

NATIONAL INSTITUTE OF GENETICS  
JAPAN

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ANNUAL REPORT

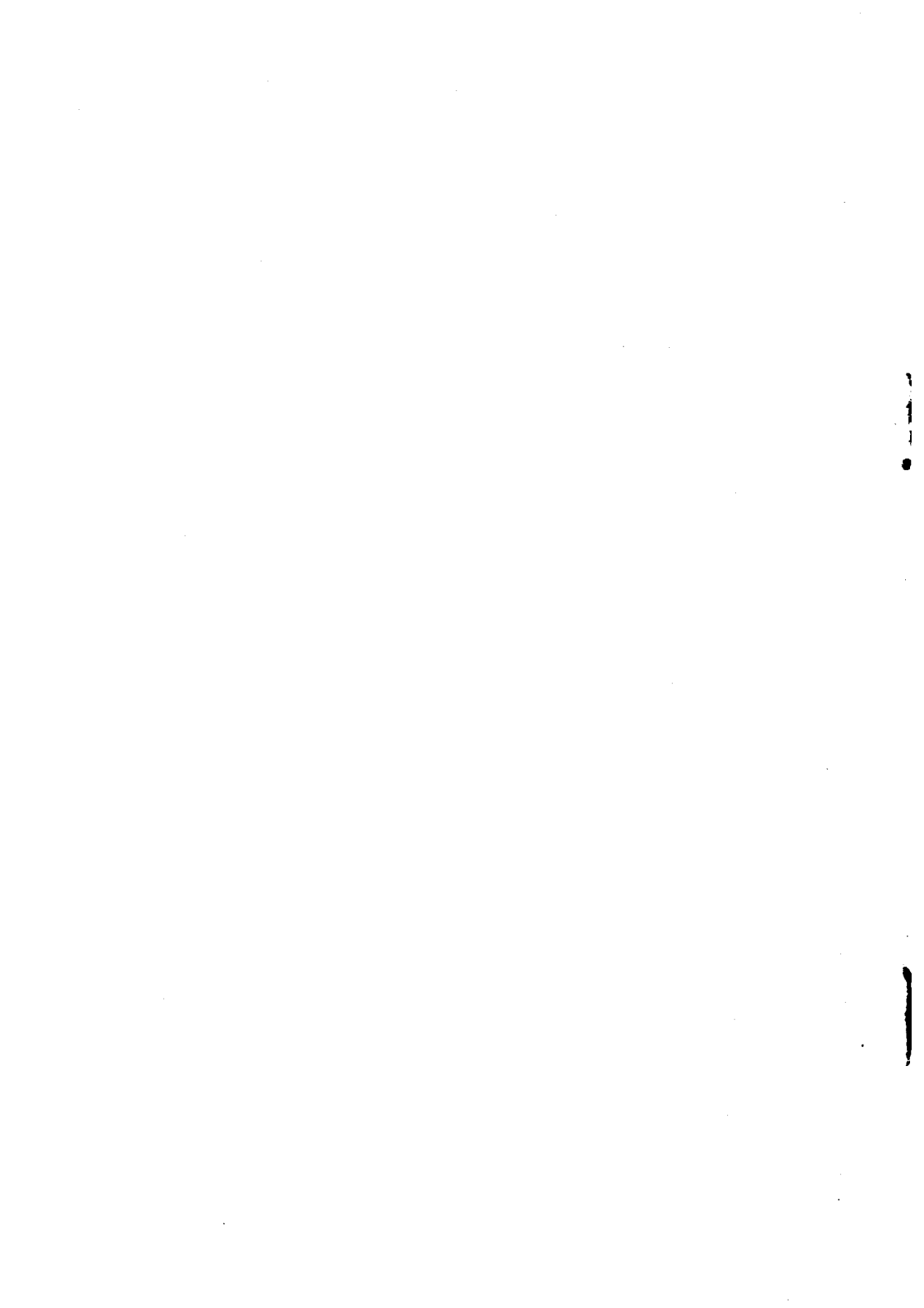
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1956

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Annual Report  
of the  
National Institute of Genetics

No. 7, 1956



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## Exhibition of Japanese Morning Glory



One of the colorful exhibits arranged for the benefit of foreign scientists attending the International Genetics Symposia was the display (by Dr. Y. Takenaka) of numerous varieties of Japanese morning glory, in which hundreds of different genotypes are being studied and maintained at this Institute. The visitors were deeply impressed by the rich and varied color patterns of flowers in full bloom. In this picture, Dr. Kihara, Director, is taking Dr. O. Winge of Sweden, around the exhibits.

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## GENERAL STATEMENT

During this fiscal year there was no change either in the number of departments or in the established number of staff members. Our institute continues to comprise six departments and fourteen laboratories. At present we have thirty-eight research members including part-time members and associates.

There were some changes in the personnel of our staff. On April 1, Dr. K. Hayashi was transferred as professor of botany to the Tokyo University of Education; he was succeeded on September 1 by Dr. Y. Ogawa. To our great regret, Dr. Y. Tanaka and Dr. T. Komai retired on December 15. We are greatly indebted to both for the many inestimable services rendered to the newly established institute, especially in the difficult period of shaping our organization. They will remain in contact with our institute as research associates. Dr. Y. Tanaka was succeeded by Dr. Y. Tajima on December 11; Dr. T. Komai's position is still vacant.

A new concrete building, eighty tsubo in floor dimensions, planned for genetic studies on microorganisms, is under construction. It will be completed in March 1957.

During the current year four staff members went to U.S.A. partly on scholarships, partly as laboratory assistants. Their names, destinations and times of departure are:

Mr. Y. Yamada, Purdue University, April,  
Dr. K. Gotoh, Iowa State College, June,  
Mr. K. Tutikawa, Jackson Laboratory, October,  
Dr. M. Tsujita, Columbia University, October.

Mr. I. Iino received a leave of absence for another year.

Dr. H. Oka was teaching in Formosa for six months; he returned in March.

Dr. M. Kimura returned in August from U.S.A. after a three year sojourn in Ames and Wisconsin; he obtained the Ph. D. degree from the University of Wisconsin. He was also awarded a similar D. Sc. degree by Osaka University.

The Rockefeller Foundation bestowed on the institute a grant of \$10,000 for the purchase of literature. Until now 28 books and 17 periodicals (with 372 back numbers) have been bought.

Dr. R. Goldschmidt has sent us 628 reprints, two books and 10 periodicals (comprising 93 issues). Since 1951, Dr. Goldschmidt continues to send us the publications which he is receiving, contributing greatly to our library.

We have had the pleasure of welcoming many guests from abroad. Many of them gave us interesting lectures on their special subjects. Their list is given on p. 7.

During the International Symposia of 1956, 120 participants visited Misima on September 11. They arrived at the institute around 10 o'clock in the morning coming from Hakone where they stayed overnight. The main event arranged for the guests was an exhibit of the Japanese Morning Glory. About 5,000 plants were kept in pots and in the field, and great care was taken to adjust the time of their flowering to this occasion. Approximately 100 mutants were on display. Later, all the participants were invited for lunch in Rakuju-En park by the mayor of Misima. Unfortunately Mt. Fuji remained invisible, since the day was at first rainy and later cloudy.

### ABSTRACTS OF DIARY FOR 1956

- Jan. 7. Meeting of Subcommittee in charge of the arrangement of lectures for the International Genetics Symposia.  
19. 45th meeting of Misima Geneticists' Club.
- Feb. 4. Public Lectures on Genetics (In the Hall of the Asahi Press, Tokyo).  
6. 46th meeting of Misima Geneticists' Club.  
15. 14th meeting of Board of Councillors.  
25. 47th meeting of Misima Geneticists' Club.
- Mar. 6. 48th meeting of Misima Geneticists' Club.  
10. Meeting of Tobacco Research Workers.  
17. 49th meeting of Misima Geneticists' Club.  
25. The radio-isotope laboratory with an irradiation room of Co<sup>60</sup>, under construction since last year, was completed.
- Apr. 14. Meeting of Preparation Committee of the International Genetics Symposia.  
21. Meeting of Cancer Research Workers.
- May 9. 50th meeting of Misima Geneticists' Club.  
25. Board meeting of Association for the Propagation of the Knowledge of Genetics.
- June 6. 14th meeting of Biological Symposia.  
28. Board meeting of Japan Association of Poultry Genetics.  
29. General meeting of Japan Association of Poultry Genetics.
- July 3. 51st meeting of Misima Geneticists' Club.  
16. Meeting of Tobacco Research Workers.
- Aug. 4. 52nd meeting of Misima Geneticists' Club.  
4. Meeting of Editorial Board of "The Heredity" (Iden).  
9. 15th meeting of Board of Councillors.  
9. 15th meeting of Biological Symposia.  
27. 16th meeting of Biological Symposia.

29. Meeting of Committee for Exhibit on Japanese Morning Glory.  
 Sep. 11. Exhibition of Japanese Morning Glory, for the attendants of the International Genetics Symposia. 120 persons visited the Institute.  
 Nov. 11. 53rd meeting of Misima Geneticists' Club.  
 Dec. 11. Three houses for official residences were completed.

## STAFF

*Director*

Hitoshi KIHARA, D. Sc.

*Members*

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

*Part-time Staff and Research Associate*

Kan OGUMA, D. Ag., Ex-Director, Emeritus Professor of Hokkaido Univ.  
 Yoshimaro TANAKA, D. Ag.

Taku KOMAI, D. Sc.  
 Yoshinari KUWADA, D. Sc., Emeritus Prof. of Kyoto Univ.  
 Yosito SINOTO, D. Sc., Professor of International Christian Univ.  
 Sajiro MAKINO, D. Sc., Professor of Hokkaido Univ.  
 Hideo ETO, D. M., Assistant Professor of Tokyo Univ.  
 Kazuo FURUSATO,  
 Flora A. LILIENFELD, Ph. D.

*Department of Administration*

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

*Misima Branch of Hatano Tobacco Experiment Station*

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

*Whole-Japan Association of Poultry Genetics*

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

*Association for Propagation of the Knowledge of Genetics*

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

COUNCIL

Yô K. OKADA, Director of National Science Museum, Chairman  
 Seishi KAYA, Professor of Tokyo University, Vice-chairman  
 Masanori NAKAIZUMI, Emeritus Professor of Tokyo University  
 Bungo WADA, Professor of Tokyo University  
 Seizo KATSUNUMA, President of Nagoya University  
 Yohichi ASAMI, Emeritus Professor of Tokyo University  
 Toshitaro MORINAGA, Director of National Institute of Agricultural Sciences  
 Takeo IRIMANO, President of Japan Monopoly Corporation  
 Toshio SAITÔ, Governor of Sizuoka Prefecture  
 Yakichi NOGUCHI, Professor of Tokyo University  
 Riichi KAWAKAMI, Head of Department of Biometry, National Institute of Public  
 Health  
 Kinichiro SAKAGUCHI, Professor of Tokyo University  
 Masatada TERADA, Professor of Jikei Medical College

Takeo SAKATA, President of T. Sakata & Company, Yokohama  
Ichiro ISHIKAWA, Commissioner of Atomic Energy Commission

## RESEARCH PROGRAM FOR 1956

### *Department of Morphological Genetics*

Studies on unstable genes in silkworm (TANAKA)  
Linkage in silkworm (TANAKA)  
Studies on the origin of wheat (KIHARA)  
Studies on substitution of nucleus in wheat (KIHARA)  
Studies on *Agropyron* (MATSUMURA & SAKAMOTO)  
Nullisomic dwarfs among the offspring of pentaploid wheat hybrids (MATSUMURA)  
Physiological genetics of the reaction of Japanese morning glory to day-length  
(SAKAMOTO)  
Human genetic studies (KOMAI)

### *Department of Cytological Genetics*

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

### *Department of Physiological Genetics*

Studies on heterosis (KOMAI et al.)  
Problems on mouse genetics (KOMAI & TUTIKAWA)  
Population genetics of some insect species (KOMAI & TAIRA)  
Analysis of genes responsible for hybrid sterility and hybrid break-down in rice  
(OKA)  
Genetic studies of some physiological and agronomic characters in rice (OKA)  
Theoretical studies of population genetics (KIMURA)

### *Department of Biochemical Genetics*

Biochemical genetics of insects and microorganisms (TSUJITA, NAWA & SAKAGUCHI)  
Embryological and biochemical studies of silkworm (TSUJITA & SAKAGUCHI)  
Biochemical studies of some mutants in silkworm and *Drosophila* (NAWA & TAIRA)  
Biochemistry of the mechanism underlying variations in flower colors in plants  
(ENDO & ABE)

Biochemical studies on the mechanism of cell division in animals (OGAWA et al.)  
 Biochemistry of the bitter substance in *Citrullus colosynthis* (OGAWA et al.)

#### *Department of Applied Genetics*

Breeding for high egg production in poultry (TANAKA, SAKAI & KAWAHARA)  
 Genetics of resistance to some diseases in poultry (KAWAHARA)  
 Genetic study of long-tailed fowl (TANAKA)  
 Experiments on the control of diapause in *Antheraea pernyi* (TANAKA)  
 Studies on polygenic inheritance (SAKAI et al.)  
 Studies on competition between plants of different genetic constitutions (SAKAI et al.)  
 Population-genetic studies of "Red-Rice" growing among upland rice (SAKAI et al.)  
 Studies on the technique of breeding in plants (SAKAI)  
 Genetic studies on "Cherry-red leaf" in tobacco plants (SAKAI et al.)  
 Genetic studies on the migration activity in *Drosophila* (SAKAI et al.)  
 Polyploidy and sterility in fruit plants (FURUSATO & MIYAZAWA)  
 Genetics of bitter-substance in *Citrullus* (FURUSATO)

#### *Department of Genetics of Induced Mutation*

Measurement of X- and  $\gamma$ -ray dosage for induced mutations (KONDO)  
 Radiation genetics of wheat and barley (MATSUMURA & FUJII)  
 Relation between the quality of radiations and mutation (MATSUMURA, FUJII & KONDO)  
 Mutations induced by irradiation of tobacco plants (MATSUMURA & FUJII)  
 Radiation genetics and its practical application (KIHARA et al.)  
 Studies on radiation-induced mutations in mice (SUGAHARA et al.)  
 Studies on the physicochemical mechanisms of radiation effects on living organisms (SUGAHARA et al.)  
 X-ray diffraction studies on muscle (SUGAHARA et al.)  
 Improvement of sugar beets by means of induced triploidy (MATSUMURA et al.)

