at Peradeniya, Ceylon, while the remaining part was done at Misima, supported by Grant RF57080 from the Rockefeller Foundation.

populations of *Oryza perennis* collected in five localities on the west coast of Ceylon. Measurements were taken for length and breadth of seed, mean values of which are presented in Table 1.

Table 1. Mean values of length and breadth of seed in six populations of wild rice.

Population	Locality	No. of seeds measured	Seed length	Seed breadth
Ky	Kirimetiana	50	7.96±0.51	2.45±0.23
Nt	Nattandiya	50	7.47±0.49	2.67±0.29
Th-(1)	Thumodara	50	7.15±0.83	2.85±0.14
Th-(2)	Thumodara	27	7.62±0.33	2.31±0.13
Kd	Kudawala	50	7.93±0.27	2.14±0.10
Md	Madampe	50	8.04±0.37	2.34±0.16

Genetic and environmental variance components as well as genetic and environmental correlations were estimated for each population, assuming that within-plant variation or covariation is environmental while between-plant variation or covariation is the sum of environmental and genetic variations. The proportion of genetic variance is presented in Table 2. Genetic and environmental correlations between seed length and seed breadth are also presented in the same table.

Table 2. Genetic variability and genetic and environmental correlations for seed size characters of wild populations of rice.

Population	Genetic variance		Mean	Correlations	
	Seed length	Seed breadth		Environmental	Genetic
Ky	0.1836	0.5835	0.3836	0.0051	0.0935
Nt	0.7892	0.6490	0.7191	0.0197	0.0267
Th-(1)	0.9688	0.7423	0.8556	−0.0544	−0.0005
Th-(2)	0.7892	0.3456	0.5674	0.2961	0.6666
Kd	0.8483	0.7026	0.7755	0.1750	0.4410
Md	0.6880	0.5598	0.6239	0.2416	0.3039

It is found from Table 1 that seed length as well as seed breadth differ markedly from population to population, the difference being often statistically significant. Table 2 shows that most populations are very heterogeneous so far as size characters are concerned.

The same investigation will be continued with other populations.

39. *Variation in anthesis in wild rice populations (Oryza sativa f. spontanea) collected in India and Ceylon*[1)]

(By Takashi NARISE and Shin-ya IYAMA)

Number of days from seeding to flowering of four wild rice populations (*Oryza sativa* f. *spontanea*) collected in India and Ceylon was investigated. The places where the material was collected are as follows.

TR	Trichur	Kerala State, India
CN	Canning	West Bengal State, India
CT-b	Cuttack	Orissa State, India
PL	Puttalam	West coast of Ceylon

Progeny lines of plants collected at random in the wild populations were grown in an experimental field at Peradeniya, Ceylon. Seeds were sown in the nursery on April 8, 1959 and the seedlings were transplanted into a paddy field on May 6. Each line contained two to ten plants on which flowering date observation was made. Within-population variability of lines with respect to number of days from seeding to flowering is shown in

Table 1. Frequency distribution of number of days from seeding to flowering in four populations of *O. sativa* f. *spontanea*.

Population	No. of lines	110–120	120–130	130–140	140–150	150–160	160–170	170–180	180–190	190–200	Average
CN	19					12	7				159.4 days
CT-b	19				1	9	3	6			163.4
TR	19	8	10		1						122.5
PT	31					9	15	5	1	1	164.5

Table 2. Analysis of variance of number of days from seeding to flowering of four wild rice populations. 1959.

Source of variation	d.f.	Mean square	Expectation of mean square	Estimate of the component of variance
Total	372			
Population	3	30,621.818**	$\sigma_w^2+4.859\sigma_s^2+92.218\sigma_p^2$	$\hat{\sigma}_p^2$ 329.204
Lines within population	84	241.401**	$\sigma_w^2+4.208\sigma_s^2$	$\hat{\sigma}_s^2$ 33.514
Within lines	285	100.375	σ_w^2	$\hat{\sigma}_w^2$ 100.375

** Significant at 1% level.

1) This work was supported by Grant RF57080 from the Rockefeller Foundation.

Table 1. The results of analysis of variance is presented in Table 2. Intraclass correlation coefficients were computed as follows.

Between strains within populations: $\frac{\sigma_s^2}{\sigma_s^2+\sigma_w^2}=0.250$

Between populations: $\frac{\sigma_p^2}{\sigma_p^2+\sigma_s^2+\sigma_w^2}=0.710$

As Table 2 shows, between population variation and between lines within population variation were statistically significant, though the latter was relatively small.

The result of this experiment indicates that wild rice populations are highly differentiated with regard to flowering time.

40. *Further note on natural crossing in wild rice*[1]

(By Kan-Ichi SAKAI and Takashi NARISE)

Occurrence of natural crossing in wild rice was measured by means of biometric analysis of seed width. Wild rices investigated were three geographical races of *Oryza sativa f. spontanea*, collected in India. One Ceylon variety of cultivated rice, *O. sativa* L., was also investigated for comparison. Lines were grown from plants collected at random and analysis of variance of seed width was made (Table 1).

Table 1. Within and between lines variances and the estimated percentage of natural crossing in wild rice populations. One variety, H 106, of cultivated rice is also included for comparison.

	H 106 variety	*O. sativa* f. *spontanea*		
		Trichur	Canning	Cuttack
Within-line variance	0.0022	0.0114	0.0179	0.0266
Between-line variance	0.0596	0.0267	0.0431	0.1413
Percentage of outcrossing	3.60	33.91	31.93	16.55

Percentages of outcrossing listed in Table 1 suggest that wild rice plants are disposed to more frequent cross-pollination under natural circumstances than domesticated varieties.

[1] This study was conducted in Peradeniya, Ceylon, supported by Grant RF57080 from the Rockefeller Foundation.

41. *Competition experiment with wild rice*[1)]

(By Takashi NARISE and Kan-Ichi SAKAI)

Oryza perennis collected at Cuttack, India and *O. sativa* f. *spontanea* at Illuppaiyadichchenai, Ceylon, were tested for their competitive ablility against two cultivated varieties of rice in Ceylon. *O. perennis* was tested in the Maha (winter) season of 1958/59, while *spontanea* was tested in two consecutive seasons of Maha 58/59 and Yala (summer) 1959. The test varieties used in this experiment were Murungakayan 302 and H 105. Plant weight and number of tillers were compared between pure-stand and mixtures with each of the test varieties. Experiments were all conducted with three replications.

Analysis of variance of data showed that the effect of competition with test varieties was highly significant for plant weight as well as number of tillers in *perennis* but not for the former in *spontanea*. Mean values of tiller number and plant weight in pure-stand and mixtures with the two test varieties are presented in Table 1.

Table 1. Number of tillers and plant weight of two wild species of rice in pure-stand and mixtures with two test varieties.

	Number of tillers				Plant weight			
	perennis	*spontanea*			*perennis*	*spontanea*		
	Maha	Maha	Yala	Mean	Maha	Maha	Yala	Mean
Pure-stand	9.38	10.62	22.37	16.50	7.05	32.42	79.77	56.10
Mixture with M 302	2.20	3.71	15.71	9.71	6.42	11.16	72.52	41.84
Mixture with H 105	1.97	2.95	17.90	10.43	5.45	7.54	93.07	50.31

Table 1 shows that number of tillers as well as plant weight of wild rice decrease if they are grown in competition with the cultivated varieties used in this experiment. It means that wild rice is a weak competitor against the test varieties.

In another experiment dealing with competition among a number of cultivated varieties of rice, it was observed that Murungakayan 302 and H 105 could be taken as representatives of competitive ability of the cultivated varieties of Ceylon. It can be concluded then that wild rice

[1)] The present study was carried out in Peradeniya, Ceylon, supported by Grant RF57080 from the Rockefeller Foundation.

may be very weak in competitive ability in comparison with cultivated rice. This result is of interest from the viewpoint of the evolution of wild plants in the presence of cultivated varieties.

42. *Propagating capacity and competitive ability of the F_1 hybrid between upland rice and "red rice".*

(By Shin-ya IYAMA)

The field of upland rice is often contaminated by the coarse "red rice" variety which has long been removed from our list of commercial varieties but is still surviving as a weed in rice fields. Spontaneous hybrids may arise between the red rice and a commercial variety of upland rice. The present experiment has been undertaken for the purpose of elucidating the fate of those hybrids in the upland rice population.

1) Propagating capacity of the F_1 hybrid.

Phenotypic comparison of "red rice", two varieties of upland rice, Nôrin Mochi Nos. 8 and 18, and their F_1 hybrids are shown in Table 1.

Table 1. Several characters of the F_1 hybrids between upland rice and "red rice" (Nôrin Mochi No. 8×"red rice" and Nôrin Mochi No. 18×"red rice") in comparison with their parental varieties.

	Plant height	Panicle length	No. of panicles	Total weight	Panicle wt. per plant	No. of seeds per plant	Fertility	
							pollen	seed
	cm	cm		g	g		%	%
Nôrin M. No. 8	90.7	20.0	7.4	22.3	9.8	333.9	98.2	—
F_1 (8×R)*	126.4	22.2	15.4	56.2	19.5	357.6	43.7	58.6
Nôrin M. No. 18	82.5	19.7	7.4	23.0	8.0	232.4	98.4	80.9
F_1 (18×R)**	127.7	22.9	15.7	60.7	17.1	314.2	33.6	48.2
"Red rice"	84.4	16.3	18.9	32.6	8.9	324.1	97.1	85.2

* F_1: Nôrin Mochi No. 8×"red rice".

** F_1: Nôrin Mochi No. 18×"red rice".

As Table 1 shows, the F_1 hybrids produce more seeds than the parental upland rice varieties although no great difference from "red rice" is recorded. It is rather surprising that the number of seeds of the hybrid is not less than that of its parents because of high hybrid sterility.

2) Competitive ability of the F_1 hybrids.

The F_1 hybrid plants were grown in a row together with their parental upland rice variety planted in alternate hills. The characters of the F_1 and the parental plants in the competition plot were compared with those

in the pure-stand plot. The difference between the two plots would be an index of competitive ability. The result of analysis of variance for competitive ability on a logarithmic scale is presented in Table 2. Gain or loss in the characters due to competition is shown in Table 3.

Table 2. Analysis of variance of competitive ability of F_1 hybrids and parental upland rice varieties (logarithmic scale).

Source of variation	d.f.	Mean square				
		Panicle number	Total weight	Panicle wt./plant	Plant height	Panicle length
					$\times 10^{-3}$	
Replication	2	.011,24	.023,11	.000,72	.1906	.001,34
Strain	3	.192,30**	.250,31**	.388,38**	2.0998	.001,96
Parents vs. F_1's	1	.554,79**	.722,96**	1.129,82**	6.1156*	.005,13
Parental group+	1	.015,77	.017,38	.025,02	.0291	.000,43
Interaction	1	.006,35	.010,65	.010,31	.1548	.000,31
Error	6	.012,17	.009,38	.026,46	.5681	.001,35

+ (Nôrin Mochi No. 8 and its F_1) vs. (Nôrin Mochi No. 18 and its F_1).
* Singnificant at 5% level. ** Significant at 1% level.

Table 3. Increment due to competition (difference between mix-planted plots and pure-stand plots). Measurements for pure-stand plots are shown in Table 1.

	Panicle wt./plant	Total weight	Panicle number	Plant height	Panicle length
	g	g		cm	cm
Nôrin Mochi No. 8	− 4.6	− 7.2	− 2.3	− 6.2	− 0.8
F_1 (8×R)	18.2	47.3	8.8	6.7	0.7
Nôrin Mochi No. 18	− 5.0	−11.8	− 3.8	− 4.8	− 1.7
F_1 (18×R)	14.1	48.4	7.6	3.6	0.6

Inspection of Tables 2 and 3 leads us to conclude that: (1) Difference in competitive ability of any of the F_1 hybrids and any upland rice variety is statistically significant. (2) F_1 hybrids show a marked increase as a result of competition with the parental varieties, while the latter show a significant decrease.

In conclusion, it may be expected that spontaneous F_1 hybrids from natural hybridization would be favored in an upland rice population due to their strong competitive ability and heterosis in spite of high hybrid sterility.

43. *Seasonal sterility of rice in Ceylon*[1]

(By Kan-Ichi SAKAI)

Three varieties of rice grown in Ceylon were planted in pots in a plant cage on the 17th of every month from December, 1957 through November, 1958. Each plot consisted of 9 plants of each variety. The seed sterility averaged on a 9 plant basis for every plot is presented in Table 1.

Table 1. Seed sterility of three varieties of rice planted at one month intervals from December 1957 through November 1958.

Treatment number	Date of planting	Seed sterility (%)			
		Mas M-24	Murungakayan 302	Murugan Samba 3081	Mean
1	17/12/57	37.31	15.97	28.10	27.13
2	17/ 1/58	22.98	9.59	24.77	19.11
3	17/ 2/58	23.96	8.28	21.48	17.91
4	17/ 3/58	24.28	9.17	22.63	18.69
5	17/ 4/58	40.18	27.02	44.06	38.75
6	17/ 5/58	42.10	20.31	18.77	27.06
7	17/ 6/58	36.01	13.01	26.21	25.08
8	17/ 7/58	11.98	2.50	22.00	12.16
9	17/ 8/58	—	—	—	—
10	17/ 9/58	29.22	5.40	53.48	29.37
11	17/10/58	30.76	33.98	14.37	26.37
12	17/11/58	—	—	—	—
Mean		29.88	14.52	27.59	

The following facts are pointed out by this experiment:

1) Seed sterility is especially high in plants planted between April and June and between September and October.
2) Varieties differ with respect to the occrrence of seed sterility.

The cause of this seed sterility is uncertain. However, the facts described above are of interest for two reasons. One is that rice plants are usually planted in April and September in Ceylon. The other is that the occurrence of seasonal seed sterility seems to be genotypically controlled.

[1] This experiment was conducted in the Division of Botany, Department of Agriculture, Ceylon.

44. *Studies on a laboratory method of testing salinity resistance in rice varieties*[1)]

(By Kan-Ichi SAKAI and P. M. RODRIGO)

"Salt resistance" of rice plants includes two aspects, the physiological resistance of the plant against saline and the overall resistance of the plant as shown by its performance under field conditions. The present study has been undertaken to detect a laboratory method for testing the physiological resistance of rice varieties against salinity. A number of varieties of rice were grown in pots without any fertilizer or manure. At the end of 40 days the plants were removed from the pots and transferred to conical flasks of water for 48 hours. Afterwards, the water was replaced with saline solutions, the salt used being unrefined common salt. The plants were left with their roots immersed in saline solutions for a definite number of days whereupon the evaluation of resistance was made by grouping the plants according to the damage caused. The damage was first observed in the rolling up of the leaf tips, followed by rolling up of the entire leaf blade, yellowing and wilting of the entire plant. The degree of damage was classified from 0 (least damaged) to 5 (most damaged).

The experiment started by treating plants with 20%, 10%, 5% and 0% saline solutions. It was found in this experiment that even 5% concentration was too high to detect varietal differences. In the second experiment, 4, 2, 1 and 0% saline solutions were used. It was found that 2% was still too high to allow an effective comparison among different varieties. In the third experiment treatment was made with 1.5%, 1%, 0.5% and 0% saline solutions and the evaluation of damage caused was made twice, 2 days and 7 days after treatment. Table 1 presents the correlation coeffi-

Table 1. Correlation coefficients between the 1st and the 2nd evaluation in the 3rd experiment.

Degrees of freedom	Percentage of salt in solution		
	1.5%	1.0%	0.5%
26	0.5581**	0.7131**	0.6390**

** Significant at the 1% level of probability.

cients between the records of the first evaluation made on the third day and those of the second evaluation made on the eighth day of the third

1) The present study was conducted in the Division of Botany, Department of Agriculture, Ceylon.

experiment.

The used varieties were divided into two groups: salt-resistant and non-resistant. The latter group includes varieties whose resistance to saline conditions is either in doubt or unknown. A chi-square test was conducted on the relation between the results of the experiment and the two groupings, resistant vs. non-resistant (Table 2).

Table 2. Chi-square test.

		Salt solution		
		1.5%	1.0%	0.5%
1st evaluation	χ^2	1.6072	7.1458	1.8046
	Probability	0.20–0.50	0.01	0.10–0.20
2nd evaluation	χ^2	2.0286	11.3000	2.0255
	Probability	0.02–0.05	0.01	0.10–0.20

Tables 1 and 2 show that the most suitable of the three concentrations of salt solution for discriminating between resistant and non-resistant varieties would be the 1% concentration and also that evaluation on the 8th day after the commencement of the treatment would give better results than evaluation on the 3rd day.

Data obtained in the second and third experiments include six treatments, 4%, 2%, 1.5%, 1% (twice) and 0.5%. Analysis of variance, estimation of repeatability, and chi-square test of the collective data were conducted. The results of these analyses can be summarized as follows:

(1) Varietal differences in response were quite distinct.

(2) The salt resistant variety group differed significantly from the non-resistant group.

(3) Differences between varieties within the "non-resistant" group were highly significant.

(4) Differences between varieties within the resistant variety group were not always significant.

(5) The repeatability of evaluation was 54% if treatment was made with a 1–1.5% solution of salt.

(6) The degree of damage observed in 1–1.5% salt solution is likely to show better agreement with the so-called salt-resistance than the damage caused by 4, 2 or 0.5% salt solutions.

45. *Variation in susceptibility to Piricularia disease in wild rice populations*[1)]

(By Takashi NARISE)

Three populations of wild rice, *Oryza sativa f. spontanea*, were investigated for inter- and intra-population variability with respect to resistance to the blast disease, *Piricularia oryzae*. Localities where wild rice populations were collected are: Chinsurah (West Bengal, India), Samalkot (Andhura, India), and Puttalam (West coast, Ceylon). Plants collected in these localities were harvested on an individual plant basis, and progenies were grown in Peradeniya, Ceylon in the summer of 1959. Susceptibility to the disease was tested by the leaf-sheath method using the leaf sheath of next to last leaf. Testing was done twice for each individual plant. The strain of Piricularia oryzea was collected in Ceylon in 1958. Average susceptibility value and genetic variability of each population are presented in Table 1.

Table 1. Average value and genetic variability of susceptibility to *Piricularia* disease in populations of wild rice, *O. sativa f. spontanea*

Population	Locality	Average susceptibility*	Genetic variability (%)
B	Chinsurah	3.2129	85.64
T-4	Samalkot	0.9991	99.56
PT	Puttalam	3.4342	95.81

*) The estimate for the most suseptible plant being 5.0.
**) Genetic variance expressed in percent of total variance.

Table 1 shows that populations differ markedly with regard to disease susceptibility. The T-4 population collected in Samalkot is as a whole highly resistant to the disease, while those collected in Chinsurah and Puttalam are highly susceptible. It is also found that within population variability is very high in all populations.

Partitioning of component of variance into environmental variance (σ_e^2), between progeny plants within lines variance (σ_l^2), between lines within populations variance (σ_s^2), and between populations variance (σ_p^2) was made, and variability on each level was estimated as follows:

Between progeny plants variability: $\dfrac{\sigma_l^2}{\sigma_e^2+\sigma_l^2}=0.9224$

1) This experiment was conducted in Peradeniya, Ceylon under the kind guidance of Dr. Yoshio Takahashi, Professor of Yamagata University, Japan. It was supported by Grant AF57080 from the Rockefeller Foundation.

Between lines variability: $\frac{\sigma_s^2}{\sigma_e^2+\sigma_I^2+\sigma_s^2}=0.1360$

Between population variability: $\frac{\sigma_p^2}{\sigma_e^2+\sigma_{Is}+\sigma_s^2+\sigma_p^2}=0.7333$

These figures suggest that plants in wild population are highly heterozygous with respect to disease susceptibility, and the inter-population variability is very high.

46. *Susceptibility of nullisomic wheat dwarfs and their respective gigas-plants to leaf rust, Puccinia triticina*

(By Seiji MATSUMURA and Keizô KATSUYA)

Dwarf plants possessing 40 chromosomes (20II), found among the offspring of the pentaploid hybrid, *Triticum polonicum*×*T. Spelta*, are nullisomics, deficient in a chromosome pair of the D-genome. Depending on which of the a~g D-chromosomes is missing, they are called a~g-dwarfs. The respective gigas-plants with 42 chromosomes found among the offspring of nullisomics are called a~g-gigas. They also may be termed D-nulli- and AB-tetrasomics.

In order to examine the susceptibility of nullisomics and their gigas-plants to leaf rust, a~g-dwarfs and a~g-gigas were tested at the first leaf stage with *Puccinia triticina* 21 B. One parent, *T. polonicum*, was susceptible, while the other, *T. Spelta*, showed resistance to rust. Because all e-dwarfs and their gigas-strains were susceptible, it has been concluded that the gene for resistance is located on the e-chromosome of the D-genome. A strain of g-dwarfs was susceptible, but a gigas-strain originating from another g-dwarf was resistant. The c-dwarfs and their gigas-plants exhibited resistance or moderate resistance and b-dwarf and its gigas-plants were resistant. Though a-dwarf was resistant, among a-gigas-plants, resistant and susceptible strains were observed. It has been assumed that the supernumerary chromosomes of resistant and susceptible a-gigas-strains were different. Among d- and f-dwarfs resistant and susceptible strains were found and also the correspondent gigas-strains showed resistance and susceptibility, respectively. We supposed that more genes for susceptibility are located on the chromosomes of the A- and B-genomes, in addition to the epistatic genes of the D-genome.

47. *Recovery of chlorophyll content in some mutants in einkorn wheat, Triticum monococcum flavescens*

(By Tarô FUJII)

Viability of the *virido-albina* mutant is poor, but the chlorophyll content recovers almost normal when the plants are placed in the phytotron (cf. Ann. Rep. No. 6). On the other hand, double recessive plants *virido-albina—basi-viridis* II look like *albina* and, even in the phytotron died without developing chlorophyll, while both parents have the ability to recover (cf. Ann. Rep. No. 8). Moreover, the new chlorophyll mutants, *basi-viridis* III, *virido-albina* II, and *xantha-alba* I, obtained from irradiation experiments, which have little chlorophyll at the seedling stage, also have shown the ability to develop almost normal chlorophyll content. From these findings, it appears that in certain chlorophyll mutants the recovery of chlorophyll content might be initiated in the presence of a small amount of chlorophyll in the leaf tips.

In an experiment, the green tips of the leaves were cut off and the plants were placed in the greenhouse and in the phytotron at seedling stage or later, when the young plants had developed 5 - 6 leaves. The batch in which the green parts were removed did not grow, and the plants died after about 2 weeks, while the batch containing uncut plants continued to develop.

On the other hand, recovery of the chlorophyll content in the *virido-albina* mutant in agar culture with WHITE's solution to which 3 percent sucrose was added was faster than in soil culture. After 20 days in agar culture the plants recovered to normal green, while it took about one month for the recovery in soil in the phytotron. Thus, it seems that agar culture is more suitable for increasing the chlorophyll content than soil culture. But, the cut batches of *virido-albina* died also in agar culture. *Albina* mutants and *albina* like plants obtained from the cross between *virido-albina* and *basi-viridis* II were also sown on agar, but they never developed any chlorophyll. It is well known that chlorophyll and radiant energy are the essentials of photosynthesis, the most important process for plant growth. The *virido-albina* mutant, mentioned above, could not survive when the green parts of the leaves were cut off. A small amount of chlorophyll was necessary for priming the process of further chlorophyll development (cf. FUJII 1959, Jap. Jour. Genet. 34).

48. *Amount of amino acids in the chlorophyll mutants of einkorn wheat*

(By Yukio ONO and Tarô FUJII)

Amino acid content in several chlorophyll mutants of *Triticum monococcum flavescens* was examined at the seedling stage. Four *albina*s, one *chlorina*, and one *virido-albina*, which has the ability to recover the chlorophyll content, were used in this examination. Normals were used as control. Free amino acid was extracted from young leaves with 75% ethanol, and the several amino acids were separated by two dimensional paper chromatography. Seven kinds of free amino acids, namely aspartic acid, gultamic acid, glycine, alanine, glutamine, phenylalanine and leucine, were detected. Four *albina*s and one *virido-albina* had all of them, while leucine and alanine were not observed either in normals or *chlorina*. *Albina* and *virido-albina* mutants showed larger amounts of all amino acids than the normals and *chlorina*. Cysteine, histidine, proline, and methionine were not observed in normals and *chlorina*, but in *albina* strains only traces of these were found. Moreover, non-identified spots (a and b in Figure 1 and 2) were observed in all strains, and the a-spot size was larger in normals than in *albina*.

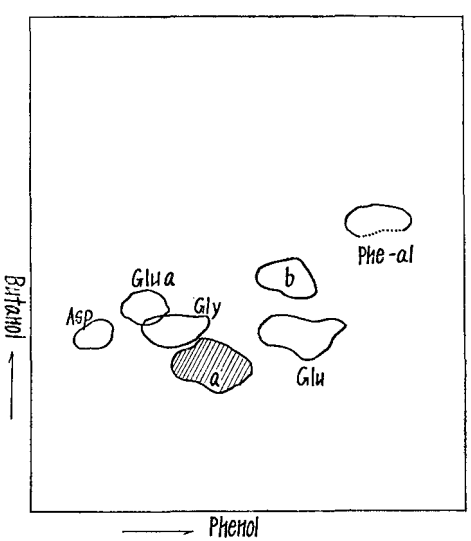


Fig. 1. Free amino acids in the normal strain.

Kinds and amount of organized amino acids were also determined by the same chromatographic method after hydrolysis of fresh leaves. About 14 kinds of organized amino acids were observed in normals, while only

10 kinds were found in *albina*. They occurred in larger amounts in normals than in *albina*. Leucine, glutamine, alanine, and the non-identified spot (a) contained in free amino acids, were not observed in organized

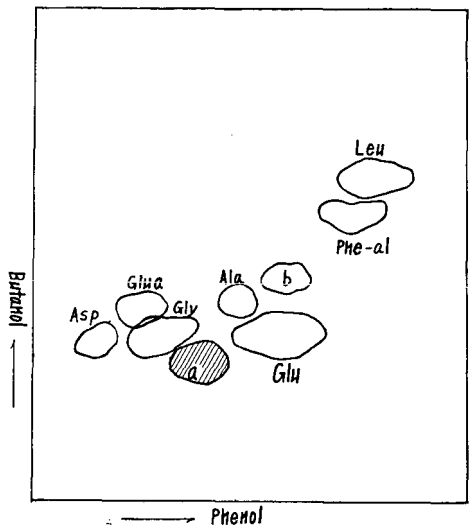


Fig. 2. Free amino acids in the *albina* strain.

amino acids. Therefore, they may be faster transferable compared with other amino acids. From these results, it appears that the *albina* strains have a larger amount of free amino acids and a smaller amount of organized amino acids than the normals.

49. *Susceptibility of wild rice to blast fungus, Piricularia oryzae*

(By Keizô KATSUYA)

In order to examine the susceptibility of wild rice to blast, we tested a strain each of *Oryza minuta* and *O. officinalis*, 3 strains of *O. perennis* and *O. sativa* var. *spontanea* and 27 strains of *O. glaberrima* in the greenhouse with 2 strains of *Piricularia oryzae*, namely P-2 and 54-04, obtained from the National Institute of Agricultural Sciences. For each trial 10 plants were kept in pots to which ammonium sulphate, calcium superphosphate and potassium sulphate were added. They were inoculated by spraying on the third or fourth leaf or by injection into one of the fourth to sixth leaves. The degree of susceptibility was determined by the types of lesions formed on the leaves. Blast readings were made on infected leaves 5 to 10 days after inoculation. The tests were repeated 2 to 3 times.

O. minuta (W0016) was resistant to the two strains of blast fungus. *O. officinalis* (W0046) showed resistance when sprayed and resistance or moderate susceptibility when injection was used. All strains of *O. glaberrima* were resistant to the two blast strains when sprayed and resistant or moderately susceptible when injection was used. A strain of *O. perennis* (W0034) was very susceptible to the two strains of blast fungus when sprayed or injected. A strain of *O. sativa* var. *spontanea* (W0150) was moderately susceptible or very susceptible to the two blast strains when inoculated by injection or spraying. Some strains of *O. sativa* var. *spontanea* (W0106, W0123) and *O. perennis* (W0032, W0033) exhibited resistance when sprayed and resistance or moderate susceptibility when inoculated by injection. Some strains of *O. perennis*, *O. sativa* var. *spontanea* and *O. glaberrima* exhibited considerably different susceptibility within the same strain.

50. *Susceptibility of the albina mutant of einkorn wheat to leaf and stem rusts, Puccinia triticina and P. graminis*

(By Keizô KATSUYA)

The susceptibility of the *albina* mutant of *Triticum monococcum flavescens* to stem and leaf rusts was tested in the phytotron (20°C daytime, 15°C at night) at the second leaf stage with *Puccinia triticina* 21 B and *P. graminis* f. sp. *tritici* 21. At first *albina* seedlings were kept in a sowing box and then they were placed in water culture with WHITE's nutrient medium (1943) to which 3 percent sucrose was added. They were inoculated by brushing. 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. The tests were repeated 2 to 3 times.

Albina exhibited susceptibility to leaf and stem rusts, while the normals were resistant to the former and susceptible to the latter. The uredosori of stem rust on *albina* were much larger than those on normal plants. Thus, susceptibility of host plants to leaf and stem rusts belonging to obligate parasites is not necessarily connected with quantitative differences in chlorophyll. A difference in another component may be the cause of a higher susceptibility of *albina* to leaf rust. Studies concerning amino acids await further experiments.

51. *Cytogenetic studies of the genus Nicotiana* XII.

(By Yô TAKENAKA)

The reduction division in PMC's were studied in 5 interspecific hybrids:

N. debneyi × *N. otophora*, *N. knightiana* × *N. rustica*, *N. repanda* × *N. palmeri*, *N. bigelovii* × *N. megalosiphon*, and *N. trigonophylla* × *N. palmeri*.

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

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 6, with the mode at 2. The frequency of PMC's with one bivalent and 3 bivalents followed that of those with 2 bivalents. PMC's with 0 or 4 bivalents were occasionally found but those with 5 and 6 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 subgenomes of *N. debneyi* which may be of amphidiploid origin.

2) F_1 of *N. knightiana* (n=12) × *N. rustica* (n=24)

So far as I know, this hybrid also has not been reported previously. MI in PMC's of this hybrid showed in most cells both 12 univalents and bivalents, except for the very rare occurrence of one trivalent. At AI in PMC's, one or two chromosome bridges were occasionally found. *N. knightiana* is considered to be closely related to *N. paniculata*, which is assumed to be one of the parents of the amphidiploid *N. rustica*. Accordingly the 12 bivalents shown in this hybrid may be supposed to be caused by the affinity between the genomes of *N. knightiana* and of *N. paniculata* which represent one of the two subgenomes in *N. rustica*. But it is thought that the genomes of *N. knightiana* and of *N. paniculata* in *N. rustica* are somewhat differentiated by inversions and other irregularities. The chromosome bridges at AI may be caused by such differences.

3) F_1 of *N. repanda* (n=24) × *N. palmeri* (n=12)

At MI of PMC's of this hybrid, the number of bivalents ranged from 0 to 9, with the mode at 6. The frequency of PMC's with 3 and 5 bivalents followed that of PMC's with 6 bivalents. PMC's with 2 and 5 bivalents were frequently found but those with 0, 1, 7, 8 and 9 bivalents were rare.

In the same hybrid, Goodspeed (1954) found that the number of bivalents ranged from 4 to 10, with the mode at 6, and assumed that *N. palmeri* was a descendant of one of *N. repanda*'s progenitors, though there is not complete pairing between the genome of *N. palmeri* and one subgenome of *N. repanda*, either or both causing the accumulation of products of translocations and other irregularities within genomes of the two species. Although there are some differences between Goodspeed's observations and mine, his speculation may be generally correct.

4) F_1 of *N. bigelavii* (n=24) × *N. megalosiphon* (n=20)

At MI of PMC's of this hybrid, bivalent chromosomes were very rare.

The number of PMC's with 0, 1 and 2 bivalents per cell were 71, 25 and 7 respectively. And in many PMC's a restitution nucleus was formed without the completion of first division.

It is difficult to speculate on the relationship between *N. bigelovii* which is an American species and *N. megalosiphon* which is an Australasian species.

Kostoff (1941–43) has studied this hybrid; my results agree well with his.

5) F_1 of *N. trigonophylla* (n=12) × *N. palmeri* (n=12)

At MI of PMC's of this hybrid, 12 bivalents formed by pairing of chromosomes of similar shape and size were observed in all cells. At AI, all bivalents divided at the same time. Not even a chromosome bridge or a stray chromosome was observed. Also all PMC's regularly completed the second division and produced normal pollen grains. Indeed, this hybrid was fertile and produced many seeds. Accordingly, *N. trigonophylla* and *N. palmeri* may be grouped together, though they differ in flower size. *N. palmeri* was considered by East (1928, 1932) to be a variety of *N. trigonophylla*, but McCray (1932) belived it to be a different species. The cytological data presented by Kostoff (1941–43) agree very well with my results.

52. *Structural changes of chromosomes of Allium scorodoprasum var. viviparum*

(By Yô Takenaka)

The somatic chromosome number of *A. scorodoprasum* var. *viviparum* has been determined as sixteen by many investigators, e. g. Katayama (1928), Morinaga and Fukushima (1931), Takenaka (1931), Y. Ono (1935) and Kurita (1951). In a previous paper (1931), the present author hinted at a possibly hybrid origin of this species on the basis of his observations of chromosome rings at meiosis and two somatic chromosomes of markedly different shape and size. He further studied in detail the meiosis of this plant, as reported in the annual report No.4 (1954). Since then three new varieties have been brought from Formosa. In the present paper, their chromosomal behavior is compared with that of the variety widely grown in Japan.

(1) From the measurement of the length of root-tip chromosomes, I can distinguish six groups, a, a', b, b', c and e, each consisting of two similar chromosomes. A Formosan variety, Nankotsu, showed no significant difference from the Japanese variety used for comparison.

The f chromosome was single; the above two varieties showed the same arm length ratio.

The other chromosomes, d, d', and g, were also single and were composed each of three parts. In their relative length, the Japanese and Formosan varieties showed a difference.

(2) The pairing of chromosomes at MI of PMC's was studied in the Japanese and two Formosan varieties, Nankotsu and Hokunin. In the Japanese variety, formation of five bivalents and one ring-shaped hexavalent was found in most cells, such configurations reaching 73% of the total number observed. In Hokunin variety, the same configuration occurred in 47% of observed cells, but cells with four bivalents and one ring-shaped octovalent were also found with 22% frequency. In Nankotsu variety, cells with four bivalents and one ring-shaped octovalent were frequently found, reaching 49% of the total cells observed, but cells with 3 bivalents and one ring-shaped decavalent were found as frequently as 24%.

The writer has assumed a hybrid origin of *Allium scorodoprasum* var. *viviparum* (1931, 1954). Under this assumption, it may be that this species has changed or is changing its chromosomal structure since its establishment. The changes might have taken place in d, d', and g chromosomes, because the relative length of three parts of these chromosomes of the above two varieties is different.

53. *Selection for high content of nornicotine*

(By Kazuo FURUSATO and Akira MIYAZAWA)

Synthetic tobacco obtained from the cross *Nicotiana sylvestris* × *N. tomentosiformis* was crossed with the commercial variety Bright Yellow. The hybrid was selfpollinated for five years. Yearly selection for nornicotine finally yielded 18 plants with high nornicotine and very low nicotine content. These will be used in our tobacco breeding program.

54. *Influence of environment on the fertility of tobacco*

(By Kazuo FURUSATO)

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

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

55. *A case of different results from reciprocal crosses (a preliminary report)*

(By F. A. LILIENFELD)

This preliminary report is confined to a few essential facts. They are briefly outlined as follows.

1. Two strains, named D and S[1], of two ecotypes of *Medicago truncatula* Gaertn., both with normal chlorophyll content, were crossed reciprocally.

2. The F_1 seedlings obtained from the reciprocal crosses were strikingly different in the coloring of the cotyledons. When D was the mother, the cotyledons of the seedlings were of a pale-yellowish, aurea, color or aurea with streaks of pale green (chlorina). In the reciprocal cross, S × D, the cotyledons were of a healthy green, approaching the intensity of color of those of the parents which made distinguishing them from the latter difficult. Sometimes, at nearer inspection, streaks of dark green on somewhat lighter background could be observed.

3. With the appearance of the first leaf the differences temporarily vanished. The first leaf in both F_1's opened as aurea and when expanded assumed a light chlorina shade producing the impression of a serious chlorophyll deficiency. Gradually the weak green tone of chlorina was gaining in intensity until a darker chlorina shade was reached. The following leaves behaved in the same way so that the new growth at the top was always before expansion aurea or very pale chlorina.

4. About two weeks later the reciprocal F_1's started to assume again a different appearance. While the great majority of the F_1 from cross D × S remained at the chlorina level, most of the plants from the reciprocal cross, S × D, started to produce more chlorophyll, turning green, and at the same time doubling their growth rate. Soon they were becoming strong, healthy plants as vigorous as the parents, of a green somewhat lighter when viewed together side by side. But the relatively pale color of the new growth at the tops of the main stem and branches remained an unmistakable indication of their hybrid origin. The plants were amply branched and flowered generously, while in the reciprocal F_1, D × S, flowering was delayed and even later only a few flower buds were initiated.

5. This newly exhibited difference in behavior was characteristic of the great majority of the reciprocal F_1's. However, in either F_1 a few plants were found which behaved as the majority of the reciprocal cross did, i.e. in D × S plants were found which turned green, became vigorous and

1) The designations D and S derive from a difference in the nuance of green of the two strains; they stand, respectively, for Dusky green and Shiny green.

flowered well. Likewise, some were found in S × D which remained chlorina and weak and could not be distinguished from the majority of the reciprocal cross.

6. Up to now carried out successive backcrosses of both F_1's to the respective male parent showed that a preponderantly either D- or S-genotype can function normally with either cytoplasm, its own or that of the other strain, effecting a development characteristic of the respective strain.

7. The F_2 generation presented a very similar aspect in both directions of the cross. The appearance, in highly variable ratios, of acute growth inhibitions and albinotic or xantha seedlings, characterizes both F_2's.

8. Conclusion: The sensitivity relations created between the hybrid nucleus and the plastids, directly or indirectly through the cytoplasm, are in either direction of the cross the cause of malfunction of the chlorophyll producing apparatus. The difference between the reciprocal crosses is merely a difference in degree. Only the difference between the two sensitivity levels can be ascribed to a difference in cytoplasm or plastids of the two biotypes. Growth inhibitions and complete chlorophyll deficiency (albinotic and almost completely chlorophyll lacking seedlings) which appear in F_2 may be attributed in first place to an intensified (as compared with F_1) plasma or plastid sensitivity of certain gene combinations and, partly, to gene segregation as such. The simultaneous occurrence of both these factors complicates the analysis. Moreover, there are indications that a gene or gene group is involved which produces some (virus-like?) agent exercising semi-lethal effects partly independently of the chlorophyll situation. The behavior, under certain conditions, of the D-strain seems to justify this supposition.

It is noteworthy that the pollen was throughout all the experiments good (in so far as the plants produced flowers).

The experiments will be reported in detail elsewhere.

56. *Cytological studies in the genus Polygonum I. Chromosome numbers in the genus Polygonum and related genera*

(By Yukio DOIDA)

In addition to the study of pollen grain formation and pollen morphology, the chromosome numbers in *Polygonum* and allied genera were examined.

The results obtained are summarised in Table 1. The following points are noteworthy.

1) A new basic chromosome number, $b=12$, was found in at least 3 species of the genus; hitherto only $b=10$, 11, and 17 have been reported by other authors.

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

Species name	PMC (n)	Root-tip cell (2n)	Previous reporter	Localities
Muehlenbeckia arisanensis Hay.		20		Tokyo*
Polygonum hydropiper Linn. var. *maximowiczii* (Regel) Makino		20	J: 22,24 So: 20	Misima
P. nipponense Makino		20		Misima
P. weyrichii Fr. Schm. var. *alpinum* Maxim.		20	Su: 20	Mt. Fuji
P. yokusaianum Makino		40		Misima
P. blumei Maisn.		40		Misima
P. persicaria Linn.		40	J: 44	Misima
P. tinctorium Aiton.		40	Su: 40	Tokyo*
P. japonicum Meisn.		40	Su: 44	Misima
P. thunbergii Sieb. et Zucc.		40	Su: 34	Misima
P. sieboldi Meisn.	20(II)		Su: 34	Misima
P. aviculare Linn.		60	L & L: 40,60	Misima
P. nodosum Pers.		22	L & L: 22,44	Misima
P. orientale Linn.		22	J: 22	Misima
P. multiflorum Thunb.		22	Sk: 22	Nagoya**
P. cuspidatum Sieb. et Zucc. (♀)		44	J: 44 So: 46	Misima
P. perfoliatum Linn.		24		
P. bistorta Linn.		24	J: 44 So: 46	Mt. Ibuki
P. tenuicaule Bisset et Moore		24		Hakone
Fagopyrum esculentum Moench.	8(II)	16	J: 16	Nagoya
F. tataricum Gaertn.	8(II)	16	Sd: 16	Nagoya

* Supplied by Koishikawa Botanic Garden, Tokyo
** Supplied by Higashiyama Botanic Garden, Nagoya
The origin of the supplied plants is not known.
J: Jaretzky (1927, 1928), So: Sokolovskaya & Sokolovskaya (1938),
Su: Sugiura (1925, 1936), L & L: Löve and Löve (1944),
Sk: Suzuka (1950), Sd: Sokad (1939).

2) The chromosome numbers for some species were different from those reported by JARETZKY (1928) and SUGIURA (1936), perhaps because of occasional aneuploidy.

57. *Developmental studies in the genus Polygonum III*

(By Yukio DOIDA)

A) Two other species producing only 4 pollen grains.

In a previous paper (1958) of this series, it was reported that *P. persicaria*

produced only four pollen grains in a pollen sac. The same has been found in *P. debile* and *P. nepalense*, as shown in Table 1.

Table 1. Number of pollen grains formed in a pollen sac of *P. debile* and *P. nepalense*.

Materials	Localities	No. of pollen grains						Total	Mode
		3+1m*	4	6+2m*	8	12	Empty		
P. debile	Foot of Mt. Fuji, Susono-cho, Shizuoka	1	92	1	26	0	10	130	4
P. nepalense	Towada-lake, Yasumiya, Akita	0	128	0	43	0	—	171	4
P. nepalense	Foot of Mt. Hakkoda, Sugayu, Akita	0	82	0	15	0	—	97	4

m*: Dwarf pollen grain

An anther of these species consists of four pollen sacs. A cross-section of a very young anther shows a mass of homogeneous meristematic cells. Following the division of the meristematic cells, the young anther becomes slightly four-lobed. In each lobe an archesporial cell differentiates from the subepidermal layer. This cell becomes the future pollen mother cell. Without undergoing mitosis the cell proceeds through normal meiosis and four pollen grains are formed. Aberrations in pollen grain formation are very few (Table 1).

The morphology of the pollen grains of both species is as follows: The pollen grains of *P. debile* have a reticulate sculpture and multi-germination pores, one in compartment of the mesh, representing the so-called "pore type". The pollen grains of *P. nepalense* have a similar reticulate but pore-less structure and three germination furrows. Thus, *P. nepalense* combines the reticulate structure of the pore type with the characteristics of the furrow type. *P. nepalense* is the only one of this kind among *Polygonum* species (Table 2).

B) Pollen morphology in the genus *Polygonum*

The external morphology of pollen grains has been used as a key character for the study of phylogeny of some plant species. WODEHOUSE, HEDBERG, NAKAI and IKUSE classified the genus *Polygonum* into several groups on the basis of their studies of pollen grains.

According to NAKAI all *Polygonum* species can be divided into two major groups, the "pore type" and the "furrow type". The grains of the former type have an almost spherical shape and a reticulate sculpture of

the exine. Many germination-pores are found. The grains of the latter type have spheroidal shape and spinose structure. They have three germination furrows. With regard to the number of pollen grains per sac, the present author has distinguished 7 classes, from class 1 with 4 to class 7 with 256 grains. As to the relation of NAKAI's to the author's classification, the pore type is found in species producing a small number of pollen grains in a pollen sac, *i.e.* it occurs in species belonging to classes 1, 2, 3 and to the majority of class 4. On the other hand, the "furrow type" is observed in species producing large numbers of pollen grains. It is found in species belonging to classes 5, 6, 7 and the remaining species of class 4, shown in Table 2.

Table 2. Relation between NAKAI's and the author's classification

Group name	Pollen morphology	Number of Pollen grains	Classes
Sub-genus Fagopyrum	B	32	4
Sub-genus Aviculare	B	32	4
Sub-genus Reynoutria	B	256	7
Sub-genus Pleuropterus	B	128	6
Sub-genus Bilderdykia	B	32?	4
Sub-genus Bistorta	B	64	5
Sub-genus Pleuropteropyrum	B	128	6
Sub-genus Ambryogonon	A	32	4
Sub-genus Tovara	A	8	2
Sub-genus Persicaria	A		
Section Dissitiflorae			
Section Didymocephalon	A*	4	1
Section Corynbocephalon			
Section Chylocalyx	A	32	4
Section Echinocaulon	A	4,8,16,32	1,2,3,4
Section Eupersicaria	A	4, 8,32	1,2,4
Genus Rumex	B	more than 1,000	?

A: pore type B: furrow type

A*: Only one species was examined (*P. nepalense*); it revealed characters of both types A. and B.

Pollen grains of the genus *Rumex*, Polygonaceae, belong to the "furrow type"; they occur in much larger numbers per pollen sac than those of *Polygonum* species.

58. *Biometrical analysis of matroclinal inheritance in rice*[1)]

(By Kan-Ichi SAKAI)

In a varietal cross of rice, matroclinal inheritance was observed for the one hundred grain weight. On a hypothetical genetic model for the co-operation of genes and cytoplasm, theoretical expectation of mean values for the reciprocal hybrid progenies were computed. Formulas for these expectations are as follows:

$$x_n = a + \frac{h}{2^{n-1}} + \left\{(x_{n-1} - a) - \frac{h}{2^{n-1}}\right\} m,$$

Table 1. Components of variation and covariation in hybrid populations.

	Notation	Components of variance or covariance
Parental varieties and reciprocal F_1 hybrids	V_{x_0}, V_{y_0}, $V_{x_0 y_1}$	E_1
Variance in F_2	V_{x_2}, V_{y_2}	$\frac{1}{2}D(1-m)^2 + \frac{1}{4}H(1-m)^2 + E_1$
Within-line variance in F_3	$V_{x_3(Wn)}$, $V_{y_3(Wn)}$	$\frac{1}{4}D(1-m)^2 + \frac{1}{8}H(1-m)^2 + E_1$
Between-line variance in F_3	V_{x_3}, V_{y_3}	$\frac{1}{2}D(1-m)^2(1+m)^2 + \frac{1}{16}H(1-m)^2(1+2m)^2 + E_2$
Variance in F_3 bulks	V_{x_3}, V_{y_3}	$\frac{1}{4}D(1-m)^2\left[3+4m(+\frac{1}{2}m)\right] + \frac{1}{16}H(1-m)^2[3+4m(1+m)] + E_1$
Within-line variance in F_4	$V_{x_4(Wn)}$, $V_{y_4(Wn)}$	$\frac{1}{8}D(1-m)^2 + \frac{1}{16}H(1-m)^2 + E_1$
Variance in two backcrosses between F_1 and P	$V_{B(X)} + V_{B(Y)}$*	$\frac{1}{2}D(1-m)^2 + \frac{1}{2}H(1-m)^2 + 2E_1$
Covariance between F_2 and F_3	$W_{x_2x_3}$, $W_{y_2y_3}$	$\frac{1}{2}D(1-m)^2(1+m) + \frac{1}{8}H(1-m)^2(1+2m)$
Covariance between F_3 and F_4	$W_{x_3x_4}$, $W_{y_3y_4}$	$\frac{1}{4}D(1+m)(1-m)^2\{3+2m(1+m)\} + \frac{1}{32}H(1-m)^2\{3+8m(1+m+m^2)\}$

* Different backrosses are divided into two groups, X and Y. X is a group of backcrosses where x_0 is included in the cross. Y is another group where y_0 is included. Variance of a cross of X group and variance of a cross of Y group can be added to give $V_{B(X)} + V_{B(Y)}$.

1) The present study was conducted in the Department of Agriculture of Ceylon and details will be published in "Heredity" (England) as a joint work with Dr. M. F. Chandraratna.

$$y_n = a + \frac{h}{2^{n-1}} + \left\{(y_{n-1} - a) - \frac{h}{2^{n-1}}\right\} m,$$

where x_n or y_n is the mean value for the n-th generation of a cross $x_0(♀) \times y_0(♂)$ or $y_0(♀) \times x_0(♂)$. The letters, a, h, and m stand for midparental value, dominance effect of genes, and cytoplasmic effect, respectively. These formulas can be rewritten as

$$x_n = m^n x_0 + \frac{h(1-m)(1-2^n m^n)}{2^{n-1}(1-2m)},$$

$$y_n = m^n y_0 + \frac{h(1-m)(1-2^n m^n)}{2^{n-1}(1-2m)},$$

if a is 0.

Components of variation in different hybrid populations have been worked out and are presented in Table 1. For explanation of the notations D, H, E_1 and E_2, refer to Mather (1949): *Biometrical Genetics*.

Genetic parameters estimated in the investigation are as follows:

Effect of cytoplasm, $m = 0.3177 \pm 0.0710$
Dominance effect of genes, $h = -0.4008$
Fixable genetic variance, $D = 0.0404 \pm 0.0392$
Unfixable genetic variance, $H = 0.2879 \pm 0.1383$
Environmental variance, $E_1 = 0.0091 \pm 0.0049$.

Observed values and theoretical expectations computed on the basis of estimations given above are shown in Table 2.