#### *Research Students and Research Items*

Chao-Hwa HU (Overseas student from Taiwan Provincial College of Agriculture):  
 Cytogenetics of haploid rice and *Nicotiana* species  
 Yasuo OTA: Polyembryony in *Citrus*  
 Setsuji KATAOKA: Cytology of interspecific hybrids in Liliaceae  
 Shinya IYAMA: Introgression between the so-called Red-Rice and Japanese upland rice varieties  
 Osamu YOSHIKAWA: Genetic studies of bacterial viruses  
 Kyozo WATANABE: Studies of nucleus in *Paramecium*  
 Yasuo SUZUKI: Population genetics of crop plants  
 Etsuo GOTOH: Population-genetic studies in *Drosophila* and domestic fowl  
 Hiroshi SO: Karyological studies on the mechanism of growth-inhibition in cancer cells  
 Keiko SATO: Genetics studies in animals

- Yutaka ONO: Cytogenetics of domestic animals  
 Yuichiro HIRAIZUMI: Studies on polygenic inheritance  
 Yoshiyuki AMANO: Effect of antibiotic substances on cell division  
 Kotoyo TSUCHIKAWA: Genetics of malformations in rat  
 Yoshihiko SUGIURA: Radiation genetics  
 Osamu KITAGAWA: Population-genetics in *Drosophila*  
 Tetsuaki HASHIMOTO: Biological effects of radiation  
 Kenjiro FUJIOKA: Immunological-chemistry of organ-specific proteins  
 Shohachi SHIMOYAMA: Cytogenetics of flowering plants

## FOREIGN VISITORS IN 1956

- Apr. 28. Dr. T. H. SHEN, and Dr. Y. T. CHANG (Joint Committee for Rural Reconstruction, China). Gave lecture on "Recent agricultural development in Taiwan".
- May 9. Prof. C. M. POMERAT (Texas University, U.S.A.). Gave lecture on "Cytological abnormalities found in tissue-culture".
- June 8. Dr. H. H. SMITH (Brookhaven National Laboratory, U.S.A.).
- Aug. 4. Dr. O. J. EIGSTI (Indiana University, U.S.A.). Gave lecture on "Present status of seedless watermelon culture in the United States".
24. Prof. A. CHOVNICK (University of Connecticut, U.S.A.).
27. Prof. I. M. LERNER (University of California, U.S.A.). Gave lecture on "Population-genetic researches in the University of California".
- Sep. 1. Dr. C. C. LINDEGREN (Southern Illinois University, U.S.A.). Gave lecture on "Genetics of yeast".
2. Prof. J. F. CROW (University of Wisconsin, U.S.A.). Gave lecture on "Method of measuring mutation rate in human populations".
4. Prof. G. L. STEBBINS (University of California, U.S.A.). Gave lecture on "Mode of speciation in plants".
11. Foreign attendants of the International Genetics Symposia (about 110 persons).
12. Dr. H. W. LI (Taiwan Sugar Corporation, China). Gave lecture on "Interspecific and intergeneric hybrids in the plant-group related to sugar-cane".
13. Dr. G. K. MANNA (Chittaranjan Cancer Hospital, India). Gave lecture on "Phylogenetic relationships among species of Hemiptera, from cytological view-point".
14. Prof. R. E. COMSTOCK, and Prof. W. C. GREGORY (North Carolina State College, U.S.A.). Gave lectures on "Statistical genetics of quantitative characters" (from Sep. 14 to Sep. 17), and on "Radiation genetic experiments in peanuts".
18. Prof. B. C. JENKINS (University of Manitoba, Canada). Gave lecture on "The ROSNER Plan in the University of Manitoba".
- Prof. E. R. SEARS (University of Missouri, U.S.A.). Gave lecture on

## FOREIGN VISITORS IN 1956

- “Transmission of rust resistance from *Aegilops umbellulata* to common wheat”.
20. Dr. R. F. KIMBALL (Oak Ridge National Laboratory, U.S.A.). Gave lecture on “Studies of mutations in *Paramecium*”.
21. Prof. S. BENZER (Purdue University, U.S.A.). Gave lecture on “Basic units of inheritance, from genetic studies in viruses”.
28. Prof. A. MÜNTZING (Lund University, Sweden). Gave lecture on “Polyploid breeding in Sweden” (at Sizuoka Agr. Exp. Sta.).
- Oct. 3. Prof. C. STERN (University of California, U.S.A.). Gave lecture on “Recent studies on mutation”.
- Dec. 28. Prof. D. C. SMITH (University of Wisconsin, U.S.A.). Gave lecture on “Breeding of forage crops in the United States”.



# RESEARCHES CARRIED OUT IN 1956

## A. HUMAN GENETICS

### 1. *Equilibrium of Human Genes*

(By Taku KOMAI)

The writer has studied the genetics of two rare human congenital abnormalities, microcephaly and van der Hoeve's syndrome which are due to a recessive gene and a dominant gene respectively. These genes seem to persist in the population in an equilibrium state by an exact balance between the new genes produced by mutation and the old genes eliminated by selection. The rate of mutation has been estimated for both genes as of the order  $10^{-5}$ . The incidence of the genes in population was calculated as of the order  $10^{-3}$  for the gene for microcephaly and  $10^{-5}$  for the gene for van der Hoeve's syndrome.

For the commoner congenital abnormalities such as hare-lip, congenital dislocation of hip joints, congenital club-foot, anencephaly and hydrocephaly, the incidence among new-borns is of the order  $10^{-4}$ — $10^{-3}$ . Our knowledge of the genetics of these abnormalities is still insufficient, and it is probable that phenocopies are mingled among the cases of real genetic origin. Hare-lip, for instance, is considered to be due to a dominant gene. If the rate of mutation ( $u$ ) of this gene is assumed to be  $3 \times 10^{-5}$  and its selection rate ( $s$ )  $5 \times 10^{-2}$ , then the incidence of the gene ( $q$ ) becomes  $q = u/s = 1/1667$  and the incidence of cases 1.2/1000. The observed incidence of cases is about 2/1000, so that the phenocopies may be about .8/1000.

For the still commoner genetic abnormalities such as taste blindness, color blindness, deficiency of palmaris longus muscle, malformation or deficiency of upper external incisors, the incidence is of the order  $10^{-2}$ . The taste-blind people occur among Japanese in frequency about 6 per cent. If  $5 \times 10^{-4}$  is taken as the value of  $s$  for this gene and  $3 \times 10^{-5}$  as that of  $u$ , then  $q$  becomes 0.245 and the taste-blind people about 6 per cent. For dominant characters of this class, if  $s$  is taken as  $3 \times 10^{-3}$  and  $u$  as  $3 \times 10^{-5}$ , then the frequency of the abnormality becomes 2 per cent.

Lastly, it is very likely that there are cases where the equilibrium of abnormal genes is maintained by heterosis, although few cases besides that concerning sickle-cell anemia have been thoroughly worked out.

2. *Population Genetics of Albinism*

(By Katumi TANAKA)

Data on the distribution of albinos in the eastern region of Sizuoka-Prefecture were analyzed. The incidence of this abnormality was 10/126,000 in Simizu and 7/59,000 in Misima, which appeared to be higher than the frequency found in the southern part of Gihu-Prefecture (16/762,500), and lower than that in Kasuga-village (5/4,000), as reported by Kurashima. It may be inferred that the frequency of albinos, and therefore the frequency of the albino gene, differs in various parts of Japan.

Of 28 albinos found in Simizu, Misima and adjacent regions, eight are married; two of the matings are between albinos. This seems to suggest a tendency to positive assortative mating.

The proportion of cousin marriages among the parents of albinos was 6/12; accordingly  $k=0.5$ . The frequency of the albino gene was estimated to be 0.0042, by using Neel's formula under the assumption that the frequency of cousin marriages in the whole Japanese population is 6%.

3. *Variation in Taste-Response to "Citibittol" among the People of Misima*

(By Yoshito OGAWA, Tôru ENDO, Yukihide ABE and Kenjiro FUJIOKA)

Personal variation in taste sensitivity to the bitter substance contained in the fruit of *Citrullus Colocynthis* SCHRAD was studied in comparison with that to P. T. C. The number of persons examined was 525. They were all people residing in Misima.

Table 1. Variation in the lowest concentration of "citibittol" solution producing a bitter taste.

Sex	Blind persons	Saturated	Concentration										Total No. of persons	
			$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$	$\frac{1}{1024}$		$\frac{1}{2048}$
Female	4	3	2	2	1	4	8	25	65	67	42	26	5	257
Male	4	2	2	2	4	9	9	65	86	44	34	5	5	268
Total	8	5	4	4	5	13	17	90	151	111	76	31	10	525

Variation in the lowest concentration of "Citbittol"\* which gave a bitter taste is listed in Table 1.

Eight persons (1.52%) showed no response even to a saturated solution of "Citbittol", and appeared to be blind to this bitter taste.

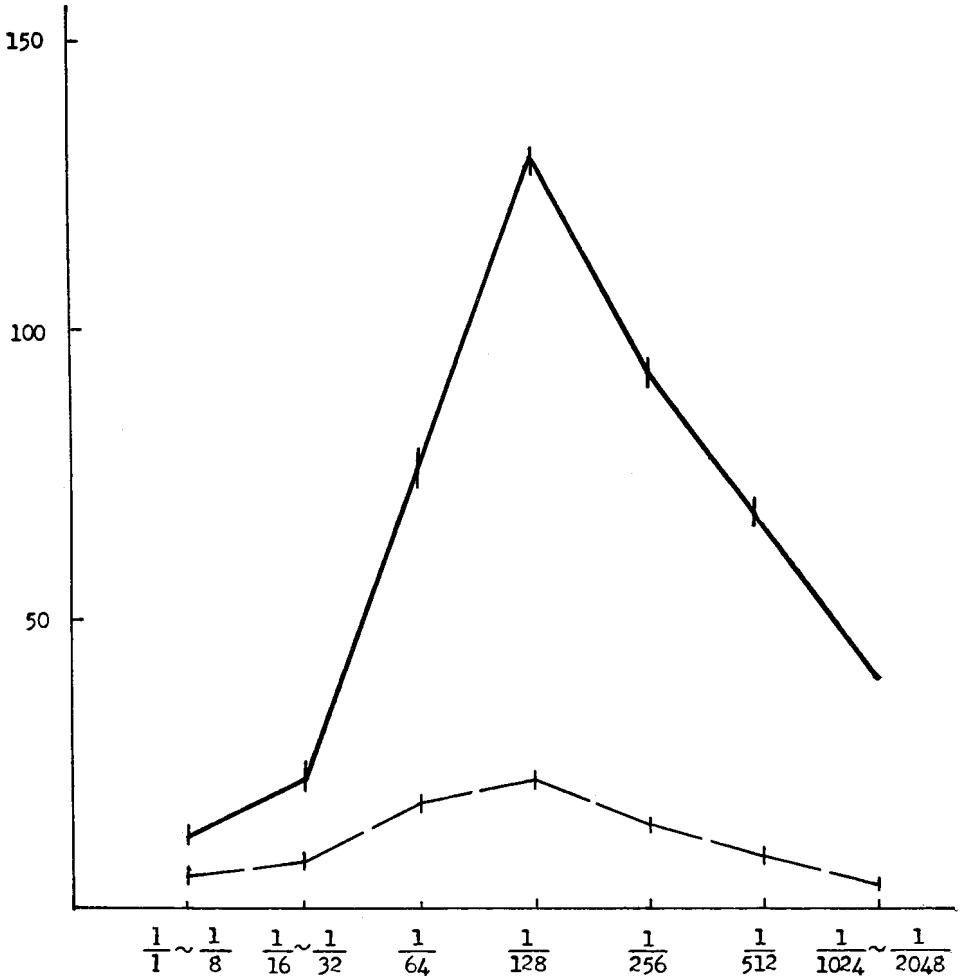


Fig. 1. Lowest concentration of "Citbittol" solution, effecting a bitter taste among P. T. C. non-tasters (Blind) and tasters (Normal).

\* Refer to Report No. 36.

In order to find a relation, if any, between the taste-blindness to "Citbittol" and that to P. T. C., the examined persons were grouped into two classes according to their reaction to P. T. C., positive or negative, as shown in Fig. 1. The group blind to P. T. C. comprised 84 persons, 4 among them blind to "Citbittol". The other group, sensitive to P. T. C., was composed of 438 persons. Among them were the remaining 4 people blind to "Citbittol". Though the percentage of Citbittol-blind people was considerably larger in the first group than in the second, their number is too small to allow for conclusions concerning the relation. Further studies are needed.

## B. GENETICS AND CYTOLOGY OF MAMMALS, BIRDS AND INSECTS

### 4. *On the Origin of the Tortoiseshell Male Cat*

(By Taku KOMAI)

As has been mentioned in the previous numbers of this report, the records of 65 specimens of tortoiseshell male cats indicate that such a cat may be produced by a mother of any color, provided that her genotype and her mate's genotype are in such relation that the gene for orange color,  $O$ , or its allele,  $O^+$ , may become heterozygous in her offspring. Since  $O$  is sex-linked, the heterozygote should be a female in ordinary cases. If a male would become heterozygous for this gene, the Y-chromosome would carry  $O$  or  $O^+$ . Such a male would be produced if crossing over took place between the X and Y of the father, and transferred  $O$  or  $O^+$  from X to Y. Next, to account for the sterility of tortoiseshell male cats, which is very usual, one seems to have to assume the presence in the Y-chromosome of a gene complex for fertility of the male, and its transfer to the X-chromosome by crossing over. Exceptionally there are fertile tortoiseshell males, as recorded by some previous authors and also demonstrated histologically by Ishihara in our Institute. The occurrence of such males might be understood by assuming only the transfer of  $O$  or  $O^+$  from X to Y without the reciprocal transfer of the gene complex for fertility from Y to X. Or else, as has been suggested by Bamber and Herdman, such a fertile tortoiseshell male is a genotypical orange in which the covering effect of the gene for orange is deficient and allows the black color to appear to some extent.

Ishihara has studied the histology and cytology of six specimens of tor-

toiseshell males. Four of these had sterile testes, in which the spermatogenesis had been arrested in the spermatogonial or spermatocytic stage, whereas two had testes which were apparently quite normal and fertile.

### 5. *Studies on the Mitotic Index in Various Organs of the Rat*

(By Takaaki ISHIHARA)

#### 1) *Mitotic indices in various organs in the early stages of development.*

It is well known that in higher animals mitotic activity in most tissues gradually decreases with the development of the individual. The present study deals with the mitotic indices of various organs of the rat (liver, kidney, lung, heart and cerebrum) in the early stages of growth. The mitotic index in those organs was generally higher in the embryonic stage than after birth, though at parturition some decrease occurred. The mitotic index in organs of new born rats rapidly rose during four days after parturition, the highest value being reached on the fourth day. Subsequently, it began to decline and reached the bottom on the eighth day. Mitotic cells were hardly observed in 12 day old cerebrums and lungs, or 30 day old kidneys and livers. It is interesting that the cerebrum showed a somewhat different pattern from that of other organs.

#### 2) *Relation between organ weight and body weight.*

The weights of various organs of the rat in the early stages of development, expressed as a percent of the weight of adult animals are set out in Table 1. The table shows that each organ markedly increases its weight in spite of a decrease in the number of mitotic cells. It may be

Table 1. Weights of various organs of young rats as a percent of the weight of the same organs in adults.