Table 2. Comparison of observed and expected values of average one hundred grain weight and variance or covariance of the same character in different hybrid populations of a reciprocal cross.

	Mean 100 grain weight (g)		Variance or covariance		
	Observed	Expected		Observed	Expected
x_0	1.78	—	V_0 and V_1*	0.0072	0.0091
y_0	3.02	—	V_2	0.0562	0.0520
x_1	2.02	2.0736	$V_{3(Wn)}$	0.0247	0.0305
y_1	2.47	2.4675	$V_{3(Bn)}$	0.0415	0.0390
x_2	2.39	2.3036	$V_{4(Wn)}$	0.0260	0.0198
y_2	2.48	2.4288	$W_{2/3}$	0.0332	0.0398
x_3	2.37	2.4451			

*) V_i stands for variance of i-th generation and $W_{i/j}$ for covariance between i-th and j-th generations.

Heritability of one hundred grain weight is 0.4009 and the number of effective factors for the same character is estimated at about 10.

59. *Effect of matroclinal inheritance on the genetical variation in a hybrid population of autogamous plants.*

(By Shin-ya IYAMA)

Matroclinal inheritance of quantitative characters of autogamous plants was reported on seed weight of rice by Sakai (Ann. Rep., No. 10, pp. 86) and the variances and covariances in early generations were computed.

The following model is proposed for matroclinal inheritance.

$$P_n=(1-m)\,G_n+mP_{n-1}\,,$$

where P_n and G_n stand for the genetically determined phenotypic value and genotypic value of an individual in the n-th generation, respectively, and m stands for the proportion of the cytoplasmic effect in the whole hereditary effect. m may take any value between zero and unity. For a pair of genes A and a, assuming the values of three genotypes AA, Aa and aa to be d, h, and $-d$, respectively, the mean value of the F_n progeny population of $AA\times aa$ or $aa\times AA$ crosses of autogamous plants may be written as follows,

$$\pm m^n d+\frac{(1-m)\,(1-2^n m^n)}{2^{n-1}\,(1-2m)}\,h$$

where $\pm$ sign means positive for $AA\times aa$, and negative for $aa\times AA$.

On the basis of the above assumption, general formulae for obtaining variances and covariances in various generations of hybrid progeny have been worked out. The details will be reported on another opportunity.

According to the result of this study, the cytoplasmic effect reduces the additive genetic variance more and more as the m value increases. The effect of cytoplasm almost disappears in a few generations when m is small but remains still effective in the later generations when m approaches 1.

Proportion if genetic variance to total phenotypic variance as well as parent-offspring correlation coefficients were computed for various values of m. Both values become smaller, as the cytoplasmic effect becomes larger. Genetic correlation, however, becomes larger as the cytoplasmic effect increases.

60. *Biometric-genetical study of seed size characters in cultivated varieties of rice*[1]

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

Six F_2 populations, six varieties of pure-line selection, five local varieties, and eight varieties of hybrid origin were investigated in Ceylon for the

[1] This study was conducted in the Division of Botany, Department of Agriculture, Ceylon.

genetic variability of seed size characters. A number of plants were selected at random from each variety or hybrid population, and ten or twenty seeds collected at random from each plant of each variety were investigated for seed length and seed width. Genetic variance and covariance together with environmental variance and covariance were estimated, and heritability and genetic and environmental correlations were computed. The frequency distribution of varieties and hybrid populations with regard to average heritability of both characters is presented in Table 1.

Table 1. Frequency distribution of F_2 hybrid populations and varieties of different origin in respect to average heritability value for seed length and seed width.

	Number of items	Heritability							
		0–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	Mean
F_2 population	6	1*					3	2	0.5913
Local variety	5			1	1	1	2		0.4503
Pureline selection	6	3	2		1				0.1509
Variety of hybrid origin	8	1	1	2	1	1	2		0.3365

* An exceptionally low heritability value of one F_2 population is not included in the computation of the mean value.

Mean values in the last column of Table 1 show that F_2 populations are highest in heritability, local varieties coming next, followed by varieties of hybrid origin, while varieties of pure-line selection are lowest. This is quite in agreement with our expectation. It should be noticed that

Table 2. Frequency distribution of varieties of different origin and F_2 hybrid populations regarding genetic correlation between seed length and seed width.

	Number of items	Genetic correlation						
		–0.65–0.46	–0.45–0.26	–0.25–0.06	–0.05–0.15	0.16–0.35	0.36–0.55	0.56–0.75
F_2 population	6			2	3	1		
Local variety	5			3		1	1	
Pureline variety	6	1			1	1		3
Variety of hybrid origin	2					1	1	

varieties of hybrid orgin are very variable with respect to heritability.

It is of interest to see in Table 2 that genetic correlation between seed width is very low or almost none in F_2 populations while it is high in a positive or a negative direction in more or less established varieties. Needless to say, genetic correlation would be the result of three causes: genetic linkage, pleiotropic effect of genes, and effect of selection. Table 2 suggests that the observed genetic correlation between length and width of seed in rice varieties is not genic but a result of natural or artificial, though probably not conscious, selection.

61. *Analyses of strains of Drosophila melanogaster selected for abdominal bristles*

(By Yukio YAMADA)

At the 26th generation of selection genetic analyses of variability were undertaken in six strains of *D. melanogaster* selected for high and low number of abdominal bristles. In each line 6 flies out of 30 were selected from each sex. Two strains for high (H_1 and H_2) and two for low (L_1 and L_2) bristle numbers underwent selection, and the remaining two served as unselected controls (C_1 and C_2), all six having the same effective population size. Results so far obtained are summarized as follows:

1. Fertility:—Mean fertility, based on the number of successful matings per 50 randomly paired matings for each line, was 69.0%, 76.5%, and 86.5% for the pair of strains comprising *H*, *L*, and *C*, respectively, and hence it was in general lower in the selected lines than in the unselected controls. However, the same tendency of phenodeviants to have lower fitness than the optimals was not observed on a within line basis in either selected and unselected groups.

2. Variability:—Phenotypic variability in terms of the coefficient of variation was substantially at the same level in all 6 strains, ranging from 6.0% to 7.5% for abdominal bristles and 11.0% to 13.5% for sternopleurals. There was no definite evidence that the phenotypic variations have been decreased or increased by artificial selection for 25 generations. Heritabilities were estimated by intra-class correlation and midparent-offspring regression. The estimates obtained by the former method were rather erratic and inconsistent, but they were estimated by the regression to be roughly 18%, 15%, and 15%, respectively, for *H*, *C*, and *L* lines. Reduction of heritability of the character selected might be ascribable to the small effective size of population in each generation.

3. Developmental stability in selected lines and their crosses:—Differences of bristle counts of right and left sides of sternopleura were used for

measuring stability in development. The difference of variances between 6 parental lines and their F_1's did not approach a statistically significant level, though the variance in H_1 was the largest and that in L_2 was the smallest. This fact does not support the general idea that stability is lost by long-term selection and that stability of crosses is superior to that of parental lines, probably because the selected lines still preserve a great deal of heterozygosity.

4. Genetic differences between lines:—Genetic differences between lines were analysed from diallel crosses and some of the results are given in Tables 1 and 2.

Table 1. Analysis of variance for genetic effects from diallel crossing

Sources	d.f.	Mean squares			
		Abdominals		Sternopleurals	
		♀♀	♂♂	♀♀	♂♂
Additive genetic	5	44.9232**	28.2309**	1.5004**	2.2165**
Non-additive genetic	9	0.4427	0.4708	0.1389	0.1964
Reciprocal effect	15	1.6980**	0.3934	0.5318**	0.5429**
Error	360	0.4587	0.3373	0.0977	0.1210

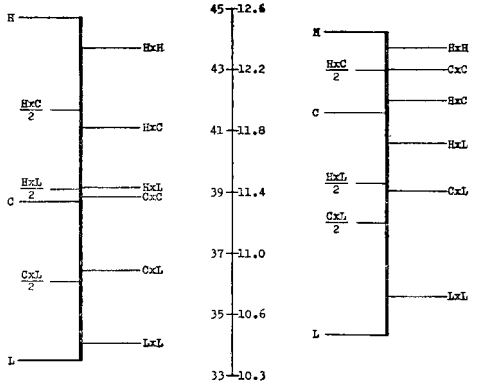
** Significant at 1% level.

Table 2. Additive genetic effects assigned to each line assuming the population mean to be zero

Strains	Abdominals		Sternopleurals	
	♀♀	♂♂	♀♀	♂♂
H_1	2.92	2.19	0.49	0.60
H_2	2.48	2.11	0.13	0.14
C_1	−0.36	−0.21	0.41	0.41
C_2	0.09	−0.39	−0.23	−0.20
L_1	−2.63	−1.84	−1.33	−0.06
L_2	−2.51	−2.22	−0.67	−0.80

As the tables show, the differences between H, C, and L are attributable mostly to the additive genetic effects, though some variance ascribable to reciprocal effect are highly significant. Means of the 6 lines and their

crosses are presented in Fig. 1. It is very interesting to see that the means of crosses between the two *H* lines and between the two *L* lines regressed somewhat to the overall population mean. This tendency seems to be general in crossings between lines selected for a character under additive genetic control.



Abdominals Sternopleurals

Fig. 1. Means of six lines and their crosses.

62. *Heritability analysis of body weight in purebreds and their crossbreds in the domestic fowl.*

(By Takatada KAWAHARA)

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 purebred and 586 crossbred females originated from 16 sires and 141 dams in WL and 16 sires and 119 dams in BPR. Mating system were such that the purebreds and their crossbreds were half-sisters, thus comparisons were possible for groups of pure and crossbred types. Measurements of body weight were taken at 4, 8, 12, 18 weeks, at laying of the first egg, and at about 48 weeks of age.

Heritability estimates based on variance components for body weight at various ages for four groups are shown in Table 1. They are 0.675, 0.637, 0.445, and 0.637, for WL, BPR, WL♀×BPR♂ and its reciprocal, respectively. There is some discrepancy between heritabilities as well as between genetic coefficients of variation estimated from sire and dam components in crossbreds at various stages, especially in the period from the 4th to the 12th week. These results suggest that dominance and/or epistasis

affect the growth rate in the reciprocal F_1 hybrids.

Table 1. Heritability estimates of body weight in purebreds and their reciprocal F_1 hybrids at various stages of growth.

		Heritabilities					
		4 weeks	8 weeks	12 weeks	18 weeks	Age at 1st egg	Adults
h_s^2	WL	0.96	0.97	0.68	0.94	0.75	0.99
	BPR	0.65	0.59	0.88	0.61	0.79	0.19
	WL♀×BPR♂	0.21	0.07	0.01	0.44	0.29	0.14
	BPR♀×WL♂	0.53	0.06	0.04	0.24	0.26	0.45
h_d^2	WL	0.92	0.87	0.36	0.06	0.45	0.14
	BPR	0.82	0.47	0.85	0.35	0.49	0.95
	WL♀×BPR♂	0.80	0.80	1.23	0.38	0.55	0.52
	BPR♀×WL♂	0.82	1.23	0.68	0.82	1.24	1.26
h_{s+d}^2	WL	0.94	0.92	0.52	0.50	0.60	0.57
	BPR	0.74	0.53	0.86	0.48	0.64	0.57
	WL♀×BPR♂	0.51	0.44	0.62	0.41	0.42	0.33
	BPR♀×WL♂	0.68	0.65	0.36	0.54	0.74	0.85

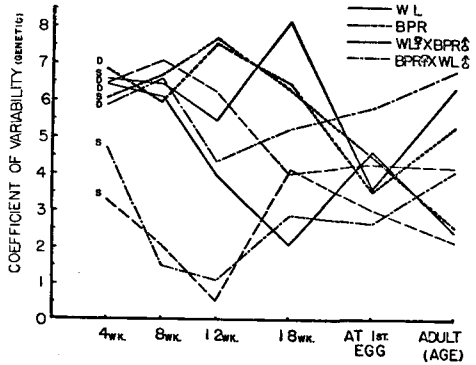


Fig. 1. Genetic coefficients of variation of body weight in purebreds and their crossbreds at various stages. $S=100\sigma_s/\bar{x}$; $D=100\sigma_d/\bar{x}$.

63. *Viability and susceptibility to a visceral form leucosis in two purebreds and their reciprocal crossbreds in the domestic fowl.*

(By Takatada KAWAHARA)

Within-breed and between-breed matings were performed using 16 sires and 141 dams of White Leghorns (WL), and 16 sires and 119 dams of

Table 1. Mortality of various types during two periods of growth.

Breed or cross	No. tested	Growing period			Maturing period			Both period		
		Dead birds		χ^2	Dead birds		χ^2	Dead birds		χ^2
		No.	%		No.	%		No.	%	
WL	362	30	8.3	WL——1.71——BPR 13.75** 7.14** 6.83** 2.25 WL♀×BBR♂ -0.93- BPR♀×WL♂	43	11.8	WL——9.29**——BPR 2.48 1.96 0.03 9.91** WL♀×BBR♂ -2.96- BPR♀×WL♂	73	20.1	WL——11.80**——BPR 12.88** 0.16 2.05 3.10 WL♀×BPR♂ -4.16- BPR♀×WL♂
BPR	253	29	11.5		53	20.9		82	32.4	
WL♀×BPR♂	281	9	3.2		44	15.6		53	18.8	
BPR♀×WL♂	227	11	4.9		49	21.5		60	26.4	
F_1 (WL♀×BPR♂ / BPR♀×WL♂)	508	20	3.9	WL——1.71——BPR 7.40** 15.83** F_1	93	18.3	WL——9.29**——BPR 6.63* 0.77 F_1	113	22.2	

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

Barred Plymouth Rocks (BPR). Mating systems were such that the purebreds and the crossbreds were half-sisters. A mortality analysis of data given in a previous report (KAWAHARA, 1959), excluding the T2 group (KAWAHARA, 1957) and accidentally dead birds, was undertaken. Data were collected from 615 purebred and 508 crossbred females. The chicks were maintained without any conscious culling up to 120 days after they laid their first egg. Mortality was recorded for two periods, *i.e.* growing period (up to the age of 18 weeks) and maturing period (from 18 weeks to 120 days after the first egg) in this experiment. A summary of total mortality data with the significance test based on Chi-square is given in Table 1. The diseases observed at death were classified according to the ten general types shown in Table 2. The results of statistical analysis of the data are as follows: 1) Total mortality in the growing period of crossbred chicks was 5.7% less than that of purebreds, and this difference was statistically significant at the 1% level. Total mortality of BPR was 3.2% greater than that of WL (11.5% vs. 8.3%), and mortality of crossbreds of BPR♀×WL♂ was 1.7% greater than that of its reciprocal (4.9% vs. 3.2%); these differences were not statistically significant. 2)

Table 2. General categories of diseases observed at the death of purebreds and their crossbreds up to 120 days after first egg. All percentage are based upon the number of hatched chicks at the start of the experiment.

Breed or cross	WL	BPR	WL♀×BPR♂	BPR♀×WL♂
No. tested / Cause of death	362	253	281	227
Leucosis	2.49	9.88	6.41	11.01
Coccidiosis and parasites	2.21	3.16	2.14	0.44
Nutritional	2.21	2.37	0	0.44
Nervous	1.38	0.79	0.36	0.44
Circulatory	0.28	0.40	0.36	0
Respiratory	0.28	1.19	0	0
Eliminatory	0.28	0	0	0
Digestive	1.10	1.58	1.08	0
Reproductive	2.21	1.98	1.08	3.08
Unaccountable cases and compound diseases	7.73	11.07	7.47	11.01
Total	20.1	32.4	18.8	26.4

Total mortality of corssbred chicks in the maturing period is greater than that of purebeds (WL=11.8%, BPR=20.9%, WL♀×BPR♂=15.6% and BPR♀×WL♂=21.5%), but it was only slightly hightly than in the maternal purebred half-sister group. The difference between purebreds and maternal crossbreds in total mortality were statistically non-significant, however. 3) It is obvious that the main cause of difference in total mortality between the growing and the maturing periods was loss from visceral form leucosis. Table 2 shows also that a larger part of the difference in mortality between the various groups was due to leucosis. Deaths in crossbreds from leucosis were actually higher than in their purebred maternal half-sisters (WL=2.5%, BPR=9.9%, WL♀×BPR♂=6.4% and BPR♀×WL♂=11.0%). Mortality from this disease in crossbreds in this case was as high as 8.5% while is was 5.5% in purebreds and the difference approached the significance level ($\chi^2=3.79$, $.10>P>.05$). The difference of 6.2% in mortality between maternal groups=4.2% *vs.* BPR maternal groups =10.4%) was statistically significant at the 1% level.

Resistance and susceptibility to visceral form leucosis are controlled, in larger part, by maternal factors and genotypes.

64. *A formula giving the effective population number based on the sampling variance in gene frequency*

(By Motoo KIMURA)

The concept of the effective population number was first introduced into population genetics by WRIGHT. Later, CROW propoed to define it from three different angles, *i.e.* (i) inbreeding effect, (ii) random extinction of alleles and (iii) sampling variance in gene frequency.

In an attempt to derive CROW's formula for the variance effective number (based on (iii)), I have obtained a slightly different result from that of CROW: Let x be the frequency of an allele A in the collection of gametes to form the $(t-1)$th generation and let x' be the similar frequency for the next generation (t-th generation), then the variance effective number of the t-th generation may be defined by

$$N_{e(\delta)}^{(t)}=\frac{x(1-x)}{2E\{(x'-x)^2\}},$$

where E means "taking expectation of". The new result obtained is as follows:

$$N_{e(\delta)}^{(t)}=\frac{2N^{(t)}}{\frac{s_k^2}{k}(1+f_{t-1})+(1-f_{t-1})}, \qquad (1)$$

where $N^{(t)}$ is the actual number of individuals in the t-th generation,

f_{t-1} is the coefficient of inbreeding and k is the number of offspring per individual of the $(t-1)$ generation such that

$$\bar{k}=\sum_{i=1}^{N^{(t-1)}} k_i/N^{(t-1)}$$

$$s_k^2=\frac{\sum_{i=1}^{N^{(t-1)}}(k_i-\bar{k})^2}{N^{(t-1)}-1}=\left\{\frac{N^{(t-1)}}{N^{(t-1)}-1}\right\}\sigma_k^2 .$$

This should be compared with CROW's formula (CROW 1954) which may be written, in the present terminology, as

$$N_{e(\delta)}^{(t)}=\frac{2N^{(t)}}{\frac{\sigma_k^2}{\bar{k}}(1+f_{t-1})+(1-f_{t-1})} . \qquad (2)$$

The difference between the above two formulae is that s_k^2 appears in formula (1) while the variance σ_k^2 appears in formula (2).

It may be noted that the difference becomes negligible when the number of individuals $N^{(t-1)}$ becomes large.

(cf. KIMURA, M. On some formulas giving the effective size of a population (in Japanese). Kwagaku Vol. 29)

65. *Some calculations on the mutational load.*

(By Motoo KIMURA)

The amount of harm done to a population by mutation may be measured by the mutational load which refers to the relative amount by which the average fitness of the population is decreased by the maintenance of deleterious genes resulting from the balance between mutation and selection. The purpose of the present report is to show the results of calculation on the mutational load under various conditions. We will designate by A_2 the mutant gene produced in each generation from its wild type allele A_1 at the constant rate of μ. We assume that generations do not overlap and designate the selective values of three genotypes A_1A_1, A_1A_2 and A_2A_2 as 1, 1-hs and 1-s respectively ($1\geqq s\geqq 0$, $1\geqq h\geqq 0$).

I. *Mutational load under random mating*

For a very large random mating population, the mutational load at equilibrium may be given by

$$\hat{L}_m=\mu\{1-\theta+\sqrt{\theta(2+\theta)}\},$$

where

$$\theta=sh^2/\{2\mu(1-2h)\}.$$

In figure 1, values of $\hat{L}_m$ are plotted against the values of h over the range of $10^{-4}\leqq h\leqq 1$, assuming $s=1$ and $\mu=10^5$.

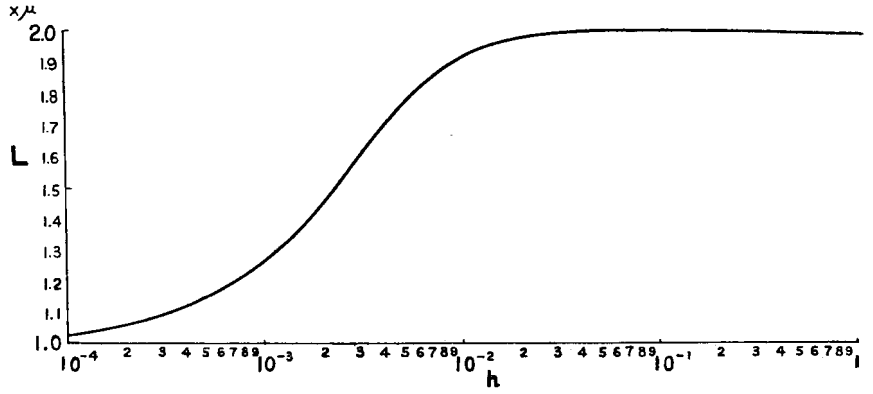


Fig. 1.

It may be seen from this figure that, if the degree of dominance of the mutant gene (h) is over 1%, the mutational load is approximately equal to twice the mutation rate, *i.e.*

$$\hat{L}_m=2\mu\,,$$

with good approximation.

II. *Effect of inbreeding*

If the population is not entirely panmictic but is slightly inbred in such a way that it keeps a constant but low level of inbreeding in each generation, then the mutational load at equilibrium becomes

$$\hat{L}_m(f)=\mu\{1-\theta_1-\phi+\sqrt{(\theta_1+\phi)^2+2\theta_1}\,,$$

where

$$\theta_1=\frac{h^2s(1-f)}{2\mu(1-2h)}\,,\quad \phi=\frac{fhs}{2\mu(1-2h)}\,,$$

and f denotes the inbreeding coefficient of the population as a whole. In Fig. 2 values of $\hat{L}_m(f)$ are plotted with a solid line against the values of f over the range of $10^{-4}\leqq f\leqq 10^{-1}$, assuming $s=1$, $\mu=10^{-5}$ and $h=0.02$.

On the other hand the broken line in Fig. 2 represents the mutational load which appears in the next generation of a panmictic population when it suddenly undergoes inbreeding of a given amount f. In this case the equation describing the load is

$$L_0(+f)=L_0+D_0f\,,$$

where

$$D_0=s(1-2h)\,\hat{x}_1\,\hat{x}_2\,,$$

in which $\hat{x}_1$ and $\hat{x}_2$ are respectively the equilibrium frequency of A_1 and A_2 in the panmictic population:

$$\hat{x}=1-\hat{x}_2, \quad \hat{x}_2=\frac{\mu}{sh}\left\{\sqrt{\theta(2+\theta)}-\theta\right\}.$$

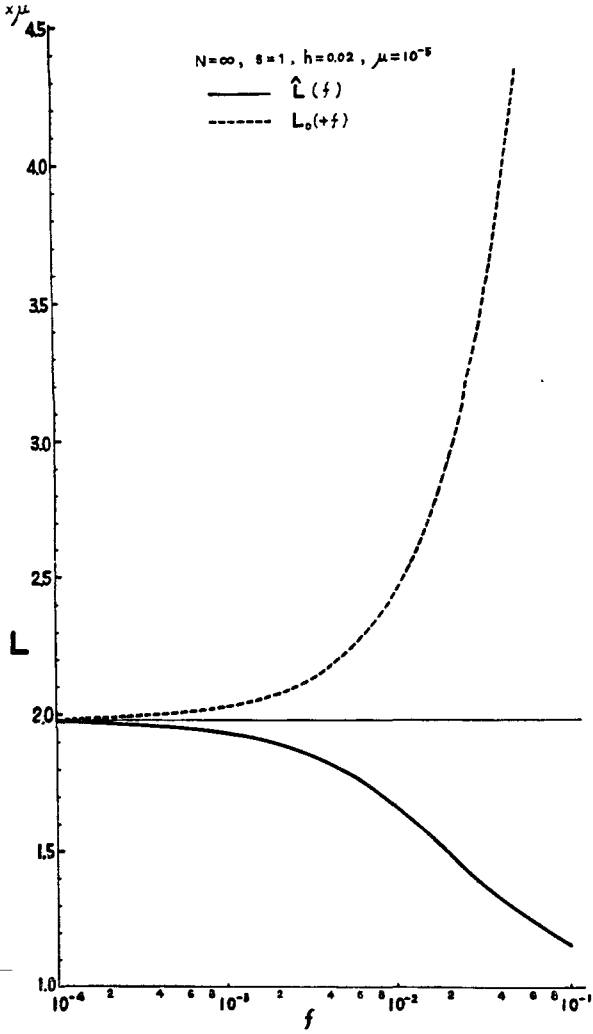


Fig. 2.

66. *On the substitutional load of a population*

(By Motoo KIMURA)

The substitutional load of a population, originally termed "the cost of natural selection" by HALDANE (1957), is the decrease of Darwinian fitness brought obout through the process of substituting for one gene (A_1) its allelic form (A_2) which is more fitted to the new environment. The amount of this decrease may be expressed in terms of loss in the geometric growth rate of the population and we may call it the substitutional load measured in Malthusian parameters.

For a population consisting of haplont organisms, the substitutional load which is expected if the substitution proceeds at the rate of one gene per generation is given by

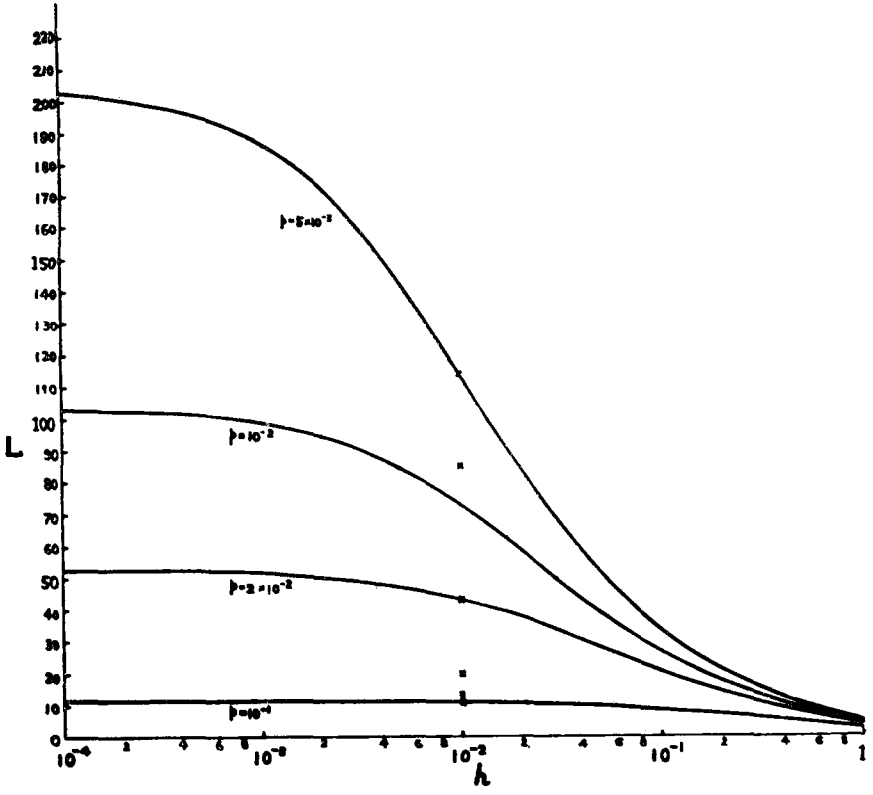


Fig. 1.

(1) $$L=-\log_e p\ ,$$

where p is the initial frequency of the gene (A_2) involved in the substitution.

For a population of diploid organisms, the corresponding substitutional load is shown to be as follows:

(2) $$L=-\frac{1}{h}\left\{\log_e p+(1-h)\log_e\frac{1-h}{h+(1-2h)p}\right\}\ ,$$

where h is the degree of dominance in fitness of the gene (A_2) which is to substitute its allele (A_1). In Fig. 1, values of the substitutional load given by (2) are plotted for several initial gene frequencies p over a wide range of h ($h=10^{-4}\sim 1$). It may be seen from this figure that L increases as p decreases, while it decreases as h increases.

67. *Substitutional load as a measure of gain in genetic information*

(By Motoo KIMURA)

In the process of adaptive evolution by natural selection, the amount of genetic information, as measured by the degree of improbability in genetic organization, is constantly being increased.

The purpose of the present report is to show that the rate of accumulation of genetic information denoted by H is directly proportional to the substitutional load, namely

$$H=\frac{L_e}{\log_e 2}\approx 1.44\,L_e \quad \text{bits per generation}\ ,$$

where a bit is a commonly used unit of the amount of information equivalent to the information content of choosing an alternative, say 0 or 1, with equal probability (0.5). The above relation may be derived from two independent courses of reasonings:

1) If those individuals which are to be eliminated by natural selection in the process of progressive evolution were kept alive and allowed to reproduce at the same rate as the favoured individuals, the population number would become, after t generations,

$$e^{L_e t}\ ,$$

times its initial value. This means that natural selection allows an incident to occur with probability one, which if without selection, could occur only with probability of

$$1/e^{L_e t}=e^{-L_e t}\ .$$

Thus information gained through t generations amounts to

$$-\log_2 e^{-L_e t}=\frac{L_e t}{\log_e 2} \text{ bits},$$

and therefore information gained per generation is

$$H=\frac{L_e}{\log_e 2}=(1.442\cdots)L_e \text{ bits},$$

as was to be shown.

2) Consider a population of haplont organisms. Let p be the initial frequency of an advantageous gene A. The probability that the gene A is ultimately established in the population is 1 under natural selection, while it is only p, if natural selection would not work and the fixation of genes were left to the action of random genetic drift. Thus information conveyed by natural selection through one gene substitution is

$$H=\log_2\frac{1}{p}=-\log_2 p \text{ bits}.$$

On the other hand we have shown in the preceding report that for one gene substitution

$$L=-\log_e p.$$

Therefore

$$H=-\log_2 e^{-L}=\frac{L}{\log_e 2}.$$

It is estimated, using the above relation, that the total amount of genetic information accumulated since the beginning of Cambrian epoch (500 million years) may be of the order of 10^8 bits, if evolution has proceeded at the standard rate (horotelic evolution).

68. *Genetical studies on the red and yellow eye pigments in Drosophila*

(By Toshifumi TAIRA)

Single and multiple eye color mutant strains of *Drosophila melanogaster* were used for this experiment. The relative amounts of eye pigments, extracted from the head without the proboscis by means of the "double extraction" method, were estimated by photometrical measurement. Several kinds of pteridines contained mainly in the eye and in the testis were separated by paper chromatography, and their relative amounts were measured fluorometrically. The results are shown Table 1.

Drosophila eye pigments are of three kinds, namely, red, yellow, and brown. Red and yellow pigments have been proved to be pteridine derivatives. A metabolic pathway from yellow to red was indicated by the

experimental results. A metabolic relationship between several pteridines was also indicated by the actions of various mutant genes affecting the pteridines.

Table 1. Relative amounts of eye pigments and pteridines in several mutant strains. (μg/100 mg in wet weight)

Strains	Head				Body			
	RP*	YP	BFP	Total	YP	BFP	IXP	Total
Ore-R	122.5	6.3	6.7	135.5	1.2	1.6	9.3	12.1
v	98.5	5.2	7.8	111.5	1.8	2.2	11.7	15.7
ca	19.1	7.9	10.6	37.6	1.2	3.1	10.1	14.4
ry	57.0	8.5	12.9	78.4	1.5	29.3	0	30.8
se	4.3	121.3	29.9	155.5	1.1	2.4	11.3	14.8
cl	29.2	54.0	24.9	107.1	1.7	2.3	11.0	15.0
Hn^{r-3}	26.1	24.2	19.7	70.0	6.6	6.8	2.7	16.1
bw	0	0	0	0	0	0	0	0
w	0	0	0	0	0	0	0	0
v; *se*	4.1	111.6	27.4	143.1	1.8	2.2	13.6	17.6
v; *ry*	21.9	7.2	10.1	39.2	1.1	27.2	0	28.3
v; *se ry*	1.0	67.7	18.1	86.8	1.0	27.0	0	28.0
se ry	0.5	97.2	32.0	129.7	1.6	27.1	0	28.7
bw; *ry*	0	0	0	0	0	0	0	0
bw; *se ry*	0	0	0	0	0	0	0	0

*) RP: red pigments; YP: yellow pigment; BFP: blue fluorescent pteridine; IXP: isoxanthopterin.

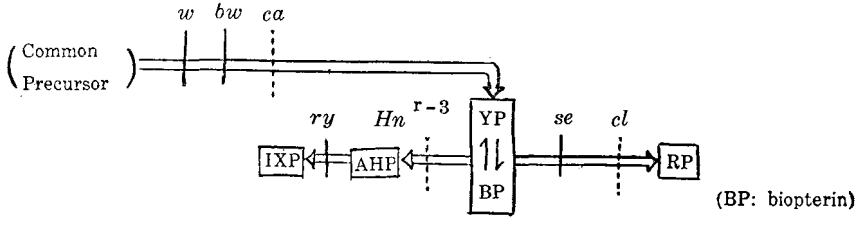


Fig. 1.

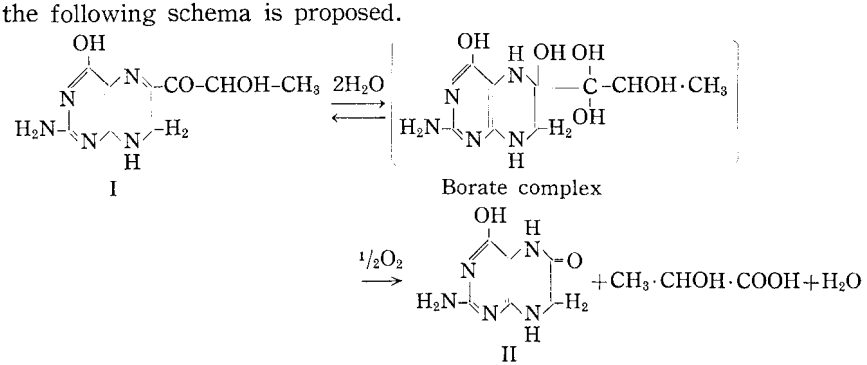
Since the pteridines are located in the eye and in the testis, a striking difference between the sexes could be detected; the total amount in the male body was about 20 times as large as that in the female body. The genic actions of both *w* and *bw* are presumed to block the common pre-

cursor of pteridine. The precursor is more or less depressed by genic actions of *ca*, *ry* and Hn^{r-3}. The homozygous *ry* fly was proved to lack xanthine dehydrogenase activity which converts 2-amino-4-hydroxypteridine (AHP) into isoxanthopterin, and the homozygous Hn^{r-3} fly was considered to have incomplete conversion of yellow pigment into AHP. The *cl* gene inhibits considerably the transformation of yellow pigment into red pigment, but the *se* gene blocks it completely. From all those results, the scheme of pteridine metabolism in *Drosophila* appears to be as shown in Fig. 1.

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

(By Saburo NAWA)

We now wish to propose a structure for the yellow pigment occurring in the eyes of the mutant *sepia* of *Drosophila melanogaster* on the basis of the following findings. Upon shaking an aqueous solution of the pigment in air in the presence of borax, the yellow color of the solution gradually disappeared. A greenish blue fluorescent compound in the reaction was identified as 7,8-dihydroxanthopterin (II) from its absorption spectrum and by chromatographic comparison with a synthetic specimen. The other reaction product was lactic acid. This reaction did not occur in the absence of oxygen. The molar ratio of oxygen consumed, and 7,8-dihydroxanthopterin and lactic acid produced in the reaction was 0.5:1:1. It is clear that the pigment carries a substituent in the 6-position of its pteridine skeleton, because 2-amino-4-hydroxypteridine-6-carboxylic acid is obtained from oxidation of the pigment. From this, it is concluded that the pigment is 7,8-dihydropteridine having a lactyl group in its 6-position. Basis on the evidence that polyhydroxy compounds form borate complexes, the following schema is proposed.



The yellow pigment in sodium bicarbonate solution consumed one mole of periodate to give acetaldehyde and a dihydropteridine, presumably 2-amino-4-hydroxy-7, 8-dihydropteridine-6-carboxylic acid. This supports again structure I. Reduction of this dihydropteridine with sodium borohydride gave a product with a spectrum characteristic of tetrahydropteridines. Furthermore, photodecomposition of the pigment gave one mole each of 2-amino-4-hydroxypteridine-6-carboxylic acid and acetaldehyde with the consumption of one mole of oxygen. This finding is again in accord with the proposed structure.

A possible biological importance of the pigment is suggested from its recognition as a hydropteridine. It was recognized that the pigment (dihydropteridine) is reduced to tetrahydro-form by the enzyme from chicken liver which catalyses the reduction of dihydrofolic acid to tetrahydro-derivative in the presence of TPNH. This suggests that *in vivo* the pigment may participate in oxidation-reduction systems.

70. *Relations between anthocyanin contents and three factors which condition the quality of flower color in pansies*

(By Toru ENDO)

The beauty of color depends upon three properties, namely, brilliance (luminosity, Y, in physical quantity), hue (dominant wave-length, $d\lambda$), and saturation (excitation purity, Pe). Flower colors of seven varieties of pansies were calibrated with Matsuda Trichromatic Colorimeter and were estimated by C.I.E. monochromatic specification (Y, $d\lambda$ and Pe). Calibrations were made using circular parts (2 cm in diameter) of the posterior petals of three plants of each variety. Varieties were chosen for having as different flower coloration as possible. The results are represented on the chromaticity diagram (Fig. 1). It was found, as expected, that the flower color of each variety has a specific place on the chromaticy diagram. Simultaneously, anthocyanin content of the posterior petals, which is the main component of flower coloration, was estimated spectrophotometrically and compared with the above three properties. The anthocyanins were extracted with 100 cc of 1% aqueous hydrochloric acid per 100 mg of dry powdered petals.

As shown in Table 1, the increase of anthocyanin content results in a considerable decrease of luminosity, but no relation between anthocyanin content and excitation purity is indicated.

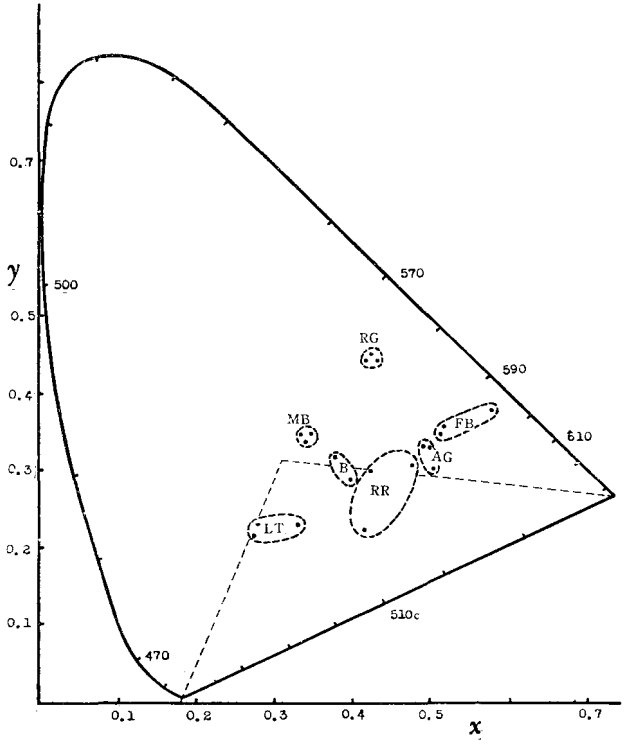


Fig. 1. Places of flower colors on chromaticity diagram.
MB (Mont Blanc): white, RG (Rhinegold): yellow, RR (Raspberry Rose): reddish purple, FB (Fire Beacon): yellowish red, AG (Alpenglow): deep red, LT (Lake of Thun): purplish blue, B (Berna): deep purple.

Table 1. Relative concentrations of anthocyanins and three factors conditioning flower color.

Flower color	$d\lambda$mμ	Y%	Pe%	Absorption maximam mμ	Optical density	Main antho-cyanidin
White	575	73.6	15.3			
Yellow	576	52.8	66.0			
Purplish red	495c	4.9	43.3	517	0.960	cyanidin
Yellowish red	598	7.0	71.3	520	0.196	cyanidin
Deep red	616	3.6	51.3	518	0.900	cyanidin
Purplish blue	555c	10.7	34.0	551	0.455	delphinidin
Deep purple	494c	1.4	23.3	523	2.580	delphinidin

71. *Anthocyanin of purplish blue and deep purple flowers of pansies*

(By Toru ENDO)

It was paper-chromatographically determined that the main anthocyanin, violanin, of purplish blue and deep purple flowers was delphinidin-3, 5-*p*-coumarylglucorhamnose (ENDO 1959). Another characterization was carried out by the measurement of the absorption spectrum, in which $E_{440m\mu}/E_{max.(540m\mu)}$ was about 8.4% when the anthocyanin was dissolved in 0.01% methanolic hydrochloric acid. This value suggests that the hydroxyl group at 5-position of the anthocyanin is substituted for sugar residue (HARBORNE 1958).

Absorption maxima of violanin and crude extracts of flowers of both strains were almost the same in the methanolic solution. However, when 1% aqueous hydrochloric acid was used as extractant, the absorption maximum of purplish blue flower (551 mμ) was shifted by about 27 mμ towards longer wave-length for violanin (524 mμ) but that of the deep purple (flower (523 mμ) was not shifted. This shift was maintained in 2 N hydrochloric acid even after the sample was heated for 30 min. at 77°C, but it disappeared after 17 hrs. when left at room temperature, and a large amount of precipitate was formed. It is assumed that the authocyanin of the purplish blue flower forms an additive complex with some part of the precipitate, most of which showed paper-chromatographically to be quercetin, and also that the complex anthocyanin is easily dissociated in methanolic solution from the quercetin-glycoside part but is comparatively stable in aqueous hydrochloric acid. In crosses between the two strains, a gene for the production of the complex anthocyanin was dominant (or epistatic) over that controlling the free anthocyanin contained in the deep purple flowers.

72. *Genes controlling flower coloration and their dosage effect in competitive expression*

(By Toru ENDO)

Eight genes responsible for the production of anthocyanins and xanthophylls have been preliminarily determined. Their fuctions may be summarized as follows:

X_1 and X_2 control the production of three xanthophylls, and have additive effect.

R controls the production of five anthocyanins, aC_1, C_3, D_4, D_5 and D_6, contained in reddish flowers.

R^L is similar to R, but produces a smaller amount of the anthocyanins. Probably a recessive allele to R.

B_1 and B_2 control the production of two anthocyanins, aC_1 and aD_2, contained in bluish flowers. Probably a gene complex.

B_1^L and B_2^L are similar to B_1 and B_2, but produces a smaller amount of the anthocyanins. Probably recessive alleles to B_1 and B_2.

Accordingly, the genotypes with respect to flower colors of the varieties can be represented as shown Table 1.

Table 1. Flower colors and their genotypes in pansies.

Flower color	Production of xanthophyll		Production of anthocyanin		
White	$x_1\,x_1\,x_1\,x_1$	$x_2\,x_2\,x_2\,x_2$	$r\ r\ r\ r$	$b_1\ b_1\ b_1\ b_1$	$b_2\ b_2\ b_2\ b_2$
Yellow	$X_1X_1X_1X_1$	$X_2X_2X_2X_2$	$r\ r\ r\ r$	$b_1\ b_1\ b^1\ b_1$	$b_2\ b_2\ b_2\ b_2$
Purplish red	$x_1\,x_1\,x_1\,x_1$	$x_2\,x_2\,x_2\,x_2$	$R\ R\ ?\ ?$		
Yellowish red	$X_1X_1X_1$?	$X_2X_2X_2$?	$R^LR^L??$		
Deep red	$X_1X_1X_1$?	$X_2X_2X_2$?	$R\ R\ ?\ ?$		
Purplish blue	$x_1\,x_1\,x_1\,x_1$	$x_2\,x_2\,x_2\,x_2$		$B_1^LB_1^LB_1^L$?	$B_2^LB_2^LB_2^L$?
Deep purple	$x_1\,x_1\,x_1\,x_1$	$x_2\,x_2\,x_2\,x_2$		$B_1\ B_1\ B_1$?	$B_2\ B_2\ B_2$?

It was observed that the production of anthocyanins was almost completely inhibited in some F_1 cross-combinations between cyanic and acyanic varieties. Such phenomenon has been so far explained by the assumption of an inhibiting gene. However, since the inhibition of the pansy anthocyanin formation is closely related to the presence of xanthophylls, the contents of which are almost the same in yellow, yellowish red and deep red varieties, the following hypothesis is proposed.

The anthocyanin and xanthophyll genes compete with one another for a common substrate. When the ratio between dose of xanthophyll genes X and that of reddish anthocyanin genes R is more than 4, only the xanthophylls are produced due to the dosage effect. In parallel, when the ratio between dose of the xanthophyll genes and bluish anthocyanin genes B is more than 1.5, only the xanthophylls are produced.

This hypothesis explains the F_2 segregations of cyanic×acyanic varieties, as shown in Table 2, in which the expected values of segregation ratios are calculated as follows:

$X/R>4$: yellow

$X/R\leq 4$: red, reddish yellow or yellowish red

$X/B\geq 1.5$: yellow

$X/B<1.5$: deep purple or purplish blue

X: X_1 and X_2, R: R or R^L, B: B_1 and B_2 or B_1^L and B_2^L

Table 2. Genotypes of F_1 hybrids and frequency of acyanic plants in F_2.

Cross-combination	Flower color of F_1	Genotype of F_1 assumed				Frequency of acyanic in F_2		
						Expected %	Observed %	Plants obtained
White × yellow	pale yellow			$X_1X_1x_1x_1$	$X_2X_2x_2x_2$	100.0	100.0	100
White × purplish red	purplish red	$R\ r\ r\ r$		$x_1x_1x_1x_1$	$x_2x_2x_2x_2$	25.0	14.3	70
Yellow × purplish red	pale yellowish red	$R\ r\ r\ r$		$X_1X_1x_1x_1$	$X_2X_2x_2x_2$	41.2	33.3	84
White × yellowish red	pale purplish red	$R^L\ r\ r\ r$		$X_1X_1x_1x_1$	$X_2x_2\ x_2x_2$	28.8	22.3	60
Yellow × yellowish red	yellow	$R^L\ r\ r\ r$		$X_1X_1X_1x_1$	$X_2X_2X_2x_2$	66.8	35.1	71
White × deep red	deep red	$R\ r\ r\ r$		$X_1x_1\ x_1x_1$	$X_2x_2\ x_2x_2$	25.0	16.7	72
Yellow × deep red	yellow	$R\ r\ r\ r$		$X_1X_1X_1x_1$	$X_2X_2X_2x_2$	66.8	70.0	20
Purplish blue × white	purplish blue	$B_1{}^LB_1{}^lb_1b_1$	$B_2{}^LB_2{}^lb_2b_2$	$x_1x_1x_1x_1$	$x_2x_2x_2x_2$	0.1	0.0	81
Purplish blue × yellow	yellow turning to purplish blue	$B_1{}^LB_1{}^lb_1b_1$	$B_2{}^LB_2{}^Lb_2b_2$	$X_1X_1x_1x_1$	$X_2X_2x_2x_2$	19.1	21.2	33
Deep purple × white	deep purple	$B_1\ B_1\ b_1b_1$	$B_2\ B_2\ b_2b_2$	$x_1x_1x_1x_1$	$x_2x_2x_2x_2$	0.1	0.0	80
Deep purple × yellow	deep purple	$B_1\ B_1\ b_1b_1$	$B_2\ B_2\ b_2b_2$	$X_1X_1x_1x_1$	$X_2X_2x_2x_2$	19.1	15.0	60

73. *Practical value of citbittol A as a reagent in the examination of human taste*

(By Yoshito OGAWA)

Citbittol A is a bitter substance extracted by the writer (1957, 1958) from the fruit of *Citrullus colocynthis*, SCHRAD. The present note gives a few data on the practical value of this substance as a reagent in the study of human taste sensitivity. The threshold value of the taste sensitivity of each individual for Citbittol A was investigated in comparison with that for Phenyl-thio-carbamide.

Aqueous solutions were made up at 20°C. in two concentrations of Citbittol A and Phenyl-thio-carbamide. Two thousand and twenty persons in the age range of 15 to 18 years were tested. They were people residing in Sapporo (Hokkaido), Hanamaki (Iwate), Matumura (Niigata), Chiba

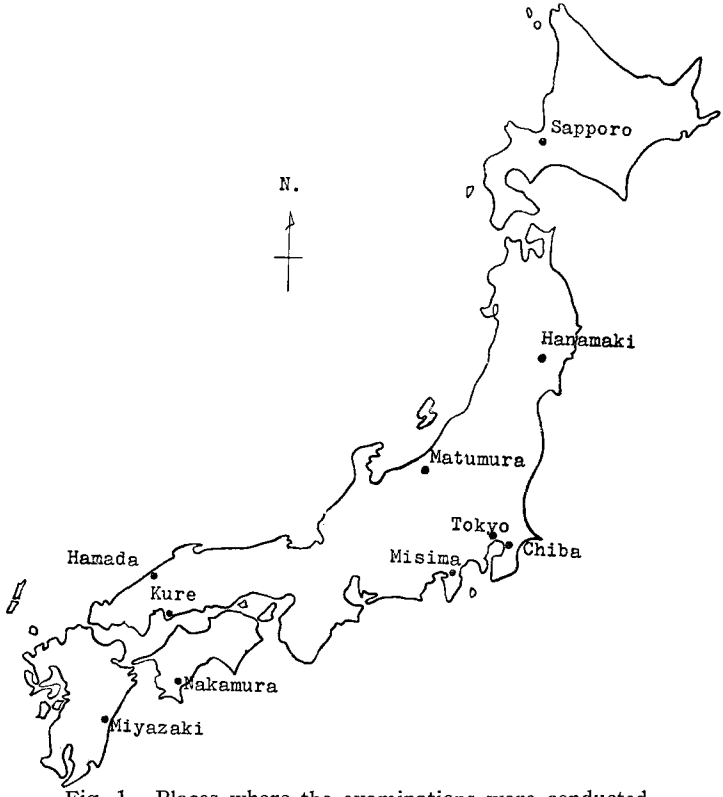


Fig. 1. Places where the examinations were conducted.