Organs	Days after parturition				Adult rats
	1 day	10 days	15 days	30 days	
Liver	3.92%	7.43%	10.02%	26.72%	7.1 gr.
Kidney		16.66	21.66	35.00	1.2
Heart		23.52	29.41	33.82	0.68
Lung		18.46	21.15	23.84	1.3
Cerebrum	12.18	48.28	66.66	72.32	1.65

recognized, therefore, that the growth of organs depends not only upon the increase in the number of cells but also on the increase in cell volume.

It may also be worthy of note that the weight of the brain remains constant irrespective of the increase or decrease of body weight, while the weights of liver, kidney and lung are proportional to body weight. It may then be said that the brain has a developmental pattern somewhat different from those of other organs.

### 6. *Resistance to Blackhead Disease of Hybrid Chickens.*

(By Takatada KAWAHARA)

Within-breed and between-breed matings were made using two males and 24 females of White Leghorns (WL), and two males and 29 females of Barred Plymouth Rocks (BP). A total of 355 female chicks were hat-

Table 1. Mortality from blackhead disease and other causes up to the eighteenth week of chickens' life.

Experiment No.	Matings	Tested No.	Total mortality (%)	Death from blackhead disease (%)	Death due to other causes (%)
T1	WL	40	10.0	0	10.0
	BP	29	10.3	0	10.3
	WL♀ × BP♂	50	2.0	0	2.0
	BP♀ × WL♂	19	5.3	0	5.3
	Pure (WL) (BP)	69	10.1	0	10.1
	Hybrid (WL♀ × BP♂) (BP♀ × WL♂)	69	2.9	0	2.9
	T2	WL	79	46.8	43.0
BP		54	35.2	31.5	3.7
WL♀ × BP♂		46	8.7	8.7	0
BP♀ × WL♂		38	15.8	10.6	5.2
Pure (WL) (BP)		133	42.1	38.3	3.8
Hybrid (WL♀ × BP♂) (BP♀ × WL♂)		84	11.9	9.5	2.4

ched. The birds were maintained without any artificial culling up to the eighteenth week, except for cases of death spontaneously occurring during this period. All of the dead birds were autopsied. The experiment was conducted on the farm of Mr. Hara.

The total mortality of female chicks during the period of the first eighteen weeks of their life was determined as growing mortality; it is represented for the two breeds and the reciprocal  $F_1$  hybrids in Table 1.

A comparison of the growing total mortality of hybrid chicks with that of the pure-bred chicks shows that the hybrids had a lower mortality. While the difference of 7.2% in mortality in the first experiment (T1) is statistically non-significant, the difference of 30.2% found in the second experiment (T2) is significant at the 1% level.

The table shows also that a larger part of the difference in mortality between the parental breeds and the  $F_1$  hybrids in the second experiment was due to blackhead disease. Mortality from this disease in pure breeds in this case was as high as 38.3%, while it amounted only to 9.5% in  $F_1$  hybrid chicks. The difference of 28.8% was also found to be statistically highly significant at the 1% level.

### 7. *Quantitative Relationship between Autosomes and Sex Chromosomes in a Few Related Species within the Coleopteran and Hemipteran Groups of Insects*

(By Tosihide H. YOSIDA)

From comparative studies of chromosomes in some groups of Coleoptera and Hemiptera, I have pointed out that a quantitative balance exists between the autosomes and the X-chromosomes, which can be expressed as  $\frac{T.A.}{X}$  (where T. A. and X are the total length of autosomes and X-chromosomes, respectively). This ratio appears to be nearly constant, so far as different species within a group are concerned. For example,  $\frac{T.A.}{X}$  in seven species of *Coccinellidae* (Coleoptera) was about 16. The same ratio in three species of *Rutelinae* (*Scarabeidae*, Coleoptera) was about 24, and in ten species of *Pentatomidae* (Hemiptera) about 12.

The relationship between autosomes and sex chromosomes was examined in a few related species of *Coreidae*, *Pentatomidae* and *Reduviidae* (Hemiptera) to find out whether a similar relationship exists in cases in which a multiple sex chromosome mechanism prevails. In these cases, the increase of the number of autosomes is accompanied by an increase in the number

of X-chromosomes. It may then be said that a quantitative balance between autosomes and X-chromosomes also exists in cases where multiple sex chromosomes are involved.

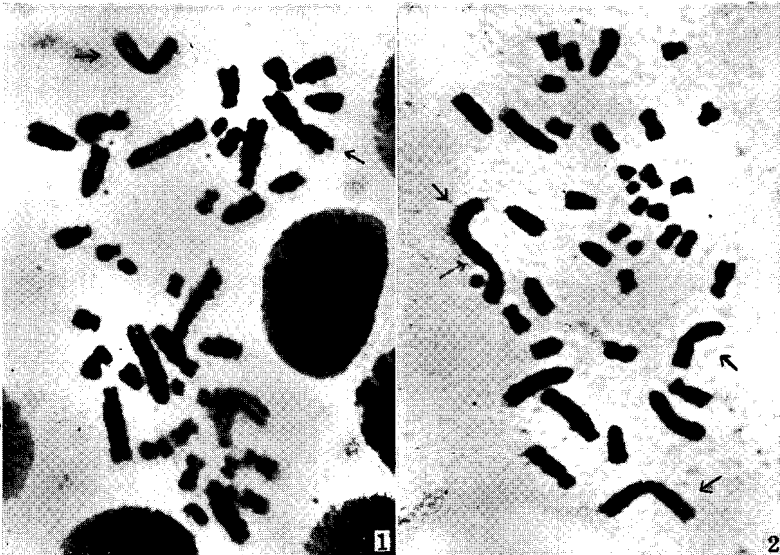
Reference: YOSIDA, T. H. 1956. Studies on the chromosomes of Coleopteran and Hemipteran insects, with special regard to the quantitative relation between autosomes and sex chromosomes. Proc. Tenth Intern. Cong. Ent. (in press).

### C. CYTOLOGY AND GENETICS OF TUMORS

#### 8. *Change of Chromosome Constitution in the Stem-line of the Yoshida Sarcoma*

(By Tosihide H. YOSIDA)

In the course of a transplantation experiment of the YOSHIDA sarcoma, I found tumor cells having a chromosome constitution different from that usually observed in this sarcoma. As stated in the previous report (YOSIDA 1955)<sup>1)</sup>, most of the cells of the original strain of the YOSHIDA sarcoma



Figs. 1-2. Photomicrographs of chromosomes in two strains of the YOSHIDA sarcoma. 1) Original strain including two large V-shaped chromosomes. 2) Substrain containing two large V- and one large J-shaped chromosomes. Arrows indicate the large V- and J-shaped chromosomes.

1) Yosida, T. H. 1955. Proc. Jap. Acad. 31: 237-242.



have 40 chromosomes, including two strikingly large V-elements (Fig. 1). The new karyotype of the tumor cells also has 40 chromosomes, but three are strikingly large, two of them V- and one J-shaped (Fig. 2). This strain was found in the YOSHIDA sarcoma obtained from the Institute for Infectious Diseases, Tokyo, and was kept in our laboratory for eleven transplant generations using W-strain rats. The W-strain rats had a strong resistance to this tumor in the first few transplant generations. However, an increase in transplantability was found after several trans-

Table 1. Frequency of the original and the new karyotypes in YOSHIDA sarcoma.

Transplant generations	Strain of rat used	No. of cells observed		Total
		2V1J type (New type)	2V type (Original type)	
9th	WK	44	13(30.2%)	57
	W	35	4(15.2%)	39
	SH	21	6(28.5%)	27
	CW	19	2(10.5%)	21
10th	WK	25	7(28.0%)	32
	W	30	3(10.0%)	33
	CW	41	2( 4.8%)	43
11th	WK	51	19(17.2%)	70
	W	23	1( 4.2%)	24
9-11th	WK	120	39(30.2%)	159
	W	88	8( 9.1%)	96
	SH	60	4( 5.9%)	64
	CW	21	6(28.5%)	27
Total		289(81.3)	57(19.7%)	346

plant generations. From the ninth to eleventh transplant generation it was found that 81.3% of cells had the new karyotype, though the remaining 18.7% showed the original karyotype of YOSHIDA sarcoma. The new strain was transplanted into three other strains. An interesting fact then

observed was that different rat strains showed a different occurrence of the two karyotypes (Table 1). In W- and SH-strains, the frequency of the original-type cells was very low, while in WK- and CW- strains it was very high.

9. *Subtriploid Chromosome Constitution Characteristic of the Tumor Stem-cells of Walker Carcinoma of Rats*

(By Toshihide H. YOSIDA)

From the karyological analysis of YOSHIDA-, MTK-II-, MTK-III- and Hirosaki-sarcomas, it was found that the stem-cells of these tumors are characterized by a subdiploid chromosome constitution, including a few striking V- and/or J-shaped elements (Yosida 1955)<sup>1)</sup>. On the other hand, the TAKEDA sarcoma is characterized by tumor stem-cells with subtetra-



Fig. 1. Chromosomes in a tumor cell of the WALKER carcinoma.

Table 1. Number of chromosomes in tumor cells of the WALKER carcinoma.

Number of chromosomes	37	51	53	57	59	60	61	62	63	98	Total
Number of cells observed	1	1	1	1	1	10	1	1	2	1	20

1) YOSIDA, T. H. 1955. Proc. Jap. Acad. 31: 237-242.

ploid chromosome constitutions, having several distinct V- and J-elements (YOSIDA 1952)<sup>1)</sup>.

Unlike these two cases, the tumor stem-cells of the WALKER carcinosarcoma are characterized by subtriploid chromosome constitutions. The numerical variation of chromosomes in this tumor is shown in Table 1. The table shows that cells with 60 chromosomes were most frequently found, while the chromosome constitution was very complicated. Of the 60 chromosomes, 26 were rod-shaped, 12 J-shaped and the remaining 22

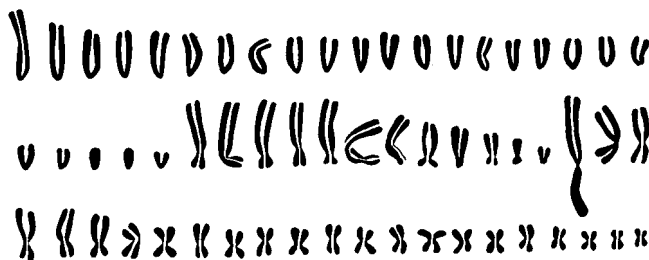


Fig. 2. Serial alignment of chromosomes in the cell shown in Fig. 1.

V-shaped. A large V-chromosome was very conspicuous as shown in Figs. 1 and 2. Such a chromosome assortment seems to be characteristic of the stem-line of this tumor.

#### 10. Occurrence of Distinct V-Chromosomes in Cells of Seven Original Rat Hepatomas

(By Toshide H. YOSIDA and Takaaki ISHIHARA)

In the course of experiments carried out in order to induce a hepatoma in white rats by administration of D. A. B. (*p*-dimethylaminoazobenzene), we (YOSIDA and ISHIHARA 1956)<sup>2)</sup> have found one consisting of four tumorous masses. From a karyological analysis, it was found that only one of them was characterized by the presence of a distinctly V-shaped chromosome. We continued the experiments and succeeded in inducing six other hepatomas in rats five months after the administration of D. A. B.. The results of our observations of those original hepatomas are as follows:

1) YOSIDA, T. H. 1952. Gann. 45: 9-15.

2) YOSIDA, T. H. and T. ISHIHARA 1956. Ann. Rep. Nat. Inst Genet. Jap. No. 6: 22-23.

In two hepatomas, designated as WK-2b and WK-1b, no V-chromosome was found in the tumor cells (Table 1). On the other hand, the other

Table 1. Chromosome constitution of tumor cells  
in the seven original hepatomas.

Strain of hepatoma	No. of cells observed	Variation in number of chromosomes		No. of V-chrom. per cell	No. of cells with V's	
		range	mode			
H-2c	32	37-88	43	2	1/32	
H-2a	15	28-61	34	1	13/15	
H-4c*	I	20	25-151	?	0	
	II	37	28-78	41	1	9/37
	III	16	34-168	81	0	
WK-2b	15	30-44	42	0		
H-1b	18	33-84	42	1	3/18	
W-1	6	54-66	46	1	4/6	
WK-1b	10	37-86	?	0		

\* Description has been given in the Annual Report of this Institute, No. 6 (1956).

four hepatomas (viz. H-2c, H-2a, H-1b and W-1) were characterized by the presence of cells with one large V-element. Two (H-2a and W-1) had a remarkably high frequency of cells with one V, while in the other two (H-2c and H-1b) their frequency was low. V-chromosomes were not found in rats which received food containing azo dye and showed no sign of malignant growth. It should be mentioned that the V-chromosomes were never found in normal cells and that though they are characteristic of tumor cells, they are not necessarily found in all cases.

The present study was undertaken with regard to the occurrence of the V-elements. But it was incidentally observed that also other small changes took place in the chromosome constitution of the hepatoma cells. These are now being investigated in our laboratory.

11. *A Study on the Transplantability of the WPY-Rat Sarcoma*

(By Takaaki ISHIHARA and Tosihide H. YOSIDA)

The WPY-rat sarcoma is a kind of fibrosarcoma which developed in a strain of rat, WAYNE pink-eyed yellow (WPY-rat), in our laboratory. In transplantation experiments of this tumor, it was found that transplantation was successful in all WPY-rats, but not in the Wistar-King-A strain (WK) (Table 1.). Transplantation was also successful in all F<sub>1</sub> hybrids

Table 1. Transplantability of WPY-rat sarcoma to WPY, WK-strains, F<sub>1</sub> and F<sub>2</sub>.

Strain	Result of transplantation		No. of rats transplanted	% of positive transplantation
	Positive	Negative		
WPY <sup>1)</sup>	126	0	126	100
WK <sup>2)</sup>	0	30	30	0
F <sub>1</sub> (WPY × WK)	30	0	30	100
F <sub>2</sub> (WPY × WK)	3	66	69	4.34

Remarks. 1) WAYNE pink eyed-yellow. 2) Wistar-King-A.

between the two strains (WPY × WK). It may be assumed that some H-genes (Histocompatibility genes) are responsible for the observed transplantability, as in mouse tumors. To determine the number of the H-genes, the variation in transplantability in the F<sub>2</sub> generation was examined. The genetic experiment showed that 4.34 percent of F<sub>2</sub>-animals were susceptible to the tumor, suggesting the possible number of H-genes to be about ten which are effective if each is represented at least by one dominant allele.

12. *Effect of Kinetin upon Division of "YOSHIDA-Sarcoma Cells"*

(By Yoshito OGAWA, Yukihide ABE and Kenjiroo FUJIOKA)

Kinetin (6-furfurylaminopurine) was isolated by Miller et al. (1955, 1956) from DNA of herring sperms. They found that it induced a high rate of cell multiplication in the callus tissues of plants. Guttman's study (1956) has shown that in onion root tip cells kinetin promoted mitotic divisions and induced polyploidy besides various forms of pycnosis. However, its effect upon the division of animal cells is not well known, though negative

results have been reported by Lettre (1956) who used animal and human cells.

We have investigated the effect of Kinetin upon the mitotic activity of YOSHIDA sarcoma cells transplanted to a strain of rat, Wister. When the solution of kinetin was injected into the abdomen, a significant increase in the frequency of mitotic figures was found in the tumor cells after about 120 hours (significant at the 1% level as shown in Tables 1 and 2).

Table 1. Frequency of mitotic figures after injection of kinetin

Hours	Kinetin (mg)							
	Control		0.03		0.15		0.75	
	Proportion	Angle*	Proportion	Angle*	Proportion	Angle*	Proportion	Angle*
48	6.25%	14.49	5.31%	13.32	3.09%	10.12	3.65%	11.01
	4.77	12.62	3.51	10.78	2.75	9.55	3.51	10.80
	4.33	12.01						
120	2.56%	9.21	2.39%	8.82	4.12%	11.73	2.73%	9.51
	2.88	9.77	2.47	9.04	3.88	11.36	2.14	8.41
	2.80	9.63						

\* Degrees after arc-sine transformation of percentage. Host: rat (wister); 2 months old. Preparation: aceto-orcein squash technique. The percentage of dividing cells was determined by observation of 10,000 cells. No dividing cell was found in the ascites if kinetin only was injected and the sarcoma was not transplanted.

Table 2. Variance analysis of the frequency of dividing cells

Source	Degrees of freedom	48 hrs. Mean sq.	120 hrs. Mean sq.
Treatment	3	4.66	3.99**
Error	5	1.52	0.1

\*\* Significant at 1% level.

However, it was found that kinetin did not change the relative frequency of different stages of mitosis.

In the control-experiment, as shown in Fig. 1, the frequency of metapha-

sic cells showed two phases of increase, two and ten days after the transplantation of YOSHIDA sarcoma. In the treated rats, the second phase was

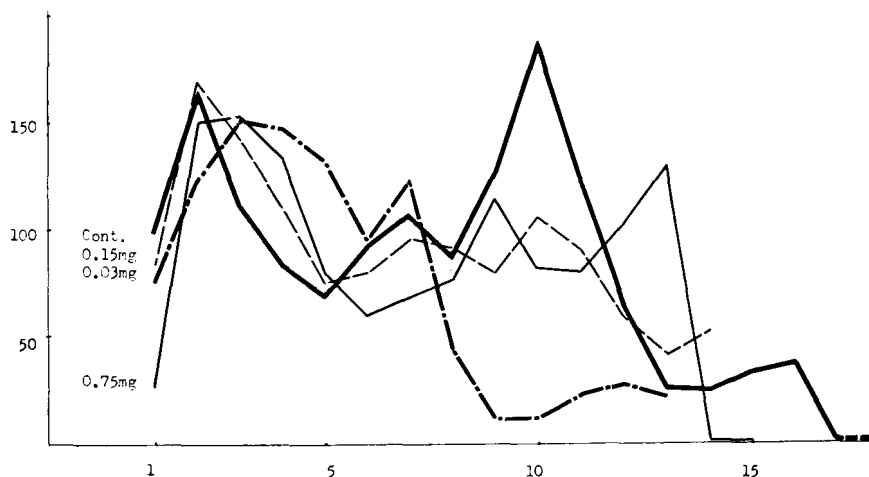


Fig. 1. Daily change of frequency of metaphasic cells (pathological figures excluded) after injecting kinetin into the abdomen of rat, 10,000 cells being observed per day. Abscisse: Days after transplantation. Ordinate: Number of metaphasic cells observed.

not apparent, probably because the animals were starved during the first increasing period, which lasted longer than in the non-treated animals. The frequency curves for treated rats, not showing any tendency to abrupt change, seem to suggest that kinetin may act upon mitotic activity directly, the effect not being due to some secondary metabolite. Thus we find a case in which kinetin acts on animal cells as on plant cells.

## D. GENETICS AND BIOCHEMISTRY OF THE SILKWORM AND OTHER INSECTS

### 13. *Genetics of the Silkworm*

(By Yoshimaro TANAKA)

#### 1) Linkage Tests.

Hereditary relationships were tested between *Se* (White-sided egg) and several other genes such as *st* (stony), *ge* (geometrid), *nb* (narrow breast)

and *so* (sooty). They were all inherited independently, no linkage being observed. This experiment was carried out under a grant-in-aid of the Ministry of Education.

2) Inheritance of "Light Eye-spot".

"Light eye-spot" is a strain with otherwise normal marking but its eye-spot is markedly lighter in comparison with the "lunules" and "stars". It was crossed with a standard normal type (race p 22), and the author found that this character was allelic and a simple recessive to normal +". The range of variability in density of the eye-spot in  $F_2$  was somewhat greater than the total range in parents. "Light eye-spot" may be designated by the symbol  $p^{le}$ .

3) Selection in No-lunule.

In the strain of *Nl* (No-lunule), some larvae are provided with small rudiments of lunules while others are entirely free from lunular marking on the second abdominal segment, though these two types are somewhat overlapping. These different forms were observed mixed among full sibs. The author tried to fix a strain with no trace of lunules on one hand, and a strain with rudimentary lunules on the other hand by selection.

After several generations, both positive and negative selections proved effective, the females from both strains giving rise to different ratios of "complete *Nl*" and "incomplete *Nl*" offspring.

14. *Effect of Day-length during the Embryonal Stage of a Wild Silkworm, Antheraea pernyi, on the Pupal Diapause*

(By Yoshimaro TANAKA)

Since 1937, the author accumulated experimental data showing that a long day during the larval life inclines pupae to diapause, while a short day makes them develop into moths without hibernating.

On the contrary, the photoperiodic effects on embryos were divergent, often even contradictory. One of the disturbing causes seemed to the author to be the influence of day length during the larval life. To lessen this influence to a minimum, he made a design of experiment to rear larvae under "neutral" day length of 14 or 15 hours a day, after exposing the eggs to different day lengths.

By this way, he could disclose that photoperiodic effects upon embryos are just opposite to those upon larvae, i.e. long day predisposes to diapause, and the short day to imaginal development.



15. *Effect of Cytoplasm upon the Expression of Quantitative Characters in the Silkworm*

(By Yataro TAZIMA, Eiichi MIDORIKAWA\* and Mieko SHIOKAWA\*)

In order to determine the effect of the cytoplasm upon the expression of quantitative characters, reciprocal crosses were carried out with regard to the weight of cocoons and of cocoon-shells.

The method adopted in this investigation was as follows. Females of strain A were crossed to males of another strain B, and this was followed by back-crossing the B male repeatedly in subsequent generations, i.e.  $A \times B$ ,  $(A \times B) \times B$ ,  $\{(A \times B) \times B\} \times B$ , ...etc.. Special attention was paid to mating the same male of B strain to females in each of the back-crosses. About 16 pairs of such crosses were performed in each generation. The heterotic vigour with reference to cocoon weight and to some other characters in  $F_1$  and in the subsequent back-cross generations decreased slightly generation after generation. Crosses were then made to an unrelated strain. In order to test whether the decrease in the expression of characters occurred with the increase of the number of back-crosses or was dependent upon the direction of the cross, all the crosses were raised in the same season. Special care was taken to mate the same male to the females of each generation in order to avoid disturbances, caused by diverse genic constitutions of the male parents. About 16 lots of the same cross of each generation were mixed together and all crosses were raised simultaneously in the same season.

The results obtained in the autumn of 1956 are shown in Table 1. Here F and  $F_m$  are Japanese type fixed hybrid breeds between Japanese and European silkworms, while 75 and 90 are Chinese type fixed breeds from hybrids between Chinese and European silkworms.

It is evident from Table 1 that 1) when a cross is made to an unrelated strain, heterotic vigour appears again irrespective of the number of back-crosses carried out before, the performance of the  $F_1$ 's being scarcely different from each other, and that 2) a marked difference is observed between both types of reciprocal crosses, independently of the number of back-crosses. Performances of the offspring when the female parents were Japanese were superior to those when they were Chinese. Similar results were also obtained in other experiments, carried out in the summer of the same year. These results agree with that of SHIMIZU (1947). He reported that in most cases Japanese type as the female parent gave offspring superior to those from Chinese type females so far as cocoon weight is concerned.

\* The Silk Science Research Institute, Tokyo.

Table 1. Comparison of cocoon weight and cocoon-shell weight in reciprocal crosses.

Cross	Cocoon wt.		Cocoon-shell wt.	
	♀	♂	♀	♂
(F <sub>1</sub> F <sub>m</sub> <sup>1</sup> ) × 90	cg 203 ± 12.0*	159 ± 10.3	cg 42.4 ± 2.8	40.0 ± 3.0
(F <sub>1</sub> F <sub>m</sub> <sup>2</sup> ) × 90	211 ± 14.6	159 ± 12.5	43.3 ± 3.1	39.6 ± 3.6
(F <sub>1</sub> F <sub>m</sub> <sup>3</sup> ) × 90	203 ± 15.2	157 ± 13.7	42.1 ± 2.8	39.1 ± 3.3
(F <sub>1</sub> F <sub>m</sub> <sup>4</sup> ) × 90	201 ± 12.8	159 ± 13.2	42.8 ± 2.8	39.6 ± 3.4
90 × (F <sub>1</sub> F <sub>m</sub> <sup>1</sup> )	179 ± 12.9	144 ± 10.9	39.3 ± 2.9	37.2 ± 3.3
90 × (F <sub>1</sub> F <sub>m</sub> <sup>2</sup> )	185 ± 12.0	148 ± 10.5	40.4 ± 3.1	37.2 ± 3.1
90 × (F <sub>1</sub> F <sub>m</sub> <sup>3</sup> )	190 ± 13.0	150 ± 12.0	41.3 ± 2.8	38.8 ± 3.0
90 × (F <sub>1</sub> F <sub>m</sub> <sup>4</sup> )	190 ± 12.5	149 ± 10.5	40.4 ± 3.2	38.7 ± 2.8
(90.75 <sup>1</sup> ) × F <sub>m</sub>	197 ± 13.9	155 ± 12.7	40.7 ± 3.3	37.9 ± 3.0
(90.75 <sup>2</sup> ) × F <sub>m</sub>	198 ± 13.7	157 ± 11.3	40.9 ± 3.1	37.8 ± 3.1
(90.75 <sup>3</sup> ) × F <sub>m</sub>	194 ± 12.4	152 ± 11.2	41.1 ± 3.0	38.0 ± 2.7
(90.75 <sup>4</sup> ) × F <sub>m</sub>	194 ± 13.1	152 ± 10.4	40.9 ± 2.9	37.6 ± 3.2
F <sub>m</sub> × (90.75 <sup>1</sup> )	211 ± 13.6	162 ± 12.0	42.6 ± 3.9	41.0 ± 2.9
F <sub>m</sub> × (90.75 <sup>2</sup> )	214 ± 16.0	160 ± 14.5	44.2 ± 3.5	39.1 ± 3.5
F <sub>m</sub> × (90.75 <sup>3</sup> )	210 ± 15.0	162 ± 12.9	41.9 ± 3.4	38.1 ± 3.1
F <sub>m</sub> × (90.75 <sup>4</sup> )	206 ± 13.8	157 ± 11.0	42.3 ± 3.1	37.6 ± 3.4

\* Standard Error

Thus it may be taken as proved that dissimilar results are obtained in this insect, depending on the direction of the reciprocal crosses. Differences could be clearly observed in the case of characters that manifest themselves early in the life history of the silkworm, as hatchability, viability of younger instars etc. (KIKKAWA 1942, TAZIMA et al. 1955). It is noteworthy that even in such late traits as cocoon weight and cocoon-shell weight the maternal effect is observable.

Between the two types of reciprocal crosses chromosomal differences should be limited only to the sex chromosomes. In this respect, the males derived from the two types of crosses do not differ genetically, having two Z chromosomes, one from the mother and the other from the father. Females might be different depending on the type of the cross since they have only one Z chromosome. Therefore, differences should be restricted to the females only.

When cocoon weight of the male for each type of reciprocal crosses is

compared, the difference between both types is obscure in some cases, but not so in others. This seems to mean that environment favors one type of reciprocal cross owing to the difference in the cytoplasmic constituents until fairly late stages of the development.

From the above we are led to believe that the causes for differences between the reciprocal matings are probably not nuclear but cytoplasmic. This suggests that the cytoplasm may function in the zygote as an activating background for the expression of the nuclear genes.

### 16. *Studies on the Complex $Nl-U-Di$ Loci in the Silkworm*

(By Mitsuo TSUJITA)

#### 1) $Nl_2$ locus.

The  $Nl_2$  mutant, produced by X-ray treatment, lacks crescent patterns (semi-lunar patterns), and resembles  $Nl_1$  which exhibits what is known as non-lunar patterns  $Nl$ . Larvae with the genotype  $+/Nl_1$ , however, often have rudimentary crescent and star-shaped patterns, which become a little more distinct after sib-mating. This phenomenon can be explained by assuming the presence of modifiers of  $Nl_1$ . On the contrary, the larvae with the  $Nl_2$  marker lack completely the lunar patterns but have normal star-shaped patterns. The  $Nl_2$  mutant had been classified at first as the  $E^{Nc}$  strain, because of its phenotype, and kept several generations. But the following experimental results of our genetical analysis have proved that this judgment was not correct.

(i) Individual  $F_1$  hybrids between  $Nl_2$  and  $U$  or  $Di$  were crossed to normal or  $Di$ . From these crosses no recombinants could be obtained.

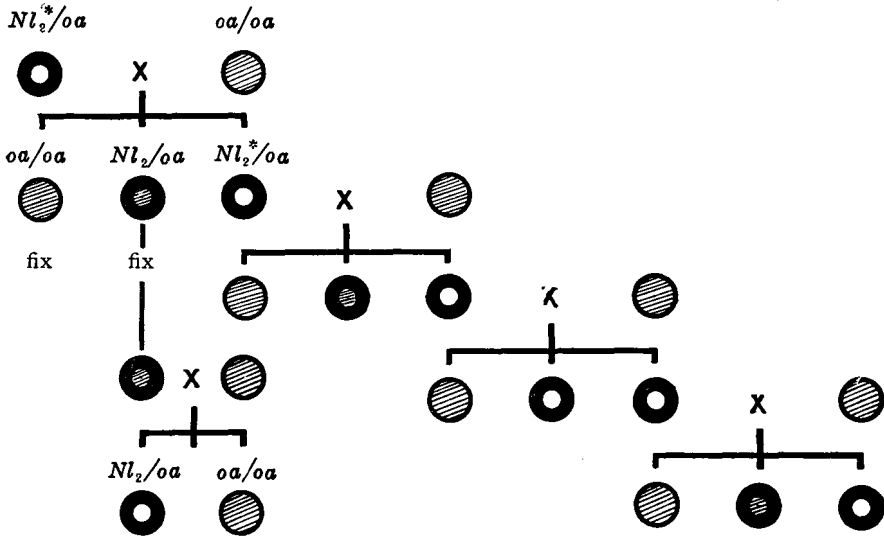
(ii) In the experiments using the original  $Nl_2$  strain, some abnormal segregation occurs. Besides, individuals homozygous for this gene are able to survive. These phenomena seem to be due to a chromosomal aberration. Individuals from which the chromosomal abnormality was removed, showed almost the same genetic behaviour as  $Nl_1$ , although their phenotypic appearance was somewhat different. Needless to say, the gene symbol  $Nl_2$  designates this genetic unit.