(Chiba), Misima (Shizuoka), Hamada (Shimane), Kure (Hiroshima), Nakamura (Koochi), Miyazaki (Miyazaki) and Tokyo.

Only 48 persons (2.37%) showed no response even to a saturated solution of Citbittol A, and 13 (0.64%) of these were also blind to Phenyl-thio-carbamide. The great majority, sensitive to Citbittol A, was composed of 1972 persons (97.73%); of these 158 (7.84%) were nontesters to Phenyl-thio-carbamide. One thousand eight hundred and fourteen persons (89.89%) were therefore normal tester to both chemicals.

Between the two classes, concerning their reaction to Phenyl-thio-carbamide, positive or negative, no significant difference was found in the variation of the threshold value of the sensitivity to Citbittol A ($P=0.9-0.8$). And no significant difference ($P=0.1$) was recognized in the variation of the threshold value of the sensitivity to Phenyl-thio-carbamide between Citbittol tester- and non-tester groups.

Another point of interest was that the detected percentage of non-tester to Citbittol A was higher in south-western than in north-eastern Japan.

Thus, Citbittol A seems to be a useful reagent in the examination of human taste sensitivity.

74. *Subunits of H_1 gene in salmonella*[1)]

(By Tetsuo IINO)

It has been known that a flagellar protein (flagellin) has complex antigenisities; that is, a flagellin molecule has several different antigenicaly active sites. A transduction experiment indicated that the specificity of the antigen complex is determined as a whole by a single gene, H_1 in phase-1 and H_2 in phase-2, respectively. Modifier genes for the antigenic specificity have not been demonstrated.

The serotypes studied most extensively on antigenic subunits of the complex are those of *g*, ···group in phase-1, as listed in the KAUFFMAN-WHITE scheme. The major components of the *g*,··· group are *f*, *g*, *m*, *s*, *t*, *p*, *q* and *u*, each of which can mutate independently of the other. Among the serotypes which carry *g*,···group antigens, all combinations of two components randomly chosen from *g*, *m*, *s*, *p* and *t* has been detected. The *u*-antigen was detected in combination with *g*, *m*, *p* and *t*, but not with *s*. The *f*-antigen accompanies either *g* or both *g* and *t*; *q* with *g* or both *g* and *m*. The presence of these antigenic combinations among the sero-

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

types and the occurrence of mutations in the antigenic subunits make it possible to devide the H_1 gene at least into five sections each of which is marked by an antigenic specificity (Fig. 1).

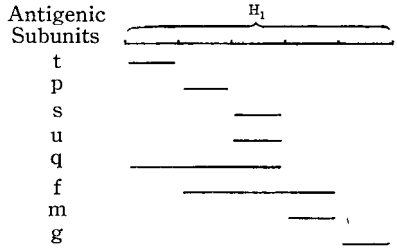


Fig. 1. A hypothetical schema on the distribution of subunit-antigen determinant in H_1.

75. *Unequall recombination in salmonella*[1]

(By Tetsuo IINO)

In a transduction experiment from *S. abony* CDC103 (*b*: *enx*) to *S. typhimurium* TM 2 (*i*: *1.2*), a recombinant type which expresses alternatively the phase-1 antigens of each of the parental strains was obtained. The frequency of $i \rightleftarrows b$ variation in this strain is the same as that of phase variation in TM 2. The antisera were prepared against each of the *b*-antigens of *S. abony* and the recombinant. The reciprocal absorption experiment with these antisera indicated that the *b*-antigens in both strains were identical. The *i*:*b* type clone does not produce any *1.2* type cells when it is incubated on semisolid plates containing anti-*i* and -*b* sera as selective agents. The gene which determines *b*-antigen type in the *i*:*b* clone is transduced into the H_2 locus; for example, *a*:*b* type is obtained in transduction from *i*:*b*—× *a*: *enx*. The transduction of Fla_1^+, which is linked to H_1, from the *i*:*b* type to *S. heidelberg* SW1092 (Fla_1^--$H_1^r H_2^{1,2}$) on semisolid screening plates produces the motile *i*:*1.2* type but not the motile *b*:*1.2*.

From these results it is inferred that the phase-1 duplicate type, *i*:*b*, is an unequall recombinant rather than a heterogenote. The stable H_1^b gene becomes unstable when it translocates to a position closely linked to Vh_2^+, the stability controller of H_2. The phenomenon is analogous with the varigated type position effect in higher organisms. These experimental results also suggest that the phylogenic differentiation of serotypic phase in

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

Salmonella has passed the following courses: duplication of H_1 in a monophasic strain, translocation of the duplicated H_1 to a region proximal to Vh_2, and the mutations of antigen type specificity resulting in the production of the diphasic types.

76. *A relation of metabolism to UV induced mutation in yeast* [1]

(By Sayaka NAKAI)

Recently it has become increasingly evident that certain celluar metabolic conditions within a critical post-irradiation period affect the frequency of mutation.

In this paper the effect on mutation frequency of controlled purine-pyrimidine and amino acid syntheses during the "sensitive period" after irradiation is reported. Using a multiple biochemical mutant of the haploid yeast strain (*Saccharomyces cerevisiae*) that requires adenine, uracil, arginine, phenylalanine, and isoleucine, it was possible to control idependently the syntheses of these substances by supplementing them in the incubating medium. Reversion of isoleucine requiring cells to non-requiring ones was used for measuring mutation frequency.

Purine-pyrimidine synthesis: In order to study the effect of purine-pyrimidine synthesis on mutation, adenine and uracil (50 μg/ml) were supplemented, in media previously supplemented with all three amino acids, at 20 minute intervals from 0 to 3 hours after irradiation. The result obtained in this experiment is shown in Fig. 1. Mutation rate showed a characteristic bimodal pattern.

When cells were supplemented with adenine and uracil immediately after irradiation, the mutation frequency decreased markedly (to about one fifth) in comparison with that obtained in the experiment without supplement.

When supplementing was delayed, the mutation frequency increased and reached the first peak at 40 minutes. A further delay caused a sharp drop of mutation frequency. The second peak appeared at 100 minutes. This result indicates that the role of nucleic acid synthesis in effecting mutations periodically changes. It seems that the two peaks observed reflect the replication process of genetic materials, though it is yet unknown whether synthesis of DNA or RNA is responsible the peaks.

Amino acid synthesis:

In order study the effect of amino acid synthesis on mutation, a similar experiment was carried out, i.e., arginine and phenylalanine (50 μg/ml)

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

were supplemented at various intervals to the incubation medium that was previously enriched with adenine and uracil. The result is also shown in Fig. 1. A bimodal change of mutation rate was found in this experiment. This result seems to indicate that mainly protein synthesis interferes with mutation between the stages when nucleic acid synthesis has the most prominent effect.

The results obtained in this experiment lead to the hypothesis that the metabolic process necessary to establish a mutation involves, at least, the following four sequential steps:

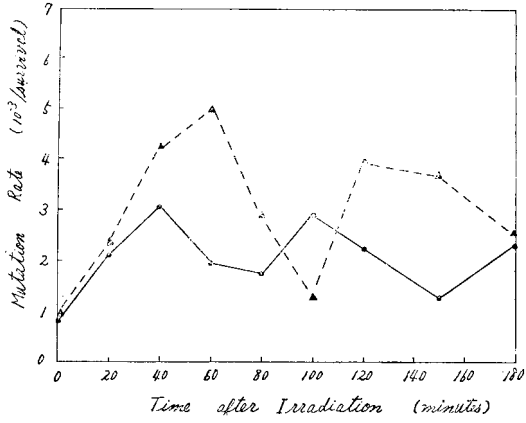


Fig. 1. Reverse mutation rate of isoleucine-requiring gene plotted against intervals between irradiation and nutritional supplement.
—— supplemented with adenine and uracil to medium pre-enriched with arginie, phenylalanine, and isoleucine.
.... supplemented with arginine and phenylalanine to medium pre-enriched with adenine, uracil, and isoleucine.

(1) Purine-pyrimidine synthesis decreases the frequency of mutation, presumably due to the process of repairing radiation damage.
(2) Purine-pyrimidine synthesis promotes the occurrence of mutation.
(3) Amino acid synthesis promotes the occurrence of mutation.
(4) Purine-pyrimidine synthesis again promotes the occurrence of mutation.

77. *Cytological studies on the nucleus of yeast*

(By Yoshiaki YONEDA)

Many problems remain unsolved in yeast cytology. Two concepts have been proposed with respect to the nucleus of yeast; one is LINDEGREN'S

nuclear vacuole theory and the other is the concept proposed by GUILLIERMOND, who considered the body located outside the vacuole to be the nucleus. Various chromosome numbers have been reported for the one species, *Saccharomyces cerevisiae* (for example, 1, 2, 4, and 5 or more as haploid number). In order to clear up the situation, the following observations were made on the nuclear structure of *S. cerevisiae*.

1) Resting nucleus: In a yeast cell, a large central vacuole to which is attached a small spherical body, is usually visible. This body was revealed by staining with Lugol's solution or acid fuchsin; it was also faintly differentiated from the cytoplasm in a living cell under the phase contrast microscope. It was revealed by the Feulgen technique that chromatin substance was never found within the vacuole but was always located in this spherical body. Thus, the latter may be assumed to be the nucleus. The resting nucleus was composed of two parts, namely Feulgen-positive substance of semilunar shape occupying one side of the nuclear cavity, and the Feulgen-negative karyoplasm. In the nucleus stained with Giemsa, a densely staining granule was observed in the center of the karyoplasm.

(2) Dividing nucleus: During budding, the Feulgen-positive chromatin gradually changed into globular chromosomal bodies. Four such bodies were observed in the haploid strain studied, which indicates that the haploid chromosome number is 4. The densely staining granule also divided simultaneously with the division of the nucleus.

3) Nucleus in sporulating stage: The author observed the meiotic division process in a tetraploid strain stained with Giemsa. At first prophase, entangled thread-like chromosomes were observed, which became gradually shortened, though it was difficult to distinguish single chromosome. At first metaphase, considerably shortened bivalent chromosomes, the number of which was probably eight, were observed. At that stage, the densely staining granule was also visible.

78. *Nature of radiation-induced mutations detected in the silk-worm by the method of specific loci* [1]

(By Yataro TAZIMA and Kimiharu ONIMARU)

According to the specific loci method, detected mutants might represent several kinds of mutations, *i. e.*, gene mutations, small deficiencies, or gross structural changes of chromosomes. It is therefore essential for the calculation of the exact mutation rate to examine what kind of mutation was obtained under a given traetment.

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

This method was employed in the silkworm in a number of irradiation experiments, but the nature of the recovered mutants has never been thoroughly exmined. Hence the present experiment was undertaken. The result seemed to indicate that mutation reaction might differ according to the locus tested. The research was, therefore, concentrated upon the cause of this seeming inconsistency between the reactions of the loci used.

a) Results with *ch* mutants

After irradiating $+^{ch}$ with X-rays and mating them to non-irradiated partners (the routine of the specific loci method), *ch* variants were picked up among F_1 individuals. They were crossed again to non-irradiated wild type. F_2 from each cross comprised two kinds of lots, *i. e.*, lots with wild type only and lots with both, wild and chocolate. The former were presumed to be the products of sib-mating of heterozygotes for the induced mutation. It was also assumed that all homozygotes might have been killed during early stages.

Among 22 mutant strains tested, 21 were found to carry embryonic lethals. The deficiency hypothesis may well explain the origin of those mutants.

b) Results with *pe* and *re* mutants

The results obtained for *pe* and *re* were markedly different from those for *ch*. In F_2 of the recovered mutants crossed to wild type, not a single lot was found to yield wild type only. It was, however, hardly possible to assume that all mutation bearing chromosomes were eliminated.

The next step of the experiment was crossing the induced mutants to + *re*/ + *re*, in order to discard all individuals carrying non-irradiated *pe re* chromosome. Thus, 20 mutant strains were tested in F_2 in homozygous condition for the mutation bearing chromosome. All of them showed segregation of three different phenotypes yielding an unexpected type *pe* as well as two expected types + and *re*.

The segregation of *pe* suggests that the mutation bearing chromosome has *pe* and that the mutation $+ \rightarrow pe$ had occurred in the irradiated chromosome of their grand parent. Among 16 mutants, those associated with embryonic lethals were 13; one was a semi-lethal and the remaining 2 were viable.

From those findings it seems that different kinds of mutation reaction exist according to the irradiated locus. However, the apparently different results in the above two cases could be explained on the same basis by assuming that the majority of mutations detected at the three specific loci were gene mutations from dominants to recessives and that the type of mutation response is determined by the time relation between the manifastation of the mutant genes and the srage of embryos which die

due to associated lethals. The egg colour genes manifest themselves in very early stages of embryonic development, which may precede the time of killing by the accompanying lethals, whereas the *ch* gene manifests its action after completion of embryonic development.

Thus it seems likely that the principal event of mutation caused by radiation is gene mutation and whether it is associated with chromosomal deficiency or not is merely incidental.

79. *X-ray induced dominant lethals from irradiation of different stages of oögensis in the silkworm* [1]

(By Yataro TAZIMA and Kimiharu ONIMARU)

By irradiating successive stages of oögenesis, we found two radiation sensitive stages in the silkworm. Sensitivity was measured in termes of lethal effects. The process from fifth larval stadium to early pupa is hypersensitive, while the late pupal stage is moderately sensitive. The former is characterized by a high number of embryos dying at an early embryonic stage, while the latter is not marked by a particular stage of the dying embryos, but, if any, it would be the late embryonic stage.

Irradiation of females at the fifth larval stadium increased the incidence of sterile moths and decreased the number of eggs formed even in fertile moths. On the contrary, irradiation of femals at the late pupal stage radiation effect was relatively slight so far as the egg production is concerned.

With the purpose of eliminating the effect of irradiated cytoplasm upon lethality, we made also observations of dying embryos from crosses between irradiated males and non-irradiated females. The incidence and stage specificity of the dying embryos were in good accord for both sexes at the stage of highest mutability, i. e., fifth larval stadium day 6 in the male and late pupal stages in the female.

From those observations it was suggested that increased incidence of dying embryos from eggs laid by females irradiated at a late pupal stage is due to radiation-induced dominant lethals, while the marked increase in early dying embryos from females irradiated at the fifth larval stadium is not attributed to dominant lethals, it is rather caused by disturbed egg plasma formation due to irradiation.

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

80. *Some observations relevant to the possible cause of radiation-induced sterility in the male silkworm*[1)]

(By Yataro TAZIMA)

An extremely sensitive stage to radiation was revealed early in the fifth stadium of the male silkworm (TAZIMA, 1958), and experiments to investigate the possible cause underlying this observation have been carried out.

When males are irradiated at this particular stage and mated to non-irradiated females, most of the eggs laid are unpigmented and dry up at a very early stage.

Two alternative hypotheses have been postulated to explain the phenomenon. One is sterility of the irradiated male due to lack or inefficiency of sperms. The other is the killing of fertilized eggs during early cleavage by dominant lethals introduced with irradiated sperms. In the discussion of radiation-induced dominant lethality, it is essential to determine which of the two may be the cause of these unpigmented eggs.

Examination of the genital organs of non-irradiated females mated to irradiated males showed that a fairly large number of sperms were ejaculated into the bursa copulatrix, while only very few sperms reached the receptaculum seminis. This indicates that though spermiogenesis, from the morphological point of view, was carried out almost normally, most of the sperms were abortive. Thus, in the majority of cases females mated to the irradiated males laid unpigmented eggs with a few exceptions of normally pigmented ones, i.e., fertilized eggs and in some cases abnormal eggs with a few pigmented serosa cells. The latter ones were also found with approximately the same frequency among the eggs laid by virgin females, which indicated that those serosa cells might have arisen from parthenogenetically developed nuclei.

This assumption was verified by double mating of irradiated males to non-irradiated + females and to *re* females. The comparison of the coloration of the serosa cells of abnormal eggs between the two crosses showed that these eggs were the products of parthenogenesis, excluding the possibility of fertilization by irradiated sperms.

The conclusion is that a high incidence of unpigmented eggs in F_1 from irradiated males is probably caused rather by inefficiency of the germ cells than dominant lethals which manifest themeselves at an early cleavage stage.

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

81. *Recovery of irradiated spermatogonia of the silkworm and the stage limit for the production of functional sperms* [1)]

(By Toshihiko SADO)

It was found that silkworm spermatogonia, especially in the later stages, are easily killed by exposure to low X-ray doses. Normally much fewer spermatogonia are found in the testes in early fifth stadium than in early fourth stadium. It may be expected, therefore, that irradiation of males at the fourth stadium affects fertility more than that given at the fifth stadium. However, according to fertility tests, irradiation of males at early fourth stadium produces less sterility than that applied at early fifth stadium.

Hence the question arose whether less sterility in the former case was due to functional sperms produced from regenerated spermatogonia after irradiation. In order to answer it, the present experiment was undertaken. The results are as follows.

When male larvae were X-rayed at early fourth stadium with 1000r of X-rays, complete depletion of spermatogonia was observed three days after irradiation but two days later regeneration occurred. However the regenerated spermatogonia were unable to develop into functional sperms by the time of the emergence of the moths. A number of primary spermatocytes at meiotic prophase, especially at synaptic and pachytene stages, were also found in the testis in early fourth stadium and most of these cells developed into functional sperms even after 1000r of X-rays. Thus it may be clear that the fertility of males irradiated in early fourth stadium with 1000r is not due to regenerated spermatogonia but due to spermatocytes irradiated at meiotic prophase I. Another experiment showed that most of these cells do not differentiate into functional sperms after 2000r of X-rays.

In groups irradiated at earlier stages, regenerated spermatogonia developed into spermatocytes 8–9 days after irradiation. These cells were histologically confirmed to differentiate into normal spermatozoa.

Fertility test showed that the recovered spermatogonia can develop into mature normal sperms, if the males are irradiated early enough in the life cycle, *i. e.*, at the first or second stadium.

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

82. *Duration of spermatogenesis in the silkwarm*[1]

(By Toshihiko SADO)

In order to elucidate varying effects of irradiation on the successive stages of spermatogenesis in the silkworm, it is important to know the onset and duration of each stage, especially when the recovery of irradiated spermatogonia is taken into consideration. No reports on this problem have been published so far either for the silkworm or for other insects.

The answer could readily be obtained if *in vitro* culture of spermatogenic cells of silkworm were successful. However, a crude estimate of the duration of cell development for each stage might be possible by recording the time of the first appearance of cells of one stage and that of the next one.

Table. Approximate timing of cell development in the spermatogenesis of the silkworm

Stage of spermatogenesis	Duration
Spermatogonia	a few days
Spermatocytes	
Meiotic prophase I	10–11 days
Metaphase I - Anaphase II	within 1 day
Spermatids	5–6 days
Spermatozoa	—
Total spermatogenesis	about 20 days

The testis of a newly hatched larva contained only a mass of primordial germ cells. Three days after hatching primary and secondary spermatogonia differentiate but spermatocytes do not yet appear. Differentiation of the spermatogonia into spermatocytes occurs first in the middle of the second instar, 5–6 days after hatching. Then the third stadium develops, and a high incidence of differentiation of spermatogonia into spermatocytes is observed. Most spermatocytes enter into a long meiotic prophase I, starting in the middle of the second stadium and lasting until the fourth molting (16–17 days after hatching). In the beginning of the fifth stadium cells in metaphase I are observed for the first time. Within a day after the onset of metaphase I spermiogenesis commences in the testis, which shows that the time between metaphase I and the beginning of spermiogenesis is very short. At the end of the fifth stadium

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

(22–23 days after hatching), spermatoza appear in bundles in the testes.

The above description is based on the observation of the most advanced among the germ cells. Most of the remaining cells develop about three or more days later.

Similar observation was made with irradiated testes. As reported previously, almost complete depletion of spermatogonia was observed three days after irradiation of male larvae with 1000r X-rays but two days later regeneration occurred from surviving primordial germ cells. Regenerated germ cells entered into synaptic stage 8–9 days after irradiation and 17–18 days after irradiation some cells were seen in meiotic metaphase I while some others were in an early spermatid stage. They developed into mature spermatozoa 23–24 days after irradiation.

Based on these observations approximate estimation was made for the duration of each stage of spermatogenesis, as indicated in the table.

83. *Studies on mutation rate by chronic irradiation of mice*[1)]

(By Tsutomu SUGAHARA, Kiyosi TUTIKAWA and Yoshiko TAKEDA)

In order to determine the dose of radiation and the scope of experiments necessary for the calculation of recessive lethal mutation rates, a pilot experiment was carried out employing three different methods. The results were quite useful for planning large scale experiments.

Progenies of mice of a multiple recessive stock, NH strain, irradiated chronically with 559 r of gamma-rays for three successive generations, as described in the Annual Report of 1958, were tested for recessive lethal mutations from three different standpoints by mating them with the CBA wild strain and studying their F_1 and F_2 progenies.

(1) Haldane's method: Recessive lethals within definite chromosome lengths from each marker gene were detected by searching for F_1 parents with abnormal segregation in F_2, according to the method proposed by J. B. S. Haldane. Recessive lethal mutation rate for the total of autosomes was calculated from the result.

(2) Litter size: The total number of recessive lethals may be calculated from the reduction of mean litter size in F_2 mice, provided that heterozygous recessive lethals do not have any effect on the viability of F_2 embryos and young animals. For example, one recessive lethal in either of P mice will reduce the F_2 litter size by a factor of 0.056.

(3) Sex ratio: Sex-linked recessive lethals would result in the decrease of sex ratio in F_1 and F_2. The mathematical relationship between the

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

shift of sex ratio and recessive lethals was mentioned in the previous report.

Results of the experiment and some analysis according to these three methods are shown in Table 1.

Table 1.

Genetical method		Irradiated	Control
Haldane's method			
No. of mice analysed		26	13
No. of recessive lethals		1	0
Total map length swept		34.24 cM	64.88 cM
Mutation rate	Observed	4.3×10^{-3}/r/total autosomes	
	Expected	$3.3\sim6.6\times10^{-3}$/r/total autosomes	
Litter size			
No. of F_2 litters analysed		296	148
At birth			
Mean litter size		7.57	7.21
Variance		6.790	5.133
At weaning			
Mean litter size		6.96	6.51
Variance		6.995	5.696
Difference at brith	Observed	+0.36	
	Expected	$-0.42 \sim -0.83$	
Sex ratio			
No. of F_2 mice analysed at birth		2240	1067
At birth		0.5335	0.5417
At weaning		0.5449	0.5389
Mutation rate	Observed	3.7×10^{-4}	
	Expected	$2.5 \sim 5.1\times10^{-4}$	

The methods of irradiation and mating applied here should result in an accumulation of recessive lethals induced in the gametes in postspermatogonial stages. The results obtained by HALDANE's method and sex ratio were qualitatively in good accordance with the expected mutation rate based on the assumption of HALDANE (1956) or the results on litter size assumed by CARTER (1957), but were statistically insignificant because of the small number of mice used. As to litter size, the difference of the means between irradiated and control progenies were statistically not significant, but the variance at birth was significantly larger in the irradiated ones than in the control. The expected result in this case was a decrease in mean litter size which could be significant even with the

number of mice used here. The unexpected result on mean litter size seems to be associated with the increased variance.

The radiation dose and number of animals sufficient to obtain significant results by each method were estimated from these data. Haldane's method seemed to be the best one among the three to determine recessive lethal mutation rate. A much larger number of animals would be necessary by the method of sex ratio. The unexpected result with regard to litter size should be followed up.

84. *Effect of X-rays on the rate of mitosis*[1]

(By Tosihide H. YOSIDA and Teiichiro TAKAHASHI)

In order to investigate the rate of mitosis in the Ehrlich ascites tumor, we X-rayed tumor bearing mice. Doses of 250 r, 500 r, 750 r, and 1,000 r were given under the following conditions: 180 kVp; 20 mA; distance of 50 cm; filter of 0.5 mm Cu + 0.5 mm Al; dose rate of 104 r/min.

In the case of 250 r, the mitotic index was markedly increased immediately after irradiation, but was reduced one hour later. 8 hours after, an increase of mitotic cells was observed again, and still 24 hours, the number of mitotic cells was much higher than before irradiation. 48 hours after irradiation, it was decreased again, and at 72 hours, the original mitotic rate was reestablished. At 500 r and 750 r, the mitotic index was increased immediately after irradiation, but the increase was not as striking as at 250 r; a marked reduction of mitotic cells was observed 30 minutes after irradiation. Their number was increased again 12 hours after irradiation, and 12 hours later the frequency of mitosis was much higher than in the control batch. The frequency of mitosis at 1,000 r irradiation was similar to that at 750 r. In this case, however, the increase of mitotic cells could not be observed immediately after irradiation.