#### 2) Abnormal segregation of the original $Nl_2^*$ strain.

From the cross of  $+/+ \times +/Nl_2$ , normal and  $Nl_2^*$  larvae segregated almost in the ratio 1:1 (sometimes the number of the latter was a little larger than that of the former). After sib-mating the  $+/Nl_2^*$  individuals,  $Nl_2^*$  and normal segregated approximately in the ratio 3:1.


\* Means that chromosome XIV has some aberration.

From the cross  $Nl_2^*/U \times +/+$  or  $+/+ \times Nl_2^*/U$ , a 1:1 ratio in the number of  $Nl_2^*$  and  $U$  was found as expected. It was further found from




 Original  $Nl_2^*$  larvae which have a chromosomal aberration



  $Nl_2$  larvae without the chromosomal aberration



  $oa$  larvae with transparent hypodermis

the results of sib-mating individuals with the genotype  $Nl_2^*/U$  that individuals homozygous for  $Nl_2^*$  are viable. This segregation suggests that this original  $Nl_2^*$  strain is somewhat different from the known  $Nl_1$  strain in its genetic behaviour. The following finding suggests that the larvae of this strain have an aberration involving chromosome XIV.

From the cross  $+/Nl_2^* \times oa/oa$ , normal and two types of  $Nl_2$  larvae arise, the first with a transparent hypodermis and the second with a non-

transparent hypodermis. Although the segregation ratio of the three phenotypes is not always the same, in some of the batches it is almost 1:1:1.

The fact that the special chromosomal aberration had been kept through several generations after the finding of this mutant and the abnormal segregation ratio mentioned above suggest that the chromosomal aberration occurs on chromosome XIV itself.

The larvae which have a transparent hypodermis showed almost the same genetic behaviour as  $Nl_1$ , namely from the cross  $oa/Nl_2 \times oa/oa$  or from its reciprocal. Oily larvae and oily  $Nl_2$  larvae segregated in the ratio 1:1. In the sib-mating of  $oa/Nl_2$ , all of the embryos homozygous for  $Nl_2$  die in an early developmental stage.

The following diagram summarizes the results obtained from crosses starting with the original  $Nl_2^*$ .

Several hypotheses can be considered as explanations of the abnormal segregation of the original  $Nl_2$  strain. Among them the following is highly plausible. In the original  $Nl_2$  individuals there is an unstable attachment of a small chromosomal fragment on which the normal genetic unit for  $Nl_2$  is located. Therefore, from this strain two types of  $Nl_2$  segregated, i.e. individuals having chromosome XIV with or without its fragment. The strain from which the fragment once was lost produces only one type of larvae which behaves normally.

3) Linkage relation between  $U$  and  $oa$ .

The question of whether crossing-over between the  $oa$  and  $U$  genetic units occurs was examined and the following experimental results were obtained.

From the cross  $oa/U \times oa/oa$ , oily and  $U$  segregated. From the reciprocal cross a very small number of normal larvae appeared in addition to the  $oa$  and  $U$  individuals. The results of several repeated experiments which were carried out during the years 1955-'56 are given in the following table.

The single oily larvae of composition  $\frac{oa}{oa} \frac{U}{+}$  (?) had side markings which resembled those of larvae with the gene constitution  $U/+$ ;  $p/p$ . Larvae resulting from the cross between this type and  $oa/oa$ , however, were all oily and did not show this character. From the cross between the normals in the above table and  $oa/oa$ , oily and normal are produced in the ratio 1:1. Therefore, it is certain that normals are produced from the cross  $oa/oa \times oa/U$ . So far as our experimental results went, we could not obtain larvae with the genotype  $oaU/oa+$ . If it were true that only one type of recombinants can be produced from this cross, there is a possibility of an unusual crossing over.

Table 1. Segregation from the cross  $oa/oa \times oa/U$ .

No.	$oa/oa$	$U/+$	$\frac{oa}{oa} \frac{U}{U}$	$\frac{oa}{+} \frac{+}{+}$	Total	Recom. Value
1	175	141	0	0	316	0
2	65	88	0	0	153	0
3	139	92	0	0	231	0
4	159	166	0	0	325	0
5	—	—	—	—	—	—
6	210	115	0	1	326	0.3
7	158	188	0	1	347	0.3
8	252	242	0	0	494	0
9	70	74	0	0	144	0
10	87	32	0	0	119	0
11	214	148	0	0	362	0
Total	1529	1286	0	2	2817	0.6

4) *Di* region.

The present experimental results seem to show that *Di*, *U*, *Nl<sub>1</sub>*, *Nl<sub>2</sub>* and *Di* form a pseudoallelic series. The crossing-over value between *odk* and *U* is 8.0 (Hasimoto '41), and that between *odk* and *Di* 12.9 (Chikushi '55). According to these experimental results, some distance on the chromosome should be considered to exist between *U* and *Di*. *U<sup>Br</sup>*, which causes a dark brownish color of the larval body, has been proved to be allelic to *U* (Tsujiata '47). Furthermore we could obtain a mutant *n* which is similar to *Di*. Namely, like *Di*, larvae homozygous for *n* completely lack crescent and star-shaped patterns, and have almost the same body color as the normals. Consequently, it can be said that they have the appearance of an extremely diluted form of *Di*. The *F<sub>1</sub>* larvae from the cross between *Di* and *n* showed the characteristics of *Di*, and in the *F<sub>2</sub>*, two types, *Di* and *n* segregated. The gene symbol *Di<sub>o</sub>* was given for *Di* and *Di<sub>a</sub>* for *n*.

From the cross *Di<sub>a</sub>/Di<sub>o</sub> × Di<sub>a</sub>/Di<sub>a</sub>* and its reciprocal, *Di<sub>o</sub>* and *Di<sub>a</sub>* segregated. In the reciprocal cross a very small number of normals were produced. From a preliminary experiment it is inferred that the phenotype of  $\frac{Di_a}{+} \frac{Di_o}{+}$  exhibits normal appearance. At any rate, it is clear from this experimental result that *Di<sub>a</sub>* and *Di<sub>o</sub>* form a complex locus.

5) Complex *Nl-U-Di* loci.

Several genes *U*, *U<sup>Br</sup>*, *Nl<sub>1</sub>*, *Nl<sub>2</sub>*, *Di<sub>o</sub>*, *Di<sub>a</sub>* and *oa*, most of which give

similar effects, form a pseudoallelic series, and they occupy a certain length of the chromosome XIV. As in multiple alleles, recombinants cannot be obtained from the crosses among them except in very rare cases. Their function is related to morphogenesis in an early developmental stage and some of them exhibit complete or partial recessive lethal action.

It has been known that terminal fusion between chromosomes VI and XIV occurs under natural conditions (Itikawa '52). This fact shows some intimate relation between these chromosomes. Both *E* and *Nl-U-Di* pseudoallelic genes which are located on each of the two chromosomes VI, XIV, resemble one another and give similar phenotypic effects. There may be some relation between these two gene series. However, we can not say anything certain without further investigation on this point.

### 17. Genetical and Biochemical Studies on the Yellow Lethal Silkworm VI. Chitin Production and Melanin Metabolism

(By Mitsuo TSUJITA and Bungo SAKAGUCHI)

1) *Chitin*:—In the yellow lethal larvae, the cuticular layer of the hypodermis, especially that of the mandible, does not develop properly, so that they cannot chew mulberry leaves and starve to death. As the amount of chitin contained in the cuticular layer of the larval hypodermis may be regarded as indicator of hardening of the cuticular layer, measurements of the amount of chitin contained in normal and mutant larvae were carried out by FRAENKEL'S method (1947). The experimental results are given in Table 1.

Table 1. Amount of chitin

Strains	Dry weight of hypodermis (mg)	Dry weight after 4.8 hours at 100°C in 5% KOH (mg)	% of Chitin
+	96.0	14.01	14.6
<i>lem</i> <sup>1</sup>	77.5	8.01	11.3

As shown in this table the production of chitin in the yellow lethal larvae is much smaller than in the normal larvae.

2) *Phenolase and phenol substances*:—By using a WARBURG manometer, the phenol oxidase activity of the epidermal tissue in the larvae was measured directly after the 1st moulting of + and *lem*<sup>1</sup> larvae. The following table shows the experimental results.

Table 2. Phenol oxidase activity

Strains	Tyrosinase		Dopa oxidase	
	O <sub>2</sub> uptake mm <sup>3</sup> /hour	Relative activity	O <sub>2</sub> uptake mm <sup>3</sup> /hour	Relative activity
+	37.8	100.0	107.5	100.0
<i>lem</i> <sup>1</sup>	32.5	86.0	75.3	70.0

A weak activity of tyrosinase was detected in the mutant larvae and the dopa oxidase activity in the normals was much stronger than in the *lem*<sup>1</sup> larvae.

Paper chromatography was used for the separation of phenolic substances and their nature was examined by the Diazo reaction and by some other methods. The experimental results obtained are given in the following table.

Table 3. Detection of phenolic substances in larval hypodermis

Strains	Tyrosine 0.45*	Dopa 0.3*	Unknown substances 0.1*
+	±	-	+
<i>lem</i> <sup>1</sup>	±	+	+

(developing solvent: 4 Butanol:  
1 acetic acid:1 H<sub>2</sub>O)

\* Rf. value

Tyrosine and dopa substances could not be detected in the normals but in the yellow lethal larvae a trace of tyrosine and a large amount of dopa could be found.

The experimental results of Table 1 and 2 suggest that in the mutant larvae a slight block exists at the step of tyrosine → dopa and a considerable block at the step dopa → x substance in the sequential reactions of melanin formation.

### 18. Genetical and Biochemical Studies on the Metabolism of Pteridines in Insects

(By Saburo NAWA, Bungo SAKAGUCHI and Toshifumi TAIRA)

In regard to the mechanism of pterin metabolism in insects such as *Drosophila* and silkworm, the following scheme has been considered by the authors:



Xanthopterin-B  $\rightarrow$  2-amino-4-hydroxypteridine-6-carboxylic acid  $\rightarrow$  2-amino-4-hydroxypteridine (AHP)  $\xrightarrow{A}$  Isoxanthopterin.

These pterin compounds in the eye-color mutants of *Drosophila* are closely related to the formation of red pigment in eyes. Furthermore, it has been shown from genetical and biochemical studies that the gene lemon lethal (*lem<sup>l</sup>*) in silkworm works in close relation to the metabolism of these substances.

LOWRY et al. (1949) have reported that xanthine oxidase-preparations from the milk of mammals were capable of catalyzing the reaction designated as *A* in the scheme. In both *Drosophila* and silkworm, an enzyme oxidizing AHP to isoxanthopterin was found and its nature investigated.

The enzyme in the silkworm was found during its whole life-cycle from egg to adult. Since a large number of silkworm eggs were available, they were used as the material for this experiment. Eggs of a normal strain of silkworm were homogenized with 0.1M phosphate buffer at pH 7.5, and the preparation was centrifuged. The supernatant solution was adjusted to 0.6 saturation with ammonium sulfate. The precipitate obtained from the centrifugation was dissolved in phosphate buffer. After incubating the enzyme solution at 37° for 4 hours with crude trypsin, the digestion mixture was dialyzed with running water for 2 days. The dialyzed fluid was reprecipitated by 0.45 saturation with ammonium sulfate. The centrifuged precipitate was dissolved in water, and the unsolved material was removed. The enzyme solution obtained by the above procedure loses as much as about 30 to 40 per cent of its activity after being kept at 5° for 24 hours. The enzymatic oxidation of AHP to isoxanthopterin (reaction *A*) was measured by the increase of optical density at 335 m $\mu$ , while that of xanthine to uric acid (reaction *B*) was measured at 290 m $\mu$ . This enzyme can catalyze both of the reactions *A* and *B* shown in the above scheme.

In reactions *A* and *B*, the aerobic activity of the enzyme was only a few per cent of what it was in the presence of methylene blue. Since the enzyme found in silkworm is not autoxidizable, it must be fundamentally different from the xanthine oxidase found in milk or in mammalian tissues, which is autoxidizable. Westerfeld *et al* (1955) have reported that the xanthine dehydrogenase found in bird tissues, such as chicken liver, was not autoxidizable to any significant extent. Thus, the enzymes from insects and birds appear to be similar.

To measure the relative ratio of reaction velocity under the optimal condition for each substrate, the pH optima were determined (Fig. 1). Using milk oxidase, the pH optima with AHP and xanthine as the substrate were in the neighborhood of 5.5 and 8.5, respectively. The maxi-

mal rate of xanthine oxidation for milk enzyme was about 5 times as high as that of AHP oxidation, while silkworm enzyme oxidized AHP at

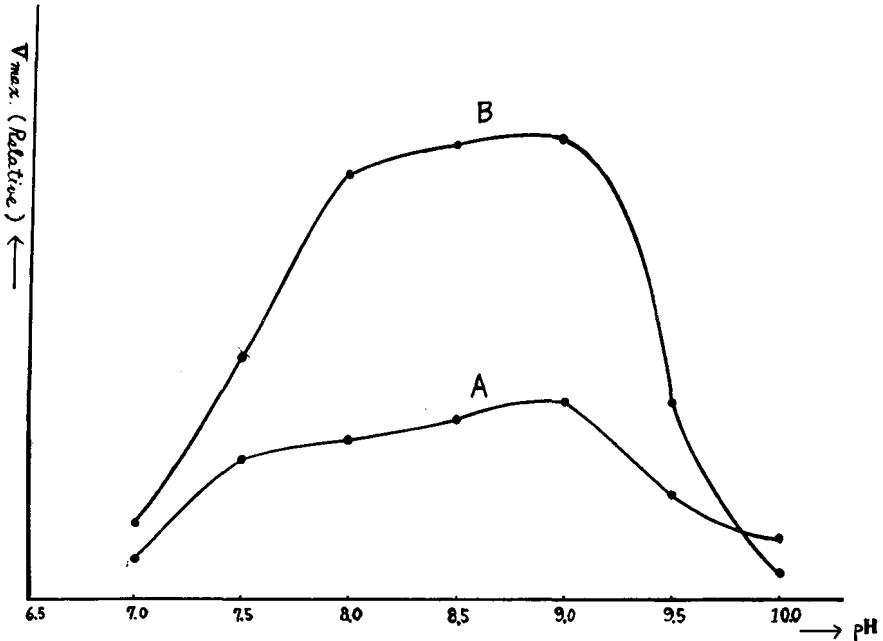


Fig. 1. The pH optima with AHP (A) or xanthine (B) as substrate in the presence of methylene blue.

approximately the same rate as that of xanthine oxidation in the presence of methylene blue. These results support the hypothesis that pterins found in insects have a more important function than those in mammals.