Two stages of mitotic cells were distinguished, i. e. prometa-metaphase and anaphase, and their frequencies were observed. Both were generally increased immediately after irradiation with lower doses.

On the basis of the above investigations, we presume that the increase of mitotic cells observed immadiately after irradiation is due to the stimulation of mitosis by X-rays.

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

85. *Chromosome breakage at metaphase and fragment formation at anaphase after X-irradiation*[1]

(By Tosihide H. YOSIDA and Teiichiro TAKAHASHI)

Tumor cells of the Ehrlich ascites carcinoma were irradiated with 750 r and 250 r under the same conditions as mentioned in the preceding paper. After irradiation by X-rays the frequencies of 1) chromosome-, 2) chromatid- and 3) incomplete chromatid-breaks were examined immediately, 30 minutes, 8, 12, 24, 48 and 72 hours after treatment. Immediatly after 750 r irradiation the frequency of chromosome breaks was not increased, while 30 minutes later all three types of breaks occurred with very high frequency, especially complete and incomplete chromatid breaks. One and 8 hours after irradiation, metaphase cells could not be found. At 12 hours, however, many mitotic cells were observed. The frequency of chromosome breaks at this time was higher than at 30 minutes after exposure, while that of complete and incomplete chromatid breaks was lower than at 30 minutes. The frequencies of the three types of breaks gradually decreased with the progress of time.

The number of chromosome breaks was higher than that of fragments 30 minutes after X-irradiation at 750 r, but become similar 12 hours later. At 24 hours after irradiation the frequencies of breaks and fragments were reversed in relation to those obtained 30 minutes after irradiation.

86. *Nature of chromosome bridges after X-irradiation*[2]

(By Teiichiro TAKAHASHI and Tosihide H. YOSIDA)

Frequencies of chromosome bridges at anaphase and chromosome abnormalities such as dicentric or ring chromosomes at metaphase were examined in the Ehrlich tumor after X-irradiation. The frequency of chro-

Table 1. Frequency of chromosome abnormalities and lagging chromosomes occurring after irradiation with 750*r*.

Type \ Hrs. after	Immed. after	0.5	12	24	48	72
Chromosome abnormalities	0	0	0.5	0.6	0.1	0
Bridges	0	2.2	3.2	2.5	1.9	1.5

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

mosome abnormalities at metaphase was very low, while that of chromosome bridges was very high (Table 1). The majority of chromosome bridges occurred through stickiness of the chromosome matrix. It is suggested that the majority of chromosome bridges resulted from physiological effects of X-rays on living material.

In the case of high doses the chromosome bridges continued to appear for a considerably longer time. Althouth it has been considered that the chromosome bridges resulting from physiological effects of X-rays are a temporary phenomenon, they continued for a longer time in the case of Ehrlich tumar cells.

87. *Relation between micronuclei and chromosome fragments after X-irradiation*[1]

(By Teiichiro TAKAHASHI and Tosihide H. YOSIDA)

Micronuclei originate from fragments or lagging chromosomes. The frequency of cells with micronuclei was not as high as that of cells with lagging chromosomes (Table 1). It is interesting that the frequency of micronuclei after irradiation with lower doses was higher than at higher doses, while the frequency of lagging chromosomes was comparable to the dose rate. For example, 84 per cent of cells had lagging chromosomes 24 hours after irradiation at 1,000 r, but at the same time only 1.4 per cent had micronuclei.

Table 1. Frequency of cells with lagging chromosomes and micronuclei after 750 *r* irradiation.

Type \ Hrs. after	Immed. after	0.5	12	24	48	72
Lagging chromosomes	0	28.0	76.0	76.0	28.0	30.0
Micronuclei	0.3	0.3	0.6	5.1	8.9	2.8

100 cells were counted in each sample.

The above observations indicate that many cells with lagging chromosomes degenerate soon after cell division.

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

88. *Transplantation experiments with spontaneous and radiation-induced leukemias in mice*[1)]

(By Yoshinori KURITA)

The present paper deals with a comparison of spontaneous and radiation-induced leukemias in mice.

A spontaneous leukemia developed in a 10 months old AKR/.Jax female mouse. It had the character of so-called generalized lymphatic leukemia and could be successfully transplanted only into AKR/Jax strain. This transplantable leukemia was named AKLA line.

A leukemia induced in a C57BL/6Ms male mouse by X-irradiation was characterized by thymic lymphosarcoma and could be successfully transplanted only into C57BL/6Ms strain. This transplantable leukemia was named BLXL-1 line.

A comparison between the two transplantable leukemias may be summarized as follows: (1) Both leukemias could be transplanted to the original strain only. They had a 100% transplantability to the respective strain of origin. (2) The average life span in mice bearing AKLA leukemia was 14 days, while it was 21 days for BLXL mice. The life span in both cases was gradually reduced in the course of transplant generations. (3) The number of white cells was the highest 12 days after transplantation for both leukemias. Leukemic cells in both leukemia lines, however, always had abnormal lymphatic forms, and their granules were always negative to peroxidase reaction. After trasplantation cells showed a daily increase in number. (4) As BLXL leukemia often showed myeloid reactions, it may belong to the so-called "stem-cell" leukemias.

Though the opinion prevails that a hematological diagnosis in radiation-induced leukemias is not reliable, the number of white cells in BLXL line have always reached about $50\times10^3/mm^3$ 12 days after transplantation and gradually reduced thereafter. Though localized BLXL leukemia began in the thymus when transplanted into intraperitoneal cavities of other mice, it later became the so-called generalized leukemia. On the contrary, AKLA leukemia developed as the generalized from but changed into a localized solid form when subcutaneous transplantations of the spleen of leukemic mice into one month old or older healthy mice were made.

The above investigations indicate that in mice there are no fundamental differences between localized and generalized, or between spontaneous and radiation-induced, leukemias.

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

89. *Relation of post-irradiation treatments to radiation-induced mutation frequency in yeast*[1]

(By Saburo NAWA and Kazuo SAITO)

A culture of *Saccharomyces sp.* 8256 a (su. ga. ma. ME. MG. adenine) was grown at 30°C in complete medium. Following incubation, the cells were held for five hours at 5°C and then incubated at 30°C for two hours to obtain synchronization of cell growth. They were removed from the medium by centrifuging and were resuspended in 0.9 per cent saline. The turbidities of cell suspensions were then adjusted to the optical density of 0.4 (1.0×10^7 cells/ml) at 525 mμ with saline. The suspensions were irradiated by ultraviolet light, diluted into various media, and incubated for a definite time. Then the cells were plated on complete media. The frequency of mutation was determined by observing the change of colony color from original pink to white after three days of incubation at 30°C.

Cell division did not occur during ten hours of incubation following irradiation. No significant changes in the level of survival were noted during the course of this experiment. When the cells were held under conditions of normal metabolism, the mutation frequency increased. Potential mutations are expected to become visible by this processes. The mutation frequency of cells held under conditions which limit normal metabolism was lower than that of cells held under conditions favoring normal metabolism. Cells incubated in complete media containing chloramphenicol showed a higher frequency of mutation than cells held in unsupplemented media. The same effect of chloramphenicol was observed in cells irradiated by X-rays. In bacteria, it has become increasingly evident that a decline of mutation frequency occurs in the cells of post-irradiation chloramphenicol treatment. If a potential irradiation induced mutation is stabilized under one metabolism and reverted under another, the effect of chloramphenicol in our case may be to prevent the reversion.

90. *Synthesis of contractile proteins in X-irradiated embryos of Triturus pyrrhogaster* BOIE[2]

(By Yoshito OGAWA)

It was found in the early developmental stages of chick (Y. Ogawa,

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

1957, 1958) as well as *Triturus pyrrhogaster*, Boie (Y. Ogawa, 1958), and in regenerating limb tissues of the latter (Y. Ogawa, 1958)[1] that the formation of actin in the skeletal muscle precedes that of myosin. In consideration of this finding the following question arose: Does the formation of actin induce that of myosin and does it directly cause myosin formation? The synthesis of contractile proteins in X-irradiated embryos of *Triturus pyrrhogaster*, Boie was examined by means of the serological technique applied before[1] in order to give an answer to this question.

G-actin and myosin, prepared from the skeletal muscle tissue of good-sized specimens of *Triturus pyrrhogaster*, were injected intravenously (four times with three day intervals, 100 mg. in tatal) into rabbits. The immunized rabbit sera was raised to serological specificity by resorption test with saline extracts of liver, spleed and skin tissue of *Triturus pyrrhogaster*. The titer of sera was adjusted to 1:512, and then the precipitin reaction with saline extract of X-irradiated embryos was carried out. A constant volume of sera and progressively decreasing amounts of antigen were used in order to prevent a failure due to an excess of antigen. Irradiation of embryos with 50r, 200r and 500r was made 108 hours after fertilization.

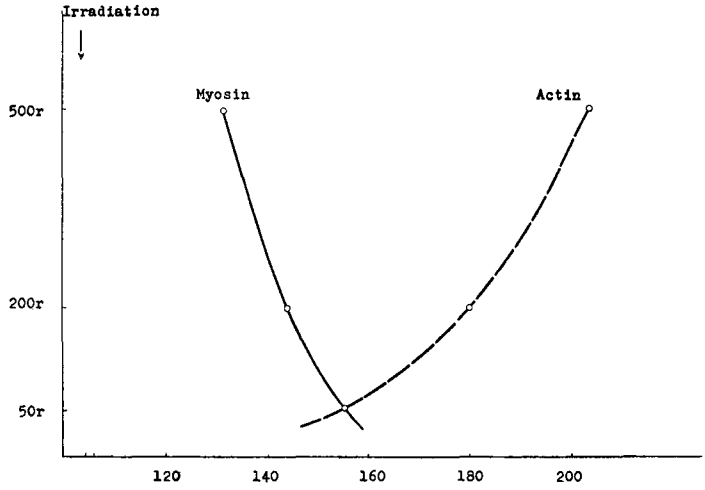

Fig. 1. Synthesis of contractile proteins in early development of the embryos of *Triturus pyrrhogaster* irradiated by x-rays 108 hours after fertilization. Abscissa: Hours after fertilization. Ordinate: Dose of x-rays.

1) Refer to Report No. 107 (p. 151).

In normal embryos, actin and myosin first become detectable 132 and 176 hours, respectively, after fertilization (Y. OGAWA, 1958). In the case of irradiation with 50r, both, actin and myosin, were detected 156 hours after fertilization. The time of actin and myosin formation after fertilization was changed to 180 and 144 hours, respectively, with 200r, and to 204 and 132 hours, respectively, with 500r, as shown in Fig. 1.

X-irradiation 108 hours after fertilization, therefore, markedly supresses the synthesis of actin and promotes that of myosin in the early developmental stages. The order of actin and myosin synthesis in the irradiated embryos at doses of 200r and 500r was opposite to that found in the non-irradiated control.

It was thus proved that during skeletal muscle differentiation in the early embryo of *Triturus pyrrhogaster*, the formation of actin does not induce that of myosin and the processes of synthesis of both proteins are independent of each other.

91. *Heterozygous effects of induced lethals and their presistence in populations*[1]

(By Chozo OSHIMA and Osamu KITAGAWA)

Male flies of an isogenic Samarkand strain of *Drosophila melanogaster* were irradiated with X-rays and the induced lethal second chromosomes were isolated by CMI method. Four hundred and twenty three chromosomes, each irradiated with 100 r, were checked and 29 lethal (6.86%) and 15 semi-lethal chromosomes (3.55%) were obtained. Two hundred and fifty three chromosomes, each irradiated with 500 r, were tested and 7 lethals (2.77%) and 17 semi-lethals (6.72%) were detected. Twenty seven of the above lethals have been retained in balanced condition with the *Cy* inversion. The identification of each lethal was examined by diallel crosses and two lethals (*l*-3, *l*-50) were proved to be allelic.

The heterozygous *Cy*/*l* female flies of each lethal strain were mated with male flies of the Samarkand strain and *Cy* and wild type offspring were scored. The genetic background was considered to be homozygous. The percentages of wild type flies (lethal heterozygotes) were obtained in each strain. These crosses were replicated ten times and the overall average percentage of lethal heterozygotes was 46.779. The *Cy*/+ female flies were mated with Samarkand male flies and the ratio of *Cy* and wild type offspring was obtained. On the basis of this ratio, the overall average percentage was corrected and the value of pre-adult viability of lethal heterozygotes was calculated to be 0.9477. The selective disadvantage of

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

lethal heterozygotes is estimated at about 5 percent.

At the same time, the heterozygous *Cy/l* female flies of each lethal strain were mated with male flies taken from an unrelated laboratory population cultured in a population cage. The laboratory population had been made up from twenty laboratory strains and has been cultured for about six months, and its genetic constitution was presumed to be heterozygous. The overall average percentage of lethal heterozygotes was 48.224. The relative viability was calculated by using the relative viability obtained

Table 1. Percentage of wild type flies (lethal heterozygotes) in offspring of the cross, *Cy/l*×Samarkand or *Cy/l*×heterozygous male. Each value is the mean of 10 replications.

Lethal	Homozygous cross	Heterozygous cross
1	47.125*	47.009
2	36.934**	47.505
3	47.936	47.649
4	49.130	48.789
5	48.061	48.268
6	47.997	48.235
7	44.330	47.106
8	42.821**	54.690**
9	47.679	48.971
10	40.922**	50.013
11	47.006*	47.820
12	48.388	46.481
13	47.704	46.156*
14	49.297	42.914**
15	46.669**	45.598*
16	44.252**	47.656
17	47.909	47.248
18	48.597	46.360*
19	49.531	46.518
20	48.784	46.858
21	47.994	51.089
22	49.504	49.634
23	49.221	50.855
24	43.144**	51.102
25	47.904	49.578
26	49.615	49.898
27	44.600**	48.069
Mean	46.779	48.224

* Significantly deviates from 50% at 5% level.
** Significantly deviates from 50% at 1% level.

above: 0.93139/0.87895=1.05966. The selective disadvantage, noted in homozygous background, seems to have disappeared. The results of crosses are shown in Table 1.

The analysis of variance of both crosses was made and the results are represented in Table 2. The differences between crosses and between lines (lethals) within a cross were highly significant. The difference between lethal strains having homozygous background was also significant, but in the case of a heterozygous background, no significant difference was detected. The deviation from 50 percent for each lethal heterozygote was examined and eight lethals differred significantly (1% level) in the direction of disadvantage, while two lethals differred significantly (5% level). As a whole, there was no correlation between pre-adult viabilities of lethal heterozygotes in homozygous and heterozygous background. From these results we can say that the selective disadvantage of lethal heterozygotes in pre-adult viability is affected by their genetic background.

Table 2. Analysis of variance

Source of variation	D.F.	M.S.	F
Total	539		
Between crosses	1	281.8978	10.9617**
Between lines within cross	52	71.1503	2.7667**
Error	486	25.7166	
Homozygous cross			
Total	269		
Between lines	26	91.6766	6.5089
Between reps	9	18.4595	1.3106
Error	234	14.0847	
Heterozygous crosses			
Total	269		
Between lines	26	50.6240	1.5841
Between reps	9	173.1142	5.4169**
Error	234	31.9584	

Eight lethals (*l*-7, *l*-8, *l*-10, *l*-18, *l*-19, *l*-22, *l*-24, *l*-26) have been kept in separate populations which were designed by pearl's method. The initial frequency of each lethal was 50 percent and the genetic background was homozygous. The state of their persistences in the populations was examined six months after starting. The frequencies of lethal chromosomes decreased as shown in Table 3. Their persistence seemed to depend on the degree of selective disadvantage represented in Table 2.

Table 3. Frequencies of lethal chromosomes in laboratory populations 6 months after starting (about 16 generations).

Lethal	Replicated population	No. of chromosomes tested	No. of lethal chromosomes	%	M
7	C	64	6	9.38	8.93
	D	59	5	8.47	
8	A	60	2	3.33	2.39
	C	69	1	1.45	
10	C	61	0	0.00	0.86
	D	58	1	1.72	
18	A	63	11	17.46	12.79
	C	74	6	8.11	
19	B	67	4	5.97	8.54
	D	63	7	11.11	
22	B	70	3	4.29	2.15
	C	69	0	0.00	
24	A	68	0	0.00	0.00
	B	70	0	0.00	
26	B	65	8	12.31	13.30
	C	70	10	14.29	

92. *Doubling dose as a common unit of measuring mutabilities in polygenes and major genes*[1]

(By Yukio YAMADA)

Mutabilities in polygenes and major genes are measured by different units and they cannot be compared with each other, because of the difficulty in estimating the number of genes belonging to a polygenic system. The present author proposes to use the doubling dose as a common unit of measuring mutability for the comparison of mutabilities in these two kinds of genes. The theoretical basis is as follows:

Assume that spontaneous and induced mutations have the same frequency distribution in genetic effect. Now, let $f(x)$ be the relative frequency of a mutation with effect x at a certain locus involving a polygenic trait, such that $\int_{-\infty}^{+\infty} f(x)\,dx=1$. Spontaneous genetic variance thus may be expressed as

$$\sigma_g^2=\int_{-\infty}^{+\infty}(x-\bar{x})^2 f(x)\mu dx,$$

where μ is the spontaneous mutation rate per locus. If n loci are involved in a metric character, total genetic variance spontaneously appearing per

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

generation is

$$\sum_{i=1}^{n} \sigma^2_{g(i)} = \sum_{i=1}^{n} \int_{-\infty}^{+\infty} (x-\bar{x})^2 f(x) \mu dx = V_{Ho},$$

where subscript i refers to values in the i-th locus, and V_{Ho} is the spontaneous mutation rate in terms of variance increase per generation. From the definition of the doubling dose, genetic variance increase per locus under doubling dose conditions is

$$\int_{-\infty}^{+\infty} (x-\bar{x})^2 f(x)(2\mu)\, dx = 2\sigma^2_g$$

And hence, the total over-all genetic variance increase involved under doubling dose conditions is

$$V_{HD_2} = \sum_{i=1}^{n} 2\sigma^2_{g(i)} = 2 \sum_{i=1}^{n} \sigma^2_{g(i)} = 2V_{Ho}$$

Accordingly, it is deduced that the doubling dose for a polygenic system is equivalent, *as an expectation*, to the amount of radiation necessary to double the number of mutants per gene per generation, as it is the case of major gene mutations. The doubling dose of a polygenic trait is obtained by the formula,

$$D_2 = \frac{m}{k},$$

where D_2, m, and k are the doubling dose, the spontaneous rate of genetic variance increase per generation, and the induced rate of genetic variation per unit dose of radiation, respectively.

93. *Doubling dose of polygenic characters*[1]

(By Yokio YAMADA and Osamu KITAGAWA)

Reports on spontaneous or induced mutability of polygenic characters, such as the number of bristles on the sternite and sternopleura of *Drosophila*, are scanty in animals as well as in plants. The mutation rate of such characters can be measured by the amount of variance increment per unit dose in case of radiation induced mutation. This method has been already reported by the present authors as well as other investigators.

The mutation rate in polygenes expressed in terms of variance increment, however, can not be compared with that of major genes, since the units of measure are entirely different, variance in the former and frequency of occurrence in the latter being used. If we want to know whether or not mutations of polygenes occur at a higher rate than those of major

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

genes, a certain common unit in estimating the rates should be used.

The difficulty in deducing mutational occurrence in polygenic characters would be avoided by the use of doubling dose, with an appropriate assumption, which would enable us to compare the mutability of polygenic systems with that of major genes, as is shown in the preceding article.

In order to estimate the doubling dose, we must know both spontaneous and induced mutation rates in bristle numbers on the sternite and sternopleura of *Drosophila*. Hitherto, the former rate was dealt with in three papers only by DURRAT and MATHER (1954), CLAYTON and ROBERTSON (1955), and PAXMAN (1957). The estimate obtained by DURRANT and MATHER seemed to be inflated as was pointed out by MATHER (1956), himself. The estimates given in the two latter reports are lower and of similar magnitude. Hence we have used tentatively the spontaneous mutation rates of those authors, 0.005 and 0.001, respectively, for the estimation of the doubling dose in abdominal and sternopleural bristle number, before our own estimates of spontaneous mutation rate in these characters are obtained.

The experimental procedure was similar as described in the previous report (1958). However, lower radiation doses, 500r, 1000r and 1500r were used in the present experiment. The means and variances of bristle numbers, which were pooled for sexes and replicates, are tabulated as follows:

Dose		0r	500r	1000r	1500r
Genomes sampled		54	42	45	50
Abdominals	$\bar{x}$	29.1155	28.6081	28.7609	28.8297
	$\hat{\sigma}^2$	0.4803	0.4404	0.7488	0.6774
Sternopleurals	$\bar{x}$	14.2714	14.6337	14.7945	14.7100
	$\hat{\sigma}^2$	0.4080	0.5584	0.4834	0.5406

From the results, our estimates of induced mutation rates are 8.7×10^{-5} for abdominal bristles and 3.5×10^{-5} per rad for sternopleurals in terms of variance increment under random mating. Thus, we obtain $D_2=57{\cdot}5$r and $D_2=28.6$r, respectively, for the abdominal and sternopleural bristle number in *Drosophila*. It may, therefore, be concluded that mutability in these polygenic systems is substantially of the same magnitude as that of major genes.

94. *Radiation effects on germ cells of rainbow trout*

(By Yusiro HARADA[1], Tosio AI[2], Tosio AI[3] and Sohei KONDO)

Three old male and female rainbow trout were irradiated with acute X-ray doses of 40 to 1200 r and the sperms and eggs were pressed out by hands.

The radiation effects on the germ cells were tested by artificially fertilizing non-irradiated eggs with irradiated sperms or irradiated eggs with non-irradiated sperms. Observations were made on the number of unfertilized eggs recovered as the ratio of the number of eggs whose embryos died before the stage of eye formation to the total number of eggs, and on the number of post-embryonic lethals recovered as the ratio of the number of unhatched eggs to that of the eggs whose embryos had reached the stage of eye formation.

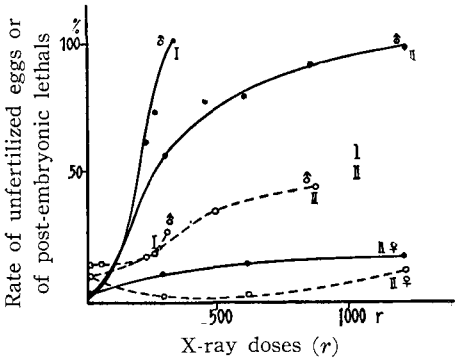

Fig. 1.
• • •: Rate of unfertilized eggs.
——: Rate of post-embryonic lethals.
Symbol I indicates irradiation of young germ cells and II irradiation of mature sperms (♂) or mature ovaries (♀).

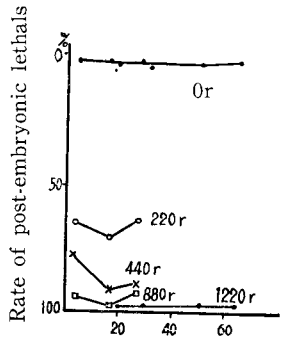

Fig. 2. Recovery tests of germ cells of male trout exposed to X-rays in the crossing season.

(I) Relation between radiation sensitivity of male germ cells and their developmental stages: Acute X-rays were given to two groups of male trouts, one so early in the hatching season that pressure on their abdomens did not release sperms and the other in the crossing season when sperms were quite easily released by a slight touch. Later these sperms were used to artificially fertilize non-irradiated eggs. 25% unfertilized

1) Prefectural Fisheries Experimental Station, Simizu and
2) Simoda.
3) Huzi Trout Breeding Station, Inogasira.

eggs were reached at about 300 r for both groups. The rate of post-embryonic lethals was, however, appreciably higher for males irradiated early in season than for those irradiated during the crossing season. For example, the dose of 300 r was strong enough to kill 100% of the fertilized eggs of the former while the same dose killed only 60% of the fertilized eggs of the latter. This means that young germ cells of male trout evaluated in terms of post-embryonic lethals are more radiation sensitive than mature sperms as is the case in the mouse and the silkworm.

(II) Comparison of radiation sensitivity between male and female trout: Radiation sensitivity of male trout in the crossing season was compared with that of female trout. The number of unfertilized eggs and post-embryonic lethals was scored. Fig.1 shows that the female germ cells proved to be much more resistant to radiation injury than the male cells.

(III) Recovery of X-rayed germ cells: To test the recovery of X-rayed germ cells, we took successive batches of sperm from the same X-rayed fish at intervals after the exposure and used them to artificially fertilize non-irradiated eggs. Fig. 2 shows that, in terms of post-embryonic lethality, there has been no male germ cell recovery from radiation injury during the period 3 to 66 days after X-ray exposure, although there has been a slight recovery of egg fertility.

The eggs of rainbow trout are much more resistant to X-rays than the sperms. Young germ cells of male trout are more radiation sensitive than mature sperms. Radiation injury of male germ cells results in a strikingly high frequency of dominant lethals at the post-embryonic stage. Only 200 r irradiation of adult males is potent enough to kill 50% of embryos during the stages between eye formation and hatching. The germ cells do not recover from these radiation injuries.