In *Drosophila*, the eye-color mutants seem to have different enzymatic activities, although the relation between the enzyme and other substances has to be further considered. For example, the experimental results for the enzyme, obtained from the supernatant of homogenized prepupae of *bw*, *sed*, and wild type strains in *Drosophila*, were as follows. The participation of methylene blue in the reaction increases the oxidative velocity in both reactions A and B. The crude enzyme preparation from wild type or *bw* strain catalyzed the reaction to some extent even if methylene blue was absent, while that from *sed* strain did not. This may be because the *sed* strain has little electron acceptor *in vivo*. So far as we know, the *sed* strain has a large quantity of xanthopterin-B in its testis, and also has some other special properties in regard to pterins.

This evidence suggests that there may be a close relationship between the metabolism of pterins and that of purines. Thus, the mutants lemon (*lem*) and *lem*<sup>1</sup> in silkworm have a large quantity of xanthopterin-B in larval epidermis, and the strain homozygous for *lem*<sup>1</sup> comes to death because it cannot eat mulberry leaves at first molting stage. The phenomena mentioned above will be better understood through further studies of the metabolism relating to purines and pterins.

19. *Microdetermination of Uric Acid in Drosophila melanogaster during Metamorphosis*

(By Toshifumi TAIRA and Saburo NAWA)

It has been assumed that the red eye pigment of *Drosophila melanogaster* could be traced back to pteridine derivatives (by Heyman et al, Hadorn et al, Forrest and Mitchell, Viscontini et al, and also the present authors), and that pterin may come from the conversion of uric acid (by Forrest and Mitchell).

The writers have been engaged in the study of the relationship between pigment formation and pterin metabolism on the basis of this hypothesis. During this year, microdetermination of uric acid in eye-color mutants of *Drosophila melanogaster* was conducted by the spectrophotometric method using uricase.

Fifty individuals, from larval to adult stage, of eye-color mutants, namely Oregon-2, *v*, *bw*, *se*, *sed* and *w*, were used as materials. After homogenizing the materials in a solution of glycine buffer (pH 9.4), the solution was boiled on a water-bath for five minutes at 100°C, and then centrifuged at 4,000 r.p.m. for two minutes. 0.5 ml. of the supernatant was mixed with 4.5 ml. of the uricase solution, which was obtained by dissolving 4 mg uricase crystalline in 100 ml. of glycine buffer.

The test solution was examined spectro-photometrically at the wave length of 292 m $\mu$ , and it was incubated for four hours at 37°C. Then the optical density was measured again by the same method. The quantity of uric acid was computed from the values of  $\Delta E$  thus obtained. The results are shown in Table 1.

The data in Table 1 suggest that (a) the quantity of uric acid in various mutants reaches the maximum at the stage of postpupae, (b) the mode of increase and decrease in quantity of uric acid in both *bw* and *w* strains, which have only a small amount of pterin compounds, is similar to that found in other strains having many pterin derivatives, and (c) there is no direct metabolic relation between uric acid and pterin.

Table 1. Quantitative change of uric acid in eye-color mutants during metamorphosis ( $\gamma$ /mg).

Strains	Third Larvae	Pre-Pupae	Mid-Pupae	Post-Pupae	Pre-Adults		Young Adults ( $\delta$ )
					( $\delta$ )	( $\varphi$ )	
Or-2	0.4	3.1	4.0	3.8	2.3	1.7	1.1
<i>v</i>	0.4	1.9	3.1	3.0	1.9	1.5	1.2
<i>bw</i>	0.6	2.6	2.8	4.7	2.6	2.4	0.8
<i>se</i>	0.4	2.0	2.8	3.0	1.9	1.8	0.7
<i>sed</i>	0.4	3.0	3.4	4.7	3.6	3.3	2.1
<i>w</i>	0.4	2.2	3.5	3.9	5.4	5.3	1.1

## E. GENETICS AND CYTOLOGY OF CEREAL CROPS AND RELATED PLANT GROUPS

### 20. *Studies on the Substitution and Restoration of the Nucleus*

(By Hitoshi KIHARA)

Two species belonging to two different genera were used in this study, namely *Aegilops caudata* and *Triticum vulgare*. Substitution and restoration of the nucleus were effected by back-crossing the reciprocal hybrids between the two species to *T. vulgare* as the male parent.

The first back-crosses ( $SB_1$  and  $RB_1$ ) had 36-52 somatic chromosomes. 49-chromosome plants were found most frequently. The  $B_1$ -plants were again pollinated with *vulgare*-pollen.

So far we have obtained  $SB_8$ - and  $RB_8$ -strains which had invariably 21 bivalents. Seed-fertility of both strains was normal, while pollen-fertility was nil in  $SB_8$  and perfect in  $RB_8$ .

At least two characters (non-waxy and black ear) were transmitted to the  $SB$ - and  $RB$ -strains from *Ae. caudata*, either by crossing-over, or by exchange of chromosomes during the meiosis in  $B_1$ .

### 21. *Cytogenetical Studies on $F_1$ Hybrids of Agropyron with Triticum and Aegilops*

(By Seiji MATSUMURA, Mikio MURAMATSU and Sadao SAKAMOTO)

The genus *Agropyron* comprises three sections, namely *Euagropyron*, *Elytrigia* and *Roegneria*. Intrasectional hybrids are easily obtained, but

intersectional crosses are difficult. In section *Roegneria*, tetraploid *A. yezoense* has a genome constitution homologous with that of *A. ciliare*, but their hybrid is completely sterile. Also, in section *Elytrigia* all 3 genomes of hexaploid *A. trichophorum* and *A. glaucum* are homologous.

Hybridization experiments between *Triticum* and *Agropyron* showed that the easiest crosses were those between the Emmer group and *Elytrigia* section, while crossing with the Einkorn group was very difficult.  $F_1$  hybrids were obtained between *A. glaucum* and several *Triticum* species, i.e. *T. polonicum*, *T. durum*, *T. dicoccum* and *T. pyramidale*. They were nearly or completely sterile. At MI varying conjugation patterns from  $3_{II}+29_I$  to  $12_{II}+11_I$  were observed and the mode of bivalents was at 7-8. The  $F_1$  hybrid between *T. vulgare* and *A. glaucum* was completely sterile and showed various kinds of chromosome conjugation from  $8_{II}+26_I$  to  $13_{II}+16_I$ , with the mode at  $11_{II}$ . Judging from the occurrence of 7-11 bivalents in these hybrids, allosyndesis of several chromosomes between the genomes of *Triticum* and those of *Agropyron* may be assumed, and moreover, the possibility of autosyndesis of a few chromosomes between the genomes of *A. glaucum* or *Triticum* is indicated.

Crossing of *A. glaucum* with three species of *Aegilops*, i.e. *Ae. longissima*, *Ae. cylindrica* and *Ae. variabilis*, was successful. All hybrids were completely sterile. Various kinds of chromosome conjugation were observed from  $1_{III}+5_{II}+15_I$  to  $10_{II}+8_I$  in the hybrid with *Ae. longissima*, from  $2_{III}+4_{II}+21_I$  to  $1_{III}+9_{II}+14_I$  in that with *Ae. cylindrica* and from  $5_{II}+25_I$  to  $10_{II}+15_I$  in the hybrid with *Ae. variabilis*. It is noteworthy that in all hybrids the average chromosome conjugation amounted to 6-7 bivalents suggesting autosyndesis between the genomes of *A. glaucum*. The wide fluctuation in the number of bivalents indicates the occurrence of some allosyndesis between the genomes of the tested *Aegilops* species and *A. glaucum*.

## 22. Complementary Dominant Lethal Genes in Rice

(By Hiko-Ichi OKA)

Cross combinations showing hybrid inviability, though a rare feature among cultivated varieties of rice, were found among several varieties from India, P.T.B. 10, P.T.B. 7, and others. In their hybrids, the seeds germinated normally, and the seedlings also grew normally up to the development of the fifth leaf. Subsequently, deterioration of chlorophyll and retardation of growth set in, resulting ultimately in the death of the plants, unless the environment was favorable. The same cross combinations at

the tetraploid level showed a similar behavior. On crossing these varieties with various others, it was found that this phenomenon was noticed only among about ten Indian varieties, which could be grouped in two classes separated by this hybrid inviability from each other.

For studying the genetical basis underlying this phenomenon, two varieties, A and B, whose hybrid is inviable, and a third variety C which produces normal hybrid plants with either A or B, were crossed in the order  $(A \times C) \times B$ , or  $(B \times C) \times A$ . The offspring showed a 1:1 ratio of normal and inviable plants. Reciprocal crosses gave similar results.

It may be inferred, therefore, that a set of complementary dominant lethals,  $L_1$  and  $L_2$ , govern this hybrid inviability, and the parental varieties A, B and C are of such genotypes as  $L_1+$ ,  $+L_2$  and  $++$ , respectively.

### 23. *Genetic Basis of Hybrid Break-down in Rice*

(By Hiko-Ichi OKA)

When distantly related varieties of rice are crossed, it is often found that, though the  $F_1$  plants grow normally, showing hybrid vigor, a part of the  $F_2$  plants show a poor growth, death or sterility, possibly due to non-adaptive recombinations of parental genes. In a typical case of this kind—a cross between 451 (an Indian variety, Continental group) and 521 (a Japanese variety, Temperature-Insular group)—, a genetic experiment was conducted to investigate this phenomenon. In this cross, though the  $F_1$  showed a vigorous growth, about one third of the  $F_2$  plants became yellowish at the beginning of the tillering stage, and did not grow any further.

The  $F_1$  of  $451 \times 521$  (or  $521 \times 451$ ) was back-crossed to the parents, and the progeny was examined. It was found that the ratio of normal to inviable plants was 3:1. The  $F_2$  ratio was judged to be 11:5. There was no difference between reciprocal crosses. These segregation ratios may be explained by assuming that a set of duplicate genes  $A_1$  and  $A_2$  are concerned, and that at least two dominant A's, i.e.,  $A_1A_1$ ,  $A_2A_2$  or  $A_1A_2$ , are necessary for normal growth. According to this assumption, the parental varieties may be considered to be of  $A_1A_1a_2a_2$  and  $a_1a_1A_2A_2$  constitutions.

These duplicate genes may be called "complementary recessive lethals," since the inviability results from the interaction of the recessive alleles.

In order to test the validity of this theory, 521 was crossed with a variety 647, which gives no aberrant hybrid progeny either with 521 or with 451, and the  $F_1$  so obtained was again crossed with 451. In the first generation

Table 1. Segregation of normal and inviable plants in  $F_2$  and back-crosses of  $451 \times 521$ .

Cross	Normal	Inviabile	Exp. Num.	$\chi^2$
			(11 : 5)	
451 $\times$ 521	42	18	41.25 : 18.75	0.044
521 $\times$ 451	47	23	28.13 : 21.87	0.085
	89	41	89.38 40.62	0.005
			(3 : 1)	
(451 $\times$ 521) $\times$ 451	32	15	35.25 : 11.75	1.199
" $\times$ 521	47	18	48.75 : 16.25	0.251
(521 $\times$ 451) $\times$ 451	26	9	26.25 : 8.75	0.010
" $\times$ 521	41	16	42.75 : 14.25	0.286
	146	58	153.0 51.0	1.281

of this cross, all plants grew normally. In the second generation, four out of nine families showed a 11:5 segregation of normal and non-viable plants. This may be explained without difficulty by the former assumption of genes if 647 had  $A_1A_1A_2A_2$ .

#### 24. Hypothesis of "Duplicate Fertility Genes" in Rice

((By Hiko-Ichi OKA))

The writer has pointed out earlier that in some hybrids between distantly related varieties of rice, though the  $F_1$  was completely fertile, sterile plants occurred in the  $F_2$ , and that this phenomenon could not be explained by the hypothesis of Gametic-Development genes, but might be explained by assuming duplicate genes whose double-recessive combinations lower the fertility of the carrier. An experiment to support this view was conducted, results of which are reported in this paper.

The varieties crossed, 1 add 719, both belonging to the continental (Indica) group, are completely self-fertile. Their  $F_1$  was also completely fertile in pollen formation as well as in seed set. However, sterile plants were found in the  $F_2$  generation; the  $F_3$  lines derived from these sterile plants appeared to be either pure for sterility or segregated into plants with varying fertility, as shown in Table 1.

It was found that in the sterile lines, the environmental variance for fertility was more than four times that found in other sterile rice plants. This sensitivity of environmental factors may suggest that these sterile

lines have a low seed-setting capacity. This may account at least partly for their wide variation in fertility, as shown in Table 1.

Table 1. Variation in fertility of two rice varieties, 1 and 719, their hybrids and back-cross progeny.

Generation	Fertility in parent	% of seed setting									Num. of plants studied	
		90	80	70	60	50	40	30	20	10		0
P <sub>1</sub> (1)		9	31	11								51
P <sub>2</sub> (719)		2	4	3	1							10
F <sub>2</sub>	90%(F <sub>1</sub> )	2	5	6	20	9		6	5	12	3	68
	(Exp.—11 : 5)			42 46.7					26 21.3			$\chi^2=1.89$ P>0.1
a F <sub>3</sub> line	32%		1	2		3	6	3	1		1	17
a F <sub>4</sub> line	40%		2	7	11	13	13	12	5	4		67
F <sub>3</sub> × P <sub>1</sub> F <sub>1</sub>				1	1	2	1					5
" F <sub>2</sub>			5	5	9	11	6	1		1	1	39
	(Exp.)		9.2	7.0	5.7	6.0	5.3	3.4	1.6	0.6	0.1	$\chi^2=9.94$ P>0.05
F <sub>3</sub> × P <sub>2</sub> F <sub>1</sub>				1	2	1	1					5
" F <sub>2</sub>		5	20	9	17	16	14	16	8	3	1	109
	(Exp.)	4.2	17.4	14.6	14.7	15.3	17.7	13.6	7.3	2.9	1.3	$\chi^2=4.28$ P>0.50

A few F<sub>3</sub> plants with low fertility were back-crossed to the parental varieties, and variation in fertility in the first and second generations were observed. The results are set out in Table 1. The back-cross hybrids were semi-sterile. In the second generation, though fertility appeared to vary continuously, the frequency of fertile plants could be judged to be about one fourth. The segregation ratio was then explained to be 1:2:1, and the distribution of fertility was computed under the assumption that the variations due to causes other than the genes under consideration, follow a normal distribution with the same standard deviation as that of the fertile parental varieties in fertile plants, and that of the semi-sterile lines in semi-sterile plants. As shown in Table 1, the observed distributions gave good fits to the expected one thus computed. The second generation of the back-crosses may then be regarded as showing a 1:2:1 or 1:3 ratio.