95. *Comparison of radiation effects of β- and γ-rays on einkorn wheat*

(By Seiji MATSUMURA)

Seeds of *Triticum monococcum flavescens* were soaked in ^{32}P and ^{131}I solutions for 2 days before sowing, to compare the radiation effects of β-rays with those of γ-rays. Radioactive solutions contained 0.15, 0.3 and 0.6 mc/gr of ^{32}P and 0.6 mc/gr of ^{131}I. Also γ-irradiation with ^{60}Co was applied at the dosages of 2.5, 5 and 10 kr immediately after soaking the seeds in water for 2 days. The growth of seedlings, height of mature plants, single-spike fertility, and chromosome aberrations of treated plants

in X_1 and chlorophyll mutations in X_2 were compared for β- and γ-irradiations. The data are shown in Table 1.

The higher the dosage of β- and γ-rays, the more delayed were the germination and growth of seedlings and the lower were the survival rate, height of mature plants, and fertility. The relation between the inhibition of seedling growth and dosage of β- and γ-radiations coincides roughly with that between the decrease of survival rate or fertility and dosage. There was poor germination and little growth of seedlings at 10 kr γ-irradiation and at 0.6 mc/gr ^{32}P β-irradiation, both irradiations being markedly effective. 5 kr γ-rays and 0.3 mc/gr ^{32}P β-rays considerably inhibited the growth of seedlings and reduced the survival rate and fertility. But 2.5 kr γ-irradiation was only slightly effective and the effects corresponded roughly to those of 0.15 mc/gr β-radiation ^{32}P and 0.6 mc/gr ^{131}I solutions. The frequency of ears with chromosome aberrations in X_1-plants and head progenies with chlorophyll mutations in the X_2-generation increased generally with the increase of β- and γ-radiation dosage. Again the effects of 0.15 mc/gr β-radiation ^{32}P and 0.6 mc/gr ^{131}I solutions corresponded roughly to those of 2.5 kr γ-radiation.

Table 1. Radiation effects of β- and γ-rays in einkorn wheat.

Dosage		Germination rate (%)	Length of seedlings* (cm)	Survival rate (%)	Plant height (%)	Fertility in X_1 (%)	Chromosome aberrations in PMC (%)	Chlorophyll mutations in X_2 (%)
Control		98.00	8.75	54.00	95.26	89.22	0.00	0.00
γ-rays	2.5kr	94.00	7.29	48.00	98.71	71.24	2.27	1.67
	5.0kr	90.00	2.06	20.00	96.30	51.80	64.00	10.00
	10.0kr	50.00	0.48	—	—	—	—	—
^{32}P	0.15 mc/gr	96.00	7.23	46.00	91.43	62.47	6.06	15.56
	0.3 mc/gr	90.00	2.69	28.00	89.79	56.52	33.33	5.00
	0.6 mc/gr	70.00	1.01	—	—	—	—	—
^{131}I	0.6 mc/gr	98.00	7.86	48.00	93.42	77.51	2.08	6.45

* The seedlings were measured 3 weeks after sowing.

These findings generally confirm the experiments performed last year. If we assume that the effects of β-radiation are confined to the embryo, we find by calculation that the 0.15 mc/gr ^{32}P solution equals about 2 krad. This, too, will account for the obtained data.

96. *Radiation effects of fast and thermal neutrons on wheat*
A) *Genetic effects of neutrons on einkorn wheat*

(By Seiji MATSUMURA)

Thermal neutron irradiations were conducted in the thermal column of the Japan Atomic Energy Research Institute's Nuclear Reactor, JRR-1. Hole No. 7 was selected to keep γ-contamination as small as possible. The thermal neutron flux was calculated to be 4.2 (V), 2.3 (IV), 1.32 (III), 0.78 (II) and 0.49×10^3 $n_{th}/cm^2\cdot sec$ (I) at the distances 4, 9, 14, 19 and 24 cm from the thermal column when the reactor was operated at 40 kilowatts. Dormant seeds of *Triticum monococcum flavescens* were treated at five different distances (I~V) for 2 weeks (actually 990.8 kWh). The thermal neutron integrated flux ranged from 4.4 to 37.5×10^{12} n_{th}/cm^2. The data are shown in Table 1. The irradiated seeds were almost uniformly injured in each

Table 1. Effects of thermal neutrons on einkorn wheat.

	Thermal neutron flux ($\times10^{12}$ n_{th} /cm^2)	Germination (%)	Length of seedlings* (Index)	Survival (%)	Plant height (Index)	Fertility in X_1 (%)	Chromosome aberrations in PMC's (%)	Chlorophyll mutations in X_2 (%)
Control	—	100	100	100	100	89.2	0.0	0.0
I	4.4	98	101.5	90	102.2	71.5	8.5	—
II	7.0	98	82.7	90	92.8	52.9	15.1	15.2
III	11.8	96	69.5	82	91.3	47.2	20.6	16.4
IV	20.6	68	27.7	36	87.3	37.1	25.0	22.6
V	37.5	0	—	—	—	—	—	—

* The seedlings were measured 14 days after sowing.

treatment. There was no germination in (V) and about 2/3 of seeds germinated but about one half of the seedlings soon died in (IV). The higher the dosage of thermal neutrons, the more delayed were germination and growth of seedlings and the more reduced were survival rate, height of mature plants, and seed fertility. The frequency of chromosome aberrations and chlorophyll mutations increased with the increase of dosage, as expected.

If we assume that γ-contamination for 10^{12} n_{th}/cm^2 is 430 r by Dr. KONDO's measurement with FRICKE's dosimeter, the 1 r equivalent effects produced by the thermal neutrons are calculated as follows, compared with the results produced last year by X- and γ-irradiation at 10 and 20 kr:

for seedling growth, $1\,r=2.0\times10^9$ n_{th}/cm^2

for seed fertility, $1\,r = 1.4 \times 10^9\ n_{th}/cm^2$
for chromosome aberration, $1\,r = 1.2 \times 10^9\ n_{th}/cm^2$
for chlorophyll mutation, $1\,r = 2.3 \times 10^8\ n_{th}/cm^2$

The exposure to 14 MeV neutrons obtained from (D, T) reaction was carried out in the Biology Division, Oak Ridge National Laboratory, by courtesy of Dr. M. L. RANDOLPH and Mr. D. L. PARRISH. Dormant seeds of *T. monococcum flavescens* were fixed at 6.3 cm from the tritium target

Table 2. Effects of fast neutrons on einkorn wheat.

Dosage		Germination (%)	Length of seedlings* (Index)	Survival (%)	Plant height (Index)	Fertility in X_1 (%)	Chromosome aberrations in PMC's (%)	Chlorophyll mutations in X_2 (%)
Control		100	100	95.9	100	—	—	—
X-ray	10 kr	94	75.6	68.0	92.6	47.2	29.4	11.7
	20 kr	40	42.3	0	—	—	—	—
γ-ray	10 kr	94	84.9	76.0	97.4	61.5	26.6	—
	20 kr	60	14.4	16.0	95.2	52.7	20.0	—
Fast neutron	0.5 krad	100	90.7	93.9	92.2	50.1	10.5	5.1
	1.0 krad	100	72.4	90.0	88.6	52.6	23.7	9.8
	1.5 krad	96	50.5	66.0	73.9	33.6	28.6	20.0
	2.0 krad	94	31.0	4.0	82.7	7.7	—	—
	2.5 krad	74	6.6	0	—	—	—	—
	5.0 krad	60	3.8	0	—	—	—	—
	7.5–20 krad	16~26	1.8~3.8	0	—	—	—	—

* The seedlings were measured 14 days after sowing.

and the exposure periods were varied from 7.7 min. to 306 min. The calculation of given doses is based on the first collision dose method developed by RANDOLPH who uses the analyzed elemental compositions and the measured neutron fluxes (cf. KONDO 1959, Ann. Rep. (in Japanese) No. 9: 135). The dosage ranged from 0.5 to 20 krad. At the same time at ORNL X- and γ-radiations of 10 and 20 kr were used for comparison. The effects of both radiations seemed to be rather stronger than those found in the same experiments last year in our institute. There was no germination or almost none at more than 2 krad of fast neutrons. At 0.5~2 krad, survival rate, height of mature plants, and seed fertility decreased, and chromosome aberrations and chlorophyll mutations increased with the increase of dosage.

Relative biological effectiveness (RBE) for fast neutrons, compared with

X- and γ-rays, is calculated as follows:

for germination rate,	3.5
for seedling growth,	10
for seed fertility,	11.8
for chromosome aberration,	8

97. B) *Relation of ploidy to chromosome aberrations*

(By Mitsuya NEZU)

Meiotic irregularities induced by various irradiations were compared in tetra- and hexaploid wheat. The experimental data are shown in Table 3.

Chromosome aberrations induced by thermal neutrons produced by JRR-1 were mostly univalents and reciprocal translocations (④ and ⑥). Univalents and ⑥ were more prominent in 6x species than in 4x species. Average number of breaks per cell increased non-linearly with the increase of dosage, when an univalent was counted as one, ④ as two, and ⑥ as three breaks. The number of breaks in 6x was about 3 times as high as in 4x species. No increase of breaks was found between treatments (II) and (III) in either species. The number of breaks obtained by these treatments was comparable to that obtained by 10 kr X- or γ-rays. Seed fertility decreased with the increasing dosage in both 4x and 6x. The 6x was slightly more susceptible than the 4x in this respect.

The plants irradiated at more than 2.5 krad of fast neutrons at ORNL did not grow. Meiotic irregularities only could be examined in plants irradiated at 2.5 krad. All chromosome configurations in 4x species were easily analyzed. At 2.5 krad the number of breaks observed was 4.47 per cell, which was about twice of as much as at X-irradiation of 20 kr. In the 6x species, a few cells had one complicated multivalent and many fragments which made an estimation of the number of breakages difficult. Estimated only from clear aberrations it was 5.62 per cell. This estimation might be somewhat too low. Both X- and γ-irradiations at 10 and 20 kr caused about 3 times as many breakages in the 6x species as in the 4x.

X-irradiation produced about 1.5 times as many breakages as γ-irradiation at 10 and 20 kr of 4x and 6x. There was no difference between sensitivities of the 4x and 6x species with regard to X- and γ-irradiation effect on fertility.

Table 3. Frequency of chromosome aberrations at metaphase-I in PMC's induced by neutrons in polyploid wheats.

JRR-1 (Thermal neutrons)

Material	Dosage ($\times 10^{12}$ n_{th}/cm^2)	Total no. of cells observed	Total no. observed						No. of cells having other aberrations	Average no. of breaks per cell
			Fragments	Univa-lents	④	⑥	⑧	⑩		
T. durum (4x)	Control	18								0.00
	I (4.4)	33			7					0.42
	II (7.0)	38		4	14				monosomic 1	0.86
	III (11.8)	34		2	12					0.76
	IV (20.6)	30	2	5	17	1	1			1.60
	V (37.5)	7			12					3.42
T. vulgare (6x)	Control	13			2					0.30
	I (4.4)	14		4	4					0.85
	II (7.0)	24		12	20	3				2.54
	III (11.8)	24		8	21	3		1		2.66
	IV (20.6)	25	2	16	27	9	1		many fragments 4	5.16
	V (37.5)	—								
ORNL										
T. durum (4x)	X-10 kr	33		2	12	1				0.87
	X-20 kr	31		8	30	2			monosomic 1	2.38
	γ-10 kr	28		2	5	1				0.53
	γ-20 kr	27			19	2				1.62
	fast neutron 2.5 krad	23		4	18	11	2	3	⑭ 1	4.47
T. vulgare (6x)	X-10 kr	23	1	11	21	5			monosomic 1	2.95
	X-20 kr	6		2	10	7		1		8.00
	γ-10 kr	29		4	30	1				2.31
	γ-20 kr	14		14	17	7	1	1		5.57
	fast neutron 2.5 krad	15	1	12	17	11			many fragments 2 complex 1	5.26

98. C) *Relation between ploidy and radiation effects*

(By Seiji MATSUMURA and Mitsuya NEZU)

Thermal neutron irradiations were conducted in the JRR-1 Reactor, as in the case of *T. monococcum*. Exposure to 14 MeV fast neutrons at ORNL was simultaneously carried out with *T. monococcum*, *T. durum*, and *T. vulgare*. The dosage ranged from 2.5 to 20 krad for *T. durum* and *T. vulgare*. After exposure to 37.5×10^{12} n_{th}/cm^2 (V) and 5 krad of fast neutrons, *T. monococcum* did not germinate, while the seeds of *T. durum* and *T. vulgare* germinated but most of the seedlings died at an early stage. At 20.6×10^{12} n_{th}/cm^2 (IV) and 2~2.5 krad of fast neutrons most of the seedlings of *T. monococcum* died, while in *T. durum* and *T. vulgare* slow growth of seedlings continued. The higher was the dosage of thermal and fast neutrons, the more delayed were germination and growth of seedlings and the more reduced were survival rate, height of mature plants, and seed fertility. These relationships were ascertained in *T. durum* and *T. vulgare*, as well as in *T. monococcum*. In general, *T. monococcum* is the most sensitive to thermal and fast neutrons and *T. durum* is unexpectedly the most resistant. There is no significant difference between *T. durum* and *T. vulgare*. Table 4 shows the thermal neutron flux having the 1r equivalent effects calculated for 2x, 4x, 6x species, and comparison is given with the results obtained by 10 and 20 kr X- and γ-irradiations at ORNL.

Table 4. Comparison of the thermal neutron flux having the 1r equivalent effects ($\times10^9$ n_{th}/cm^2) in 2x, 4x and 6x wheats.

Ploidy	Length of seedlings	Seed fertility	Chromosome aberrations	Chlorophyll mutations
2x	1.6	2.6	4.1	0.73
4x	3.6	2.7	2.4	—
6x	4.9	5.1	2.0	—

Table 5. Comparison of fast neutron RBE in 2x, 4x and 6x wheats.

Ploidy	Germination rate	Length of seedlings	Seed fertility	Chromosome aberrations	Chlorophyll mutations
2x	3.5	10.0	11.8	8	10
4x	3.3	5.5	20.0	26	—
6x	3.0	5.5	10-17	~10	—

RBE of fast neutrons in comparison with X- and γ-rays is calculated as shown in Table 5. RBE is lower for the early stage characters (germination rate, length of seedlings) than for those of mature plants (seed fertility, chromosome aberrations), especially in polyploids.

99. *Radiosensitivity in polyploid plants*

(By Tarô FUJII and Seiji MATSUMURA)

Radiosensitivity of plants under the same physiological conditions varies according to the species or variety. Moreover, it is well known that radiosensitivity also varies according to the chromosome number, polyploid or heteroploid, within the same genus or species. Dry dormant seeds of *Beta*, *Raphanus*, *Astragalus*, *Capsicum* and *Citrullus* and their autotetraploids were irradiated by γ-rays from a ^{60}Co source. The behavior of autotetraploids was clear. In general, they showed a higher tolerance to radiation than did the respective diploids. Tetraploid germination and survival rates in all materials were higher than diploid rates.

In *Triticum* and *Aegilops*, diploid species were the most sensitive to radiation but no clear difference in sensitivity was found between allotetra- and hexaploid wheat species. Among *Oryza* species, *O. officinalis* (n=12) was the most sensitive, while *O. sativa* f. *spontanea* (n=12) was the most resistant to radiation. Four tetraploid species, *O. alata*, *O. eichingeri*, *O. minuta* and *O. latifolia* (the last two surely alloploids), showed moderate sensitivity. *Nicotiana* species with low chromosome numbers (*N. alata* n=9, *N. Langsdorffii* n=9, *N. longiflora* n=10) seemed to be very sensitive and the allotetraploid species (*N. rustica* n=24, *N. tabacum* n=24) were the most resistant among the American species. Four Australian species with different chromosome numbers, namely *N. suaveolens* (n=16), *N. Gossei* (n=18), *N. megalosiphon* (n=22) and *N. rotundifolia* (n=22) showed relatively high radioresistance and their germination rates were similar to those of the tetraploid American species. On the contrary, *N. Debneyi* (n=24) with the highest chromosome number among the Australian species was the most sensitive. Furthermore, the amphidiploid *N. tabacum*×*N. Debneyi* showed high susceptibility, though having the highest chromosome number of all materials. In *Nicotiana* as in *Oryza*, no clear relation between chromosome numbers and radiosensitivity was recognized. These findings show that radiosensitivity depends not noly on the number of genomes but also on the genome constitution, especially in allopolyploid plants (cf. FUJII and MATSUMURA 1959, Jap. Jour. Breed. 9).

100. *Radiosensitivity in vegetatively propagated plants*

(By Seiji MATSUMURA and Tarô FUJII)

Radiosensitivity is different according to the species or genus, and is also dependent on physiological conditions; it is strongly affected by water content, dormancy, and metabolic activity. The determination of radiosensitivity of cuttings, tubers or bulbs of vegetatively propagated plants is interesting to the radiobiologist. Moreover, breeding from bud or somatic mutations is widely used in these plants.

Irradiation experiments were carried out with several vegetatively propagated plants, and growth inhibition and morphological abnormalities in the irradiated generation were observed.

Cuttings of lemon trees were subjected to X- and γ-ray treatments of 1~20 kr. The percentage of sprouting decreased roughly with the dosage increase, but a promoting effect in sprouting ability was shown at low doses of X- and γ-rays. Stolons of two commercial varieties of strawberries were irradiated with X- and γ-rays of 0.5~4 kr. There was almost no difference in growth between the untreated and the irradiated groups. Only a slight inhibition was observed at a higher dosage. Bulbs of several commercial varieties of tulips were irradiated with γ-rays of 1~3 kr. The percentage of flowering bulbs in the irradiated generation decreased with the increase of dosage, while the irradiation effect was not clear with respect to sprouting rate. In all flowers of the variety Spring Field obtained from irradiated bulbs small red spots appeared over the whole surface of the petals and their density increased with the increase of dosage. A red flowering variety Marshal Haig when irradiated had split petal margins, especially on the tips, the splitting increasing with the increase of dose. Bulbs of four commercial varieties of gladiolus were irradiated with 1 to 5 kr of γ-rays. Irradiation did not affect either the percentage of sprouting and flowering plants or the average plant height.

According to our experiments, the optimum dosage of radiation for genetical study and plant breeding from cuttings, tubers, and bulbs is about 1/10 of that commonly applied to dormant seeds. In seed propagated plants the direction of mutation in most cases is from dominant to recessive. Therefore, detection of mutated homozygotes is observable only in X_2 or successive generations. But recessive mutation is sometimes detectable in the X_1 generation of vegetatively propagated plants, because they are generally heterozygotes. The flower color was altered by radiation with relatively high frequency and relatively simple biochemical change. Thus, irradiation of shoots, tubers and bulbs may be useful as

a method of plant breeding in vegetatively propagated plants (cf. MATSUMURA and FUJII 1959, Rep. Kihara Inst. Biol. Res. 10).

101. *Cytological studies on X-rayed rice*

(By Chen-seng HUANG[1])

The purpose of this work was to find out if, during the growth of rice, a competition takes place between cells with chromosome aberrations and normal cells. Dormant seeds of rice were irradiated with 5, 10, 20, 30 and 40 kr. Root tips and pollen mother cells of X_1 plants were fixed for chromosome observation. The former were stained by FEULGEN's reaction and the latter with aceto-carmine. Chromosome bridges at mitotic anaphase were not clear and appeared to be caused by stickiness; therefore observations were made mainly at metaphase. Most of the chromosome aberrations observed at metaphase were fragments and dicentric or more complex chromosomes, and the frequency of cells with aberrations increased linearly with the increase of dosage. Chromosome aberrations in pollen mother cells of X_1 plants were mostly double, single and reciprocal translocations. The frequency of translocations also increased linearly up to 20 kr but remained constant between 20 and 30 kr. No competition between cells with translocations and those without was found in 5 kr plot, when root tip and PMC data were compared. In 10 and 20 kr plots, about 8% of cells with translocations were eliminated during successive somatic divisions, and about 40% were eliminated in 30 kr plot. The seed fertility per pancile of X_1 plants, which ranged from zero to 100 percent, was also investigated. Three peaks—*i.e.*, fertile, semisterile and sterile—were found in 20 and 30 kr plots, while only 2 peaks were found in 5 and 10 kr plots. The three peaks can be explained as due to normal behavior, single and double translocations, respectively.

It is noteworthy that no somatic selection was found at as low dosage as 5 kr. Generally, the frequency of spontaneously occurring translocation is very low, but cells with translocations may survive. Under natural conditions, translocations of chromosomes occur in the somatic stage of rice and result in mutations in the reproduction stage.

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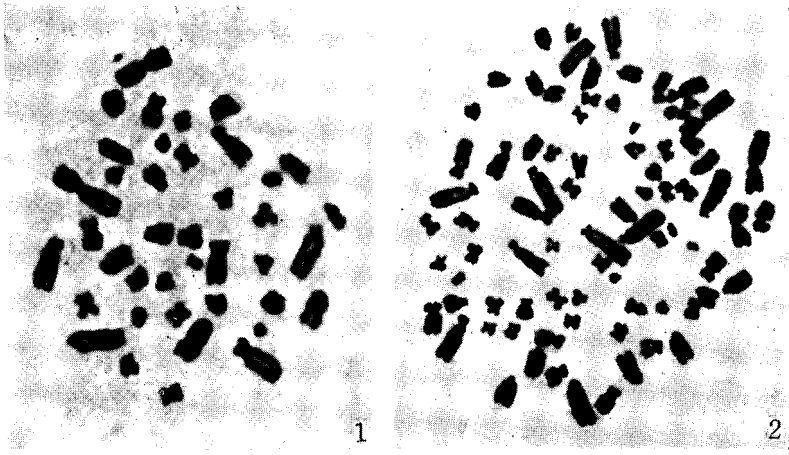
102. *Variation in the frequency of tetraploid cells in the course of transplantations of the Yoshida sarcoma*

(By Tosihide H. YOSIDA)

The Yoshida rat sarcoma obtained from the Pharmacological Research Institute of Tokyo was characterized by tumor stem cells containing 40 chromosomes and only 3.4 per cent near-tetraploid tumor cells (Figs. 1 and 2). The frequency of the tetraploid cells in this tumor has never changed in the couse of serial transplantation to W-strain rats. Surprising was the fact that about 51 per cent of the tumor cells which had developed by inoculation of a rat of B (Buffalo)-strain showed tetraploidy. The number of tetraploid cells increased to 61.1 per cent at the next transplant generation in rats of the SH-strain. Moreover, it increased also in N- and W-strains in the following transplant generation, while it markedly decreased



Figs. 1 and 2. Chromosomes of Yoshida sarcoma cells.
1, near-diploid cell ($s=40$) 2, near-tetraploid cell ($2s=80$).

when transplanted to the LE-strain. Since the frequency of tetraploid cells differed considerably according to the strain, rats of various strains, such as WKA, W, N, WPY, LE, B, CW, were used for transplantation of a tumor which had developed in the N-strain. The frequency of tetraploid cells in these tumors decreased generally to 25–35%. They decreased gradually in the course of successive transplant generations to rats of all strains; in the 9th to 10th transplant generations they amounted to about 10 per cent. Although a higher percentage of tetraploid cells occurred in the tumor transplanted to the B-strain at the first transplant generation,

they showed no increase in this strain at the 5th and 6th transplant generations.

The chromosomes in 100 near-diploid and near-tetraploid cells were counted exactly. Cells containing 40 chromosomes were at the highest frequency (90%) in the diploid cell group, while those containing 80 chromosomes were most frequent (36.0%) in the tetraploid cell group. The range of chromosome number variation in the latter group is much wider than that in the former. A comparative idiogram analysis of diploid and tetraploid cells showed that the latter were derived from chromosome duplication of the original diploid assortment.

103. *Invasive ability of diploid and tetraploid tumor cells of the Yoshida sarcoma*

(By Tosihide H. YOSIDA)

As shown in the preceding paper of this report, two kinds of Yoshida sarcoma cells, with near-diploid and near-tetraploid chromosome numbers, were observed after transplantation to a B-strain rat.

The percentages of near-diploid and near-tetraploid cells in the ascites tumor at the 3rd transplant generation were in the ascites form 26 and 69, respectively. The ascites tumor cells were transferred into the peritoneal cavities of W-strain rats by usual transplantation technique, and small pieces of the lung of the same animal were implanted into the peritoneal cavities of rats of the same strain. On the other hand, small pieces of solid tumor developed in the peritoneal cavity were transplanted into the peritoneal cavities of other animals of that strain.

In the case of transplantation of the ascites tumor, the number of tetraploid cells was reduced to 37.5 per cent, while that the diploid cells was increased to 57.0 per cent. One among four rats inoculated with lung tissues developed an ascites tumor in the peritoneal cavity. The frequency of diploid and tetraploid cells in the ascites tumor, thus developed, was 8.2 and 91.0 per cent, respectively. On the other hand, the ascites tumor developed by transplantation of tumor pulp taken from the solid tumor showed that the frequencies of diploid and tetraploid cells were 93.0 and 7.0 per cent, respectively.

The results of the above investigation indicate that the infiltration-ability of the tetraploid cells of the Yoshida sarcoma was much stronger than that of the diploid cells.

104. *Double inoculation of hyperdiploid and hypotetraploid cell strains of the Ehrlich ascites carcinoma and their invasion into organs*

(By Tosihide H. YOSIDA and Kurao ITO)

A hyperdiploid strain of the Ehrlich ascites carcinoma was obtained from the Children's Cancer Research Foundation, Boston, in December, 1958. Since that time the tumor has been transferred from mouse to mouse for about 30 transplant generations. In addition, a hypotetraploid strain of the Ehrlich ascites carcinoma was obtained from the Zoological Institute, Tokyo University.

Table 1. Frequency of hypotetraploid cells from double inoculation with hyperdiploid and hypotetraploid Ehrlich tumor cells.

Transpl. gener.	1	3	6	7	8	9	10
% of 4X tumor cells	40.4	37.1	15.0	9.5	8.5	7.5	7.0

200 cells were counted in each sample.

Simultaneous inoculation with the two different tumors, hyperdiploid and hypotetraploid Ehrlich ascites carcinoma, was undertaken in order to find out which cells have a stronger competitive ability. Almost the same number of hyperdiploid and hypotetraploid tumor cells were inoculated simultaneously into the peritoneal cavity of the same host. In order to estimate the activity of the tumor cells, the number of metaphasic cells was calculated on the basis of 200 tumor cells per each transplant generation. It was evident that the number of tetraploid tumor cells gradually decreased in the course of transplant generations. In the 10th transplant generation the frequency of the tetraploid cells decreased to about 7 per cent (Table 1).

In the 6th transplant generation following double inoculation, lung, spleen, and liver were removed from the tumor-bearing mouse, and small pieces of those organs were inserted separately into the peritoneal cavities of new hosts. This method made possible intra-peritoneal proliferation of tumor cells which had invaded these organs. In this way, three ascites tumor cell populations in the 7th transfer generation were obtained from those inoculations. As shown in the table (Table 1), the frequency of hypotetraploid cells is 9.5 per cent in the case of usual transfer technique, while it increased to 18.0, 19.0, and 16.0 per cent in the case of lung, spleen, and liver inoculation, respectively. In the next transplant generation which was obtained by serial organ transfer, the number of

hypotetraploid tumor cells increased to 32.0 and 26.2 per cent, respectively, in the case of lung and liver transplantations.

Based on the above investigations the conclusion may be drawn that the hyperdiploid tumor cells in the ascites tumor are much stronger in competition than the hypotetraploid, but the latter have a higher ability to invade than the former.

105. *Effects of extract from Gentiana upon living plant tissue*

(By Yô TAKENAKA, Yoshito OGAWA and Tôru ODASHIRO)

Japanese Gentian and Gentiana Japonica Pulverata, which are sold in Japanese drug stores, consist mostly of dried roots and rhizomes of *Gentiana scabra* Bunge var. *buergeri* Maximowicz. Gentiana Japonica Pulverata is powdered while Japanese Gentian is not.

Both materials were treated with methanol. After evaporation of the solvent, a dark brown oil residue remained. The extract solutions at a concentration of 1g/2ml. were used in the following experiments.

Water solutions at various concentrations (0.5-10%) were prepared from the extract (50% concentration), and living roots of *Allium scorodoprasum* var. *viviparum* and *Vicia faba* were placed in the solutions for from 2 to 30 hours. Some living root-tips taken from the solutions were squashed immediately by the hydrochloric-acetic acid-orcein method, while others were washed and soaked in tap-water for various lengths of time before squashing. In this report, results of observations of materials treated in 5% solutions of this extract for 2, 4, 6 and 24 hours are reported.

In general, the metabolic nucleus did not show structural changes as a result of short-time treatments, but with the increasing time of treatment the nucleoli appeared to be enlarged like vacuoles.

Short-time treatments at prophase showed chromosome stickiness. In long-time treatments the spiral structure of prophase chromosomes became conspicuous, and the chromosomes seemed to revert to the metabolic stage. At late prophase, the chromosomes of short-time treatments appeared to be somewhat sticky. In late prophase of long-time treatments double structures became clearly recongnizable and some nuclei seemed to have reverted to the metabolic stage.

At metaphase, "c-pair figures" were seen, but the chromosomes were never shortened and thickened as after colchicine treatment. The longer was the treatment time, the more c-pair figures appeared, and the c-pair chromosomes tended to become metabolic nuclei. Toward late metaphase, the number of nuclear plates increased gradually, and two sets of sister

chromosomes showed loss of polarity, a comb-like appearance and a tendency toward the metabolic nucleus.

At anaphase, many chromosome bridges appeared and sometimes multipolar nuclei were observed. Even at telophase, chromosome bridges occasionally were observed. From short-time treatment, faint connecting fibres were observed, but they were not present after long time treatment.

In many instances, the two daughter nuclei were close to each other with no cell membrane between, thus giving rise to binuclear cells. In other instances, there were some metabolic nuclei with doubled chromosome number.

From the results of observations mentioned above, it may be concluded as follows:

(1) This extract decreases the visibility of the chromosome matrix.
(2) It weakens the function of the centromere.
(3) It disturbs the polarity of chromosomes.
(4) It decreases the visibility of the spindle and connecting fibres.
(5) It weakens the repulsion between sister chromosomes.
(6) It produces chromosome bridges at anaphase.
(7) It checks or retards the development of cell membranes.
(8) It produces binuclear or polyploid cells.
(9) It suppresses cell division.

In spite of the fact that *Gentiana scabra* var. *buergeri* is not poisonous to the human body, the extract acts radiomimetically on root-tip cells of plants. These phenomena suggest the action of arresting tumor-cell division.

106. *Effect of glucuronic acid on the growth of the animal embryo*

(By Yoshito OGAWA)

The effect of glucuronic acid on the growth of animals has been reported for rats (MATSUOKA, *et al.*, 1957, 1858: NAKAYAMA, *et al.*, 1957, 1958) and chick embryos (TUNOO, *et al.*, 1957: USIZIMA, *et al.*, 1958), but the relationship between glucuronic acid and animal growth has not been made clear. In this experiment, the growth promoting effect of glucuronic acid on the early developmental stages of the embryos of *Triturus pyrrhogaster*, BOIE was examined. Embryos of *Triturus pyrrhogaster* are a useful material for this kind of test since the developmental process is typical and is suitable for treatment with chemicals.

Immediately after fertilization, the embryos were raised in glucuronic acid (containing Lacton 20%) or sodium glucuronate (1.0%, 0.1%, 0.01%, 0.001% and 0.0001%) solution at 20°C, and the body weight and length of the developing embryos were measured. Furthermore,

the growth progress with respect to organ formation was investigated histologically. Glucose treatment at the same concentrations and non-treated groups were prepared for control.

It was found that sodium glucuronate and glucuronic acid showed a significant promoting effect on the early development of embryos keeping a normal balance in organ formation. Sodium glucuronate appeared to be more effective than glucuronic acid in promoting growth and its most effective concentration was 100 mg./1000 cc. This effect of sodium glucuronate was observed approximately on the 13th day after fertilization (Stage-32), but it did not last longer than two weeks. The difference between sodium glucuronate-treated and non-treated plots seemed to correspond to five developmental stages, representing the time equivalent to 132 hours at 20°C, but no remarkable effect of this chemical was found when the treatment was started on the 18th day after fertilization (Stage-36).

It is noteworthy in this experiment that the stage at which sodium glucuronate become effective falls always approximately on the 13th day after fertilization (Stage-32), irrespective of the dosage and the developmental stage at which the treatment was started. This experimental result is very similar to the effect of Kinetin (6-Furfuryl-aminopurine) on the growth of the embryo of *Triturus pyrrhogaster* (Y. Ogawa, 1959). A remarkable promoting effect of sodium glucuronate and Kinetin on animal cell division was already observed by the writer two years ago. The relation between the above two chemicals with respect to growth promoting effect during early developmental stages is now being examined.

107. *Development of muscle protein in the regenerating limb tissue of Triturus pyrrhogaster*, Boie

(By Yoshito Ogawa)

DeHaan (1956), using actomyosin anti-serum, reported a treansient appearance of actomyosin in the regenerating limb tissue of *Amblystoma mexicanum* 20 days after the operation. His finding is very interesting since it indicates the existence of a precursor of actomyosin. On the other hand, the formation of action precedes that of myosin in the early developmental stages of both the chick (Y. Ogawa, 1957, 1958) and *Triturus pyrrhogaster* (Y. Ogawa, 1958, 1959).

An experiment was carried out in order to find out whether the appearance of actin in the developing skeletal muscle before the formation of myosin is limited to early developmental stages or is a general phenomenon. The formation of actin and myosin in regenerating limbs of *Triturus pyrrhogaster* after amputation at the knee was investigated essentially by the same

methods as those employed in the experiment with the early embryonal stages.

G-actin and myosin were isolated from the skeletal muscle of *Triturus pyrrhogaster* by the method of STRAUB and SZENT-GYÖRGY (1951). Both proteins at a concentration of 25 mg/ml. 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 was raised to serological specificity by a resorption test with saline extracts of liver, spleen and skin tissue. Titration with homologous antigen was then carried out and the titer of sera was adjusted to 1:512 before preparing the precipitin reaction with a saline extract of granulated tissue of amputated limbs. A constant volume of sera and progressively decreasing amount of antigen were used for preventing a possible failure due to excess of antigen.

It was found that actin and myosin first become detectable in the granula of regenerating limbs 20 and 27 days after operation, respectively, as showen in Table 1.

Table 1. Determination of proteins in the muscle of the regenerating limb of *Triturus pyrrhogaster* BOIE

Days after operation	No. of granules extracted	No. of reaction series	Lowest antigen concentration giving positive results*	
			Actin	Myosin
16	10	2	—	—
18	10	2	—	—
20	10	2	1 : 20	—
22	10	2	1 : 160	—
25	10	2	<1 : 160	—
27	10	2		1 : 10
29	6	2		1 : 40
31	6	2		1 : 160

* Antigen concentrations used ranged from 1:10 to 1:160 in tissue weight.

DeHaan's result may be due to a cross-reaction of his anti-serum with developing actin, because the actomyosin anti-serum often reacts with actin, as already pointed out by KESZTYUS *et al.* (1949).

Therefore, it is probably generally true that in the skeletal muscle actin appears before the formation of myosin, both in early developmental stages and in regenerating tissue.

108. *Invariance of X-ray spectra at various depths in phantom**

(By Sohei KONDO and Takesi KATO)

To make a perfect interpretation of the relative biological effectiveness of X rays in terms of linear energy transfer, it is necessary at the present stage of radiation dosimetry to know precisely the X-ray spectra in the exposed materials. For this purpose, measurement of the X-ray spectrum in acrylic acid resin plates of 0 cm, 1 cm and 5 cm thick has been carried out with a scintillation counter. The plates were placed in front of a sodium iodide crystal $1\frac{3}{4}''$ in diameter and $1\frac{1}{2}''$ in length. The X-ray KXC-18 generator was operated at very low filament voltage, ca. 0.2 V, so that the counter was able to count incident photons with no appreciable coincidence losses. The spectra shown in Fig. 1 were obtained at 7.3 m from the tungsten target of the X-ray generator operated at 170 kVp with no external filter.

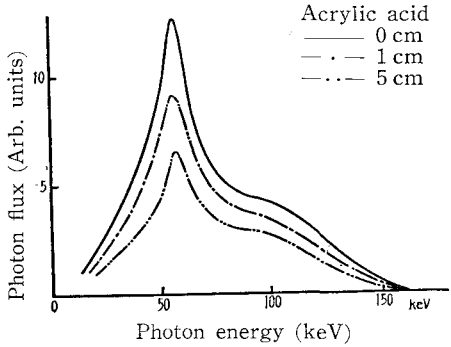


Fig. 1. Change of measured spectra of 170 kVp X-rays with various thicknesses of absorbers placed in front of the scintillation counter.

We may conclude from Fig. 1 that the spectral distribution of X-rays remains almost invariant at various depths in the phantom. This also will be the case in living things except for points near bone or some other high atomic number material. Therefore, the *LET* spectrum of the absorbed dose in X-rayed biological material is obtainable by calculation from the X-ray spectrum obtained outside the exposed material.

Fig. 1, however, shows only the relative change of the X-ray spectrum. Corrections for scintillator escape effects and for statistical spread of pulse hights from the photomultiplier must be made in the measured spectrum

* This research has been partly supported by a grant from the Rockefeller Foundation.

to obtain the true X-ray spectrum. Since the contribution of the latter correction to a continuous spectrum is rather small, we have made only corrections for escape effects using the Rawson-Cormack Matrix[1]. The peaks of the spectra shown in Fig. 1 are due to characteristic X rays of tungsten used as the target of the X-ray generator. Fig. 2 represents

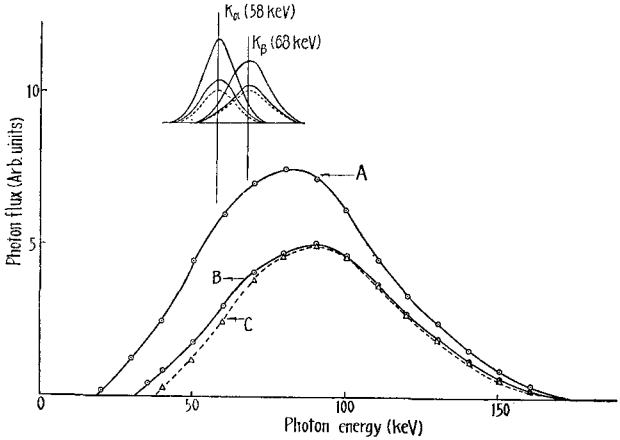


Fig. 2. Comparison of corrected spectra of primary 170 kVp radiation from Toshiba KXC-18 generator. *A*: filter 0.3 mm Cu+1.0 mm Al. *B*: filter 1.0 Cu+1.0 mm Al. *C*: spectrum for filter *B* calculated from spectrum *A*.

some of the corrected spectra for external filters *A* (0.3 mm Cu+1.0 mm Al) and *B* (1.0 mm Cu+1.0 mm *A*l). In Fig. 2, the spectra of characteristic X rays of tungsten are shown separately, since the effects of pulse height spread due to such monoenergetic photons are appreciable. The dotted curve *C* is the curve calculated from the observed spectrum *A* by taking account of the increase in absorption coefficients of filter *B* compared with filter *A*. From the good agreement of the calculated curve *C* with the observed *B*, we may conclude that the spectra shown in Fig. 2 are close to true spectral distributions of X-rays.

1) E. G. Rawson, D. V. Cormack: A matrix to correct for scintillator escape effects. *Nucleonics* **16**, No. 10, 92–97 (1958).

109. *Silver-phosphate glass dosimeters and their application to X-ray dose measurements inside X-rayed mice and to separate measurements of thermal neutron fluxes and gamma doses in the JRR-1 Reactor*[1)]

(By Sohei KONDO)

Rods (1 mm in diameter and 6 mm in length) and plates ($10\times10\times0.2$, 1.0 and 3.00 mm) of silver-phosphate glass were made by Dr. Ryosuke Yotota, Toshiba Co., Kawasaki, and a fluorimeter (see Fig. 1) was built by Toshiba Company following the author's plan given schematically in Fig. 2. As is

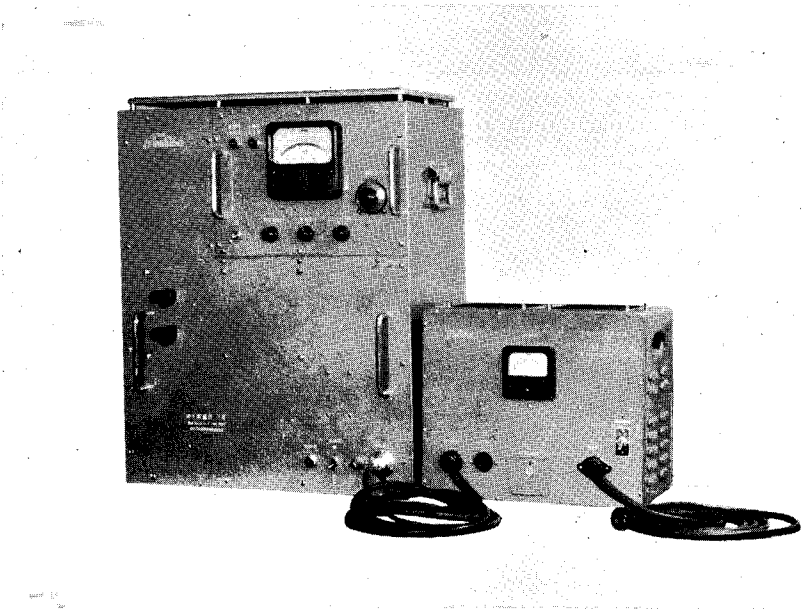


Fig. 1. Fluorimeter.

well-known, this special glass exposed to any kind of ionizing radiations acquires the ability to fluoresce when excited by ultraviolet rays. The amount of fluorescence is linearly proportional to the exposure dose. The fluorescence fades very little at room temperature in over a month.

The above rod dosimeters are useful for conventional X-rays in the range from 5 r to 10^3 r with better than $\pm5\%$ accuracy.

These rods were implanted in various organs of two mice for measure-

1) This research has been supported by the Education Ministry of Japan for Trial Manufacture Research and the Rockefeller Foundation.

ment of internal exposure dose distribution and exposed to X-rays of 170 kVp with no external filter. The results are shown in Table 1. The certainty that the glass readings can be taken as they are, in spite of the sensitive dependence of the glass reading per roentgen on X-ray energy, has been assured through our finding[1] that the X-ray energy spectra in an acrylic acid resin phantom at various depths have an almost invariant shape. Thus, we may conclude from Table 1 that within error of ±5% the dose distribution in X-rayed mice is constant.

Table 1. Relative exposure dose inside various organs of mice exposed to 50 r of X rays of 170 kVp with no external filter.

Organ	Back bone	Testis	Intestine	(under the abdomen)	Kidney
Relative dose	1.04±0.03	1.02±0.03	1.02±0.03	1.01±0.03	1.01±0.05
Organ	Liver	Lung	(on the back)	Brain	Spleen
Relative dose	1.00±0.05	1.00±0.03	0.99±0.03	0.98±0.04	0.94±0.05

Note: The average of the fluorescence readings of all the rods for two mice is taken as 1.00.

The measurements of thermal neutron fluxes and gamma contamination doses were made with the use of glass plates of 10×10×3 mm with

Table 2. Measured thermal neutron fluxes and gamma ray does-rates in Reactor JRR-1.

Position and power level	No. 7 Hole (at 11.8 cm from graphite surface); 40 kw		No. 16 Hole; 5 kw
	by glass dosimeter	by gold foils and ion chamber*	by glass dosimeter
Thermal neutron flux ($n \cdot cm^{-2} \cdot sec^{-1}$)	$2.0(1\pm0.19)\times10^{8}$	2.5×10^{8}	5.2×10^{10}
Gamma contamination dose-rate (r/h)	460(1±0.12)	500	7.0×10^{4}

* These figures reported by the personnel of JAPAN Atomic Research Institute were based on measurements carried out several months before the present work was done.

different $AgPO_3$ concentrations (8%, 10% and 12%) in No. 7 Hole (thermal column) and No. 16 Hole of the JRR-1 Reactor, the latter being close to the center of the reactor. The principle of the measurement was given in the previous abstract[2]. The observed results are given in Table 2. The agreement between the results obtained by independent methods is satisfactory. The advantage of this method is that the dosimeters are so small that they can be placed among the substances to be irradiated and, in addition, that the absolute values of thermal neutron fluxes and of gamma contamination doses can be obtained simultaneously simply by calibrating the glass readings with a known gamma field.

The No. 7 and No. 16 Holes have been most often used for radiobiological studies and so the data on Table 2 will be useful for determination of thermal neutron fluxes and gamma contamination doses in such experiments.

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2) S. Kondo: Ann. Report Natl. Inst. Genetics, Japan **9**, 134-136 (1959).

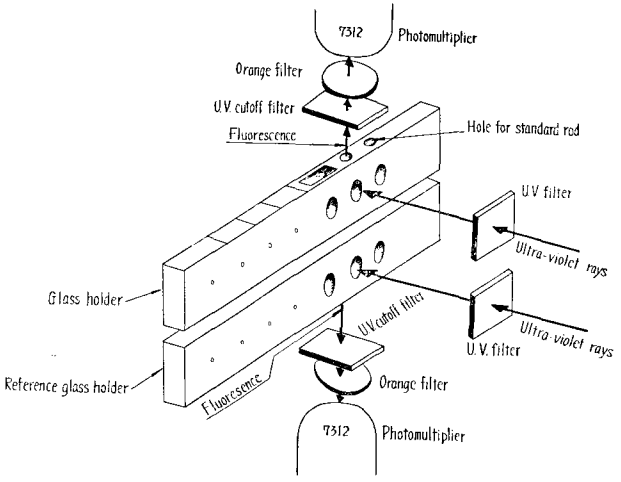

Fig. 2. Schematic diagram of fluorimeter.

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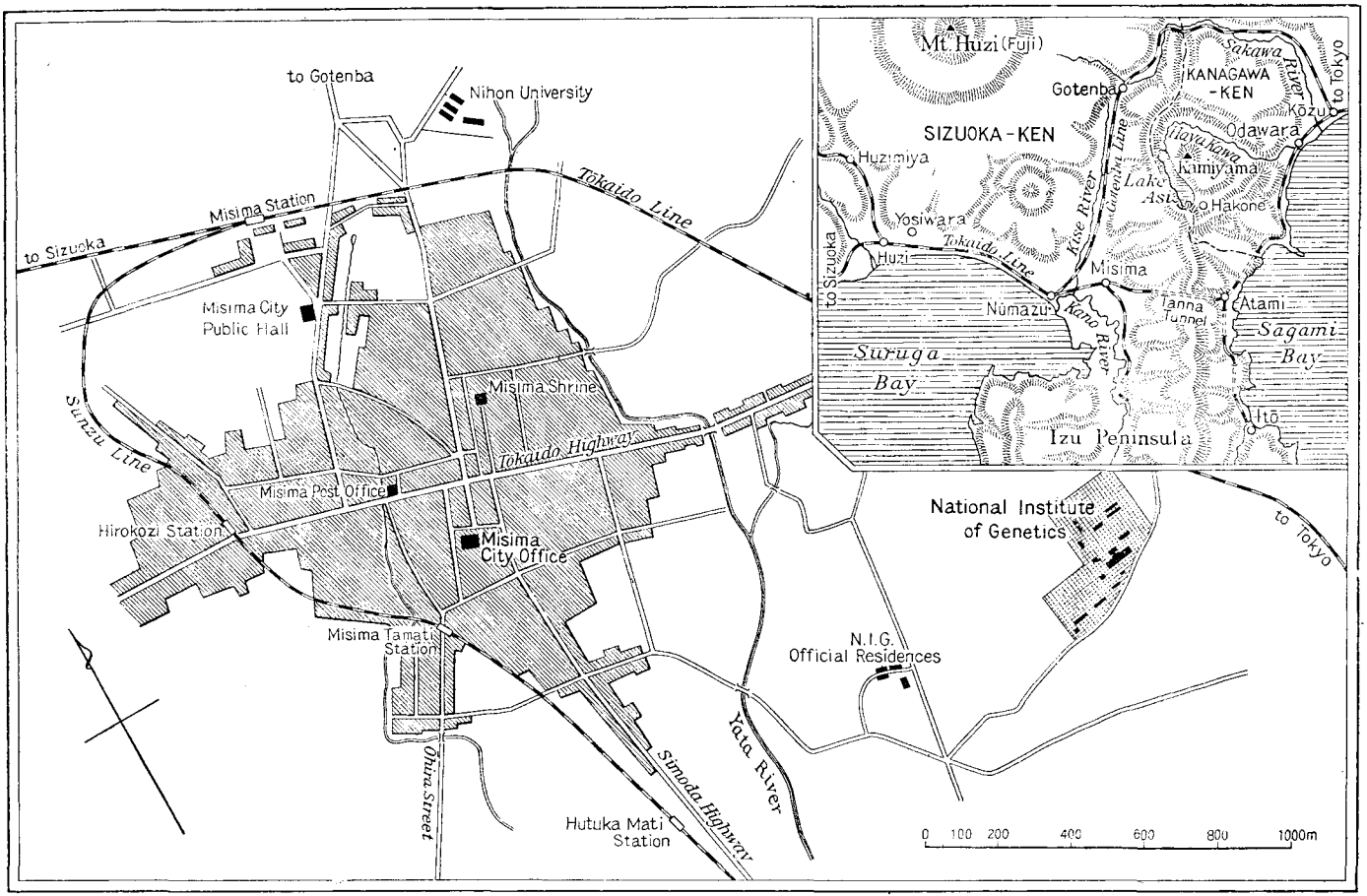
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