The results of this crossing experiment may lead to the following assumption of underlying genes: A set of duplicate genes, A<sub>1</sub> and A<sub>2</sub> (on different loci, independent from each other), are concerned, and plants with two or more dominant A's (A<sub>1</sub>A<sub>1</sub>, A<sub>2</sub>A<sub>2</sub> or A<sub>1</sub>A<sub>2</sub>) are fertile, while plants with one or none have a low fertility. The parental varieties, 1



and 719, might have such genotypes as  $A_1A_1a_2a_2$  and  $a_1a_1A_2A_2$  respectively. Their  $F_1$ , carrying  $A_1a_1A_2a_2$ , would be fertile, but the  $F_2$  would segregate into 11 fertile and 5 semi-sterile plants. The  $F_3$  sterile lines (not showing a segregation) would have  $a_1a_1a_2a_2$  constitution, and a fertile to 3 sterile plants segregation would result if back-crossed to either parent.

The assumed role of the duplicate genes is exactly the same as that proposed in the foregoing paper regarding inviability of a part of  $F_2$ . In both cases, it was assumed that two dominant alleles of a set of duplicate genes were necessary either for normal growth or for seed setting. It however appeared that there was a slight difference between  $A_1a_1a_2a_2$  and  $a_1a_1a_2a_2$  plants, the former showing a better growth or a higher fertility than the latter. Further studies may be needed to confirm the validity of this assumption. If one dominant gene was enough for maintaining viability, only the double-recessive plants,  $a_1a_1a_2a_2$ , would be inviable or sterile, giving rise to a 15:1 instead of 11:5 ratio. The writer had expected this situation when starting these experiments. Such a situation may also actually be present among the commercial rice varieties.

Duplicate genes as pointed out in this paper seem to be widely spread among rice varieties. They might bring about the so-called hybrid breakdown in hybrid populations, and might work as an isolating barrier between distantly related varieties of rice.

## 25. *Karyosystematic Studies in Poaceae, IV*

(By Tuguo TATEOKA)

An examination of chromosome configurations has been carried out in several Poaceae. In 1956, twelve species listed in Table 1 were examined cytologically. Also, some genera whose systematic position has been disputed were submitted to investigation from the standpoints of systematic cytology and anatomy.

1. Genus *Phaenosperma*—Although in several respects the external morphology of *Phaenosperma* suggests its systematic position in Eragrostoideae, this genus is more closely related to some Festucoideae than to any Eragrostoideae according to the results of the examination of chromosomes and leaf structure.

2. Genus *Garnotia*—To refer *Garnotia* to Eragrosteae or Agrostae has been conveniently adopted by many systematists, but there is no reliable basis for such views. The anatomical features of the leaf of some *Garnotia* species are clearly different from those of the typical members of Eragrosteae and Agrostae, and at the same time they are suggestive of an

Table 1. Chromosome numbers of some Poaceae.

Species	2n
<i>Brylkinia schmidtii</i> OHWI	40
<i>Diarrhena japonica</i> FRANCH. et SAV.	38
<i>D. fauriei</i> OHWI	38
<i>Astrebala lappacea</i> DOMIN	40
<i>A. pectinacea</i> F. MUELL.	40
<i>Tripogon japonicus</i> OHWI	20
<i>Mosdenia phleoides</i> STENT	40
<i>Coelachne japonica</i> HACK.	40
<i>Cleistachne sorgoides</i> BENTH.	36
<i>Themeda triandra</i> FORSK.	30
<i>Schizachne purpurascens</i> SWALLEN	20
<i>Ehrharta calycina</i> SM.	24

affinity between *Arundinella* and *Garnotia*. However, the spikelet structure of the two genera is clearly different. It seems correct to place *Garnotia* near Arundinelleae as an independent tribe, Garnotieae.

3. Genus *Thysanolaena*—The examination of leaf structure of *Thysanolaena* supports C. E. HUBBARD's (1934) opinion that this genus should be treated as an independent tribe. The observed characteristics, as well as the morphological features, show that *Thysanolaeneae* and *Arundineae* are closely related.

## F. CYTOLOGY AND GENETICS OF NICOTIANA AND SOME OTHER FLOWERING PLANTS

### 26. Cytogenetic Studies on the Genus *Nicotiana*, IX

(By YŌ TAKENAKA, Yoshiyuki AMANO and Tuguo TATEOKA)

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

1) F<sub>1</sub> of *N. gossei* (n=18) × *N. longiflora* (n=10).

At MI in PMC's of the F<sub>1</sub> of *N. gossei* × *N. longiflora*, from 0 to 3 chromosome pairs were found, with the mode at 1. The frequency of PMC's with only univalents followed that of PMC's with one bivalent and

that of cells with two bivalents totalled 20%. PMC's with 3 bivalents were very rare. KOSTOFF (1943) also found univalents or 1-2 bivalents and very rarely 3 bivalents in this hybrid combination. Considering the small number of bivalents it is difficult to determine whether the few chromosomal affinities are allosyndetic between the genomes of both parents or autosyndetic between the two genomes of *N. gossei*.

2)  $F_1$  of *N. gossei* (n=18) × *N. plumbaginifolia* (n=10).

Concerning this hybrid or its reciprocal, no report has been yet published, so far as we know. At MI in PMC's of this hybrid, the bivalents ranged from 0 to 4, with the mode at 2. PMC's with 1 and 3 bivalents were frequently observed but those with 0 and 4 bivalents were very rare. Since *N. plumbaginifolia* is a species closely related to *N. longiflora*, meiosis in  $F_1$  of *N. gossei* × *N. plumbaginifolia* should be similar to that found in  $F_1$  of *N. gossei* × *N. longiflora*. But at MI of PMC's, the former had one more bivalent on the average than the latter.

3)  $F_1$  of *N. paniculata* (n=12) × *N. tabacum* (n=24).

At MI in PMC's of this hybrid, from 0 to 4 bivalents were found, with the mode at 1. PMC's without bivalents and with 2 bivalents were very frequently observed, but those with 3 and 4 pairs very rarely.

GOODSPEED (1954) studied the same hybrid and found from 0 to 4 bivalents, with the mode at 0. His observations generally agree with our results. But in the same hybrid, KOSTOFF (1943) found usually from 2 to 12 bivalents, and one or sometimes more trivalents and multivalents. The cause of the difference between KOSTOFF's on one hand and GOODSPEED's or our own results on the other is not determined. But KOSTOFF also found that the reciprocal hybrid, *N. tabacum* var. *macrophylla* × *N. paniculata*, had most frequently 2~5 bivalents. This result approaches GOODSPEED's and our own findings.

It appears from the results of GOODSPEED and from our own observations that chromosomal affinities in this hybrid exist rather between the genomes of *N. tabacum* than between the genomes of both parents, since the number of bivalents is approximately the same as in haploid tobacco (TAKENAKA and TANAKA 1956).

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

As far as we know, no report on this hybrid has yet been published. At MI of PMC's of this hybrid, the bivalent range observed was from 1 to 8, with mode at 3 to 4. This pairing is probably due to allosyndetic affinities between the genomes of both parents. Otherwise, if we assume that *N. megalosiphon* is of amphidiploid origin like *N. tabacum*, the chromosome pairing observed in this hybrid can also be interpreted as caused by autosyndetic affinities between the genomes of each parent. The number

of bivalents found in this hybrid could be, of course, also interpreted as due to the former as well as to the latter affinities.

27. *Cytological Studies in Euphorbiaceae. I. Chromosome Numbers of Some Species Euphorbiain*

(By Shohachi SHIMOYAMA)

Chromosomes of *Euphorbia* have been examined by HARRISON (1930, 1931), MOYER (1934), PERRY (1943), and D'AMATO (1947) among others. Their studies have revealed 6, 7, 8, 9, and 10 as basic numbers for this genus.

The author's observations of somatic chromosomes of root tip cells in some species of *Euphorbia* found in Japan have shown the following results.

1) *E. maculata* L.

The materials were collected at Mishima, Shizuoka Pref. The chromosome number is  $2n=12$ . All chromosomes are J-shaped.

2) *E. pseudochamaesyce* FISCH., MEY., et LALLEM.

Plants examined were obtained at Mishima. Twenty chromosomes were counted in root tip cells; all chromosomes are of J-shape.

3) *E. pekinensis* RUPR.

This species is very polymorphic. The materials were collected at Yamanaka, Yamanashi Pref., and Nikkô, Tochigi Pref. as well as at Mishima. The chromosome numbers found are  $2n=28$  (plants at Mishima), and  $2n=56$  (plants at Yamanaka, Nikkô and Mishima). One pair of large chromosomes with a secondary constriction was found in the nucleolus plates of the individuals having  $2n=28$ . Individuals having  $2n=56$ , had two pairs of such chromosomes.

4) *E. Sieboldiana* MORR. et DENCE.

The materials were collected at Mishima. The chromosome number observed was  $2n=20$ . The chromosomes, being clearly larger than those of the above three species, were V- or J-shaped.

28. *Genetics of the Photoperiodic Behavior of the Japanese Morning Glory, Pharbitis Nil* CHOIS. II. *Sensitivity of  $F_1$  Plants to Dark Period*

(By Sadao SAKAMOTO)

In order to investigate the genetical background of photoperiodic behavior in *Pharbitis Nil* CHOIS. (a short-day plant) two strains with different responses, namely a wild strain No. 856 collected in Nepal and a strain

called Tendan from North China, have been crossed reciprocally. Tendan is more sensitive to dark period than No. 856. The sensitivity of  $F_1$  plants to dark period was measured by the position of the node on the main axis bearing the first flower bud. The materials were grown in a temperature- and humidity-conditioned greenhouse kept at 30°C and 80% relative humidity under the natural day-length in Misima (35°N). Ten experiments were carried out successively from March to December 1956.

The average positions of the node bearing the first flower bud in  $F_1$  plants and parental strains are shown in Table 1.

Table 1. Variation in the average position of the node on the main axis bearing the first flower bud under natural day-length in Misima (35°N).

No. of experiment	I	II	III	IV	V	VI	VII	VIII	IX	X
Date of sowing	Mar. 1	Apr. 1	May 1	June 2	July 3	Aug. 1	Sep. 1	Oct. 1	Nov. 2	Dec. 1
Parents										
856	2.9	4.4	∞	23.1	15.3	7.4	3.2	2.8	2.4	2.6
Tendan	2.9	2.7	3.0	4.4	5.1	3.6	3.0	2.4	2.2	1.9
$F_1$										
856 × Tendan	2.9	3.5	4.2	16.3	6.6	4.0	3.0	2.8	2.4	2.2
Tendan × 856	3.0	3.1	5.3	17.0	6.9	4.7	4.0	3.0	2.8	2.3

∞: No flower bud was found below the 20th-30th node.

There was no significant difference between the reciprocal crosses. The sensitivity of  $F_1$  plants was nearer to that of Tendan than to the intermediate values between the parents. Only in experiment IV (sown June 2), was the value found for  $F_1$  plants higher than the mean of the parental values.

Judging from this photoperiodic behavior of  $F_1$  plants, it seems that Tendan's sensitivity to dark period is prevalent over that of No. 856.

## 29. Crossing Experiments with *Citrullus vulgaris* SCHRAD. and *Citrullus Colocynthis* SCHRAD.

(By Kazuo FURUSATO and Akira MIYAZAWA)

A cross between *Citrullus vulgaris* and *C. Colocynthis* was carried out with the following results.

1. When *C. Colocynthis* was the male parent, the seeds did not develop to maturity and did not germinate. However, parthenocarpic fruits were

formed which grew to the same size as the fruits of self-pollinated *C. vulgaris*. It has been reported (Кносноо 1955) that germinating seeds had been obtained from this direction of the cross. It may be that other varieties of *C. vulgaris* behave differently in this respect.

When the reciprocal cross was made, i.e. with *C. vulgaris* as the male parent, a great majority of the seeds germinated well, and fruits of normal size were produced. The  $F_1$  hybrids obtained from this cross direction showed at first metaphase regular chromosome pairing with  $11_{II}$  (both parents have  $n=11$ ).

2. When diploid *C. vulgaris* was crossed with a tetraploid derived from the same variety, only non-germinating seeds were produced. But when diploid *C. Colocynthis* was crossed with tetraploid *C. vulgaris*, triploid germinating seeds were obtained which developed into plants of normal growth and size and yielded seedless fruits like the triploid seedless watermelons. The triploids showed at MI variable chromosome pairing with  $11_{III}$  when it was complete, and other, secondary, configurations with trivalents, bivalents, and univalents.

3. When tetraploid *C. vulgaris* was crossed with the original diploid strain, germinating triploid seeds were obtained. Also, when tetraploid *C. vulgaris* was crossed with diploid *C. Colocynthis* as the male parent, triploid seeds were produced which gave triploid plants with seedless fruits (Table 1).

Table 1. Viability of seeds and chromosome conjugation found in the above described experiments.

Cross combination	Seeds	Chromosome conjugation
<i>C. vulgaris</i> (2x) × <i>C. Colocynthis</i> (2x)	empty	
<i>C. Colocynthis</i> (2x) × <i>C. vulgaris</i> (2x)	viable	$11_{II}$
<i>C. vulgaris</i> (2x) × <i>C. vulgaris</i> (4x)	empty	
<i>C. Colocynthis</i> (2x) × <i>C. vulgaris</i> (4x)	viable	$11_{III}$
<i>C. vulgaris</i> (4x) × <i>C. vulgaris</i> (2x)	viable	$11_{III}$
<i>C. vulgaris</i> (4x) × <i>C. Colocynthis</i> (2x)	viable	

4. The  $F_2$  generation and the test cross ( $F_1 \times C. vulgaris$ ) showed a segregation represented in Table 2. *C. Colocynthis* had fruits with white flesh, white seeds and bitter taste; *C. vulgaris* had fruits with pink flesh, black seeds and sweet taste. In the  $F_1$  the flesh was white, the taste bitter and the color of seeds was intermediate.

The figures of Table 2 are small but we may assume (1) for flesh color two genes and the segregation ratio of 9:7, (2) for taste one dominant

Table 2. *C. Colocynthis* × *C. vulgaris* F<sub>2</sub> and test cross (F<sub>1</sub> × *C. vulgaris*).

Flesh color		Taste		Seed color	
White	Not white	Bitter	Not bitt.	Black and interm.	White
F <sub>2</sub> 19	17	25	11	34	2
V. cr. 18	86	50	54	104	0

gene and the segregation ratio of 3:1, and (3) for seed color two or more duplicate genes.

### 30. *Dextrality and Sinistrality in Plants*

(By F. A. LILIENFELD and Hitoshi KIHARA)

In *Medicago tuberculata* WILLD. and *M. litoralis* ROHDE, both plants with clockwise coiled (right-handed) and counterclockwise coiled (left-handed) pods occur. Both kinds are constant. Crosses between right- and left-handed plants were carried out.

In F<sub>1</sub> right dominates over left, and in F<sub>2</sub> monohybrid segregation takes place.

Ours is the first experiment of this kind carried out with plants. In the well known animal counterpart, *Limnaea peregra*, right too dominates over left but the segregation is delayed and does not manifest itself until F<sub>3</sub>, owing to the maternal influence upon coiling direction.

### 31. *Attempt to Use Disease-Resistant American Varieties in Triploidy Breeding of Sugar Beets*

(By Seiji MATSUMURA)

Triploid seeds of sugar beet were obtained in 1954 from intervarietal crosses between Hon-iku No. 398-4 $x$  (No. 4398) and three American 2 $x$  varieties with higher resistance to diseases (US 226, GW 304, GW 443). Seeds, called 3 $x$ -A and 3 $x$ -B, were collected from the 4 $x$  and 2 $x$  mother beets respectively, planted in the ratio of 3:1. Comparative studies of yield, sugar content and disease resistance were carried out in several triploid combinations and the diploid GW 443 in the experimental fields of Hokkaido in 1955 (Table 1). GW 443 was more resistant to diseases and gave a higher yield (with a 10-15% increase in sugar content) than Hon-

Table 1. Comparison of disease resistance, yield and sugar content in several triploid combinations.

Variety	Susceptibility (August)		Susceptibility (October)		Yield per Tan*		Sugar %		Sugar content per Tan*	
	** Index	Rate	** Index	Rate	*** Kin	Rate	%	Rate	*** Kin	Rate
1) GW 443	0.17	100	0.99	100	5,510	100	14.93	100	820	100
2) 4398 × HI <sup>†</sup> 162	1.20	701	4.25	429	5,236	96	14.09	94	734	90
3) " × HI 399	1.43	841	4.78	483	5,145	93	14.55	97	748	91
4) " × HI 401	1.17	630	3.28	331	4,848	88	16.00	107	779	95
5) " × HI 390	1.01	595	4.06	410	5,619	102	15.36	103	866	106
6) " × HI 192	0.89	524	2.88	291	5,491	100	15.70	105	867	106
7) " × US 226	0.47	275	2.43	245	5,726	104	14.88	100	848	103
8) " × GW 304	0.22	129	0.57	58	5,824	106	14.88	100	864	105
9) " × GW 443	0.45	265	1.25	126	5,568	101	15.83	106	880	107
L. S. D. 5%	0.41		0.79		315		0.71		44	

\* Tan=ca. 0.1 ha.

\*\* Index=The larger the index, the more susceptible were the leaves.

\*\*\* Kin=0.6 Kg.

† HI=Hon-iku Number.

iku No. 192, the most widely grown variety in Hokkaido. The sugar yield of four triploid combinations of tetraploid No. 4398 with two Hon-iku Nos. 192 and 390 and two American GW 304 and 443, was rather better than that of diploid American GW 443, although the Hon-iku combinations were markedly more susceptible to diseases than GW 443.

### 32. How to Improve Seed Production of Triploid Sugar Beets

(By Seiji MATSUMURA and Sadao SAKAMOTO)

In practice, production of so-called "triploid" seeds according to a certain planting arrangement (f. inst.  $4x$  and  $2x$  mother beets mixed in the ratio of 3:1) is difficult, inconvenient and uneconomical for a large scale seed production by farmers. In order to find a simpler method to produce triploid beets, mixed sowing of  $2x$  and  $4x$  seeds in several ratios, 3:1, 5:1 and 8:1, has been studied in  $3n$ -No. 1, Hon-iku No. 398- $4x$  × No. 162- $2x$ . The somatic chromosome numbers of the offspring were determined.

Triploid seeds, obtained from mixed sowing in the ratio of 8:1, had a very low germination and could not be used in practice. There was no



significant difference in germination between the seeds obtained from mixed sowing either in the ratio of 3:1 or 5:1. The frequency of  $3x$  seedlings obtained from the so-called triploid seeds was 45.6%, 46.5% and 42.4% for the ratios 3:1, 5:1 and 8:1, respectively. The frequency of  $2x$  seedlings decreased with the increase of the numerical ratio of  $4x$  to  $2x$  seeds in mixed sowing; namely 50.4%, 44.1% and 39.4% in the same order of ratios. On the other hand, the frequency of  $4x$  seedlings showed the inverted relation, namely 4.0%, 9.4% and 18.2% in the same order. From this experiment the ratio of 5:1 gave the best results. Therefore, it is advisable to mix  $4x$  and  $2x$  seeds in the ratio of 5:1 for a larger scale production of triploid seeds by farmers.

Moreover, the relation between the frequencies of  $2x$ -,  $3x$ - and  $4x$ -seedlings and the rate of germination of triploid seeds were studied in mixed sowing using the ratio of 5:1 mentioned above. The  $2x$  seeds germinated generally earlier than the  $3x$  and  $4x$ . Among the seeds, which germinated within 6 days after sowing 73% were  $2x$ , while only 36% were  $3x$  and 40%  $4x$ . But, 63% of all  $3x$  seedlings were found from the seeds, which germinated later between the 7th and the 11th day after sowing. Thus, to thin out the small seedlings in practical cultivation of triploids, we should call the farmers' attention to this fact of late germination of  $3x$  seeds. Furthermore, to promote the percentage of  $3x$  plants from triploid seeds, selection of seeds and seedlings with the help of dominant marker genes and an increase of self-incompatibility of the parental strains should be attempted.

## G. GENETICS AND BIOCHEMISTRY OF PIGMENTS AND OTHER SUBSTANCES IN PLANTS

### 33. *Anthocyanin in the Fruit-coats of Two Varieties of Egg-plant, Burma and Black Beauty*

(By Yukihide ABE and Kanji GOTOH)

It was shown earlier that the formation of acylated delphinidin glycoside (Burma-type) was effected by a dominant gene, whose recessive allele governs the production of delphinidin 3-glucorhamnoside (Black Beauty-type). This view is based upon the results of paper chromatographic examination of the pigments of both varieties and the  $F_1$ - and  $F_2$ -generations of their hybrids.

In order to obtain further information, the anthocyanin of the fruit-coat of Burma has been isolated in a crystalline state (hair-fine needles) as picrate. A series of chemical analyses of the pigment converted into chloride has shown that it is a derivative of *p*-hydroxycinnamoyl delphinidin 3, 5-diglucoside combined with rhamnose (probably one mol.). On the other hand, the anthocyanin of Black Beauty obtained in a crystalline form has proved to be identical with delphinidin 3-glucorhamnoside (Shibata 1956).

Consequently, it may be concluded that the difference between the pigments of the two varieties of egg-plant with regard to chemical constitution is controlled by a single pair of alleles.

34. *Further Studies on Paper Chromatography of Anthocyanins:  
Examination of Glycoside Types by Partial Hydrolysis*

(By Yukihide ABE and Kôzô HAYASHI)

The method of paper chromatography of natural anthocyanin pigments was developed by BATE-SMITH (1950, 1954) and HAYASHI et al. (1953). This method proved to be effective for the identification of aglycones, but further studies were still required for identifying the types of glycosides.

In this study, several different types of glycosides, e. g. 3-monoglucoside, 3-monogalactoside, 3-glucorhamnoside, 3-glucoxyloside, 3, 5-diglucoside and acylated glycosides, were successfully separated by the additional use of several solvent mixtures. On the other hand, it has been found that a stepwise degradation of anthocyanins into lower glycosides, depending upon the nature of the original glycoside, is clearly demonstrated by paper chromatograms when the glycoside is mildly hydrolysed.

It is expected that the test of the chromatographic behavior of glycosides in different solvent-mixtures together with the above-mentioned partial hydrolysis will make the identification of the naturally occurring anthocyanins more reliable (Bot. Mag. Tokyo. 69: 577-587, 1956).

35. *Dominance Relationships of Flower Color in Varietal F<sub>1</sub> Hybrids  
of Swiss Giant Pansy, Viola tricolor maxima HORT.*

(By Toru ENDO)

Forty-two crosses were made among seven varieties of pansies differing in flower color. The parental varieties were Mont Blanc (white), Rhinegold (yellow), Raspberry Rose (reddish purple), Fire Beacon (yellowish

red), Lake of Thun (light purplish blue) and Berna (deep purple). All except the variety Berna showed almost the same pattern in the blotched parts of the lateral and anterior petals. Reciprocal crosses showed no difference in flower color.

In the cross within the acyanic group (Mont Blanc × Rhinegold), the flower color was pale yellow. This shows that yellow is incompletely dominant over white.  $F_1$  hybrids between the acyanic and cyanic groups generally showed that the anthocyanin-forming ability of the latter group was dominant over the lack of this ability in the former. In some exceptional cases, however, the dominance relationship was reversed. For instance, the yellow color of Rhinegold appeared to be dominant over the reddish color of two varieties, Alpenglöw and Fire Beacon. Among the varieties of the cyanic group, it was found that bluish was always dominant over reddish and the deeper colors were generally dominant over the fainter ones. The experimental results so far obtained are not sufficient to determine whether epistatic relations are involved or not in addition to the dominance relationship of the genes. In order of decreasing dominance, the varieties may be arranged as follows: Berna > Lake of Thun > Raspberry Rose > Rhinegold > Alpenglöw ≥ Fire Beacon ≥ Mont Blanc.

### 36. *Chemistry of the Bitter Substance in the Fruits of Citrullus Colocynthis*, SCHRAD.

(By Yoshito OGAWA, Toru ENDO and Yukihide ABE)

*Citrullus Colocynthis* is a wild species of Cucurbitaceae distributed in South Arabia and Western India. The fruits have a pronounced bitter taste to a great majority of humans. The inheritance of the bitterness was studied by other members of this Institute, the whole project being initiated and guided by H. Kihara. A chemical purification of the bitter substance was carried out.

The flesh of the fruit was mashed and treated with ether or methanol. The bitter substance could be completely transferred into either ether or methanol. After evaporation of the solvent a dark green oil remained, which tasted very bitter. The bitter substance of the oil was stable either in acidic or in alkalic medium, and stood heating up to 100°C. It was again treated with ether, and then washed in basic (2N.NaOH) and acidic (2N.HCl) media to remove impurities. In this way the bitter substance was obtained in the ether solvent. This crude material was purified by chromatography on alumina. The result is shown in Table 1.

Table 1. Chromatography on alumina of "Citbittol".

No.	Solvents	Amount of outflowing solvent (cc.)	Taste	Comment
1	Chloroform	10	None	Green fatty subst.
2	"	5	"	"
3	"	"	"	"
4	"	"	"	"
5	"	"	"	"
6	Chloroform+ether	"	"	
7	ether	"	"	
8	"	"	"	
9	ether+methanol	"	"	Colorless needle crys.
10	"	"	"	"
11	methanol	"	Bitter	Yellow oil
12	"	"	"	"
13	"	"	"	"
14	"	"	"	"
15	"	"	"	"
16	"	"	"	Colorless fine grain crys.
17	"	"	None	"
18	"	"	"	Yellow fine grain crys.



*P*-nitro-benzoyl-citbittol  
 m. p. 102°C  
 (150 ×)

The purified bitter substance was oily (b. p. 81°C), and consisted only of C, H and O. By SCHOTTEN-BAUMANN-SKRUP's method, it was crystallized as a *p*-nitro-benzoyl-ester, composed of colourless needles (m. p. 102°C from ethanol) as shown in Fig. 1.

The oily bitter substance was named "Citbittol".

## H. POPULATION GENETICS AND STUDIES ON QUANTITATIVE CHARACTERS

### 37. *Genetic Basis of Polymorphism*

(By Taku KOMAI)

Polymorphism (or morphism in Huxley's terminology) usually develops in relation to a life in a variable environment or under a variable ecological or physiological requirement, especially when the environment or requirement varies from extreme to extreme, such as, from high to low temperature, from dry to wet air, from light to dark surrounding, from concealment to display, as well as for concealment in surroundings of different colors, and mimicry to models bearing different markings. The polymorphisms are controlled by a simple genetic mechanism. The dimorphisms so far studied have been shown to be controlled by a single set of alleles which show a complete dominance-recessiveness relation. If the heterozygote has an intermediate phenotype, trimorphism may result.

Tetramorphisms seem to be controlled by two sets of para-alleles which produce allied but distinct phenotypes. Polymorphisms of still higher orders seem to be due to more than two sets of such para-alleles. Heterosis is apparently an essential property of all polymorphisms. The numerical ratio among the morphs is kept constant as long as there is no change in environment or in the physiology of the species. If any change in the latter takes place, the ratio changes accordingly. If a polymorphic species inhabits a wide area within which a climatic gradient occurs, a cline may develop in the relative incidence of the morphs and genes. The para-alleles seem to have a heterozygous inversion as the cytological back-ground.

### 38. *Statistical Studies on the Breeding Behavior of Wild Populations of *Setaria pumila**

(By Kan-Ichi SAKAI and Shinya IYAMA)

This report deals with an investigation of the breeding behavior of *S. pumila* by the method of analysis of variance. This method of investigation, which is described below, will be useful for determining the frequency of occurrence of natural cross-pollination in wild as well as cultivated plant species.

Assume that from a population of a plant species we collect  $l$  plants, each bearing a number of seeds, at random. If we raise  $m$  progenies from each of  $l$  plants and investigate  $n$  seeds from each offspring with regard to their size, we can construct the following table of analysis of variance.

Source	d.f.	Mean square	Expectation of mean square
Total	$lmn - 1$		
Between plants	$l - 1$	$M_1$	$\sigma_e^2 + n\sigma_0^2 + mn\sigma_p^2$
Between offspring within plants	$l(m - 1)$	$M_2$	$\sigma_e^2 + n\sigma_0^2$
Within offspring	$lm(n - 1)$	$M_3$	$\sigma_e^2$

From this table we can get the estimated values of  $\sigma_e^2$ ,  $\sigma_0^2$  and  $\sigma_p^2$ .

If we denote the frequency of  $A$  and  $a$  genes in a population as  $x$  and  $1-x$ , the frequency of  $AA$ ,  $Aa$ , and  $aa$  may be written as  $x-y$ ,  $2y$  and  $1-x-y$ . Let the frequency of occurrence of cross-fertilization be  $q$ , then the components of genetic variance included in  $\sigma_0^2$  and  $\sigma_p^2$  will become

$$\sigma_p^2: [(2-q)^2(x-x^2-\frac{1}{2}y)] D$$

$$\sigma_0^2: [qx(1-x)(2-q) + \frac{1}{2}y(2-2q+q^2)] D,$$

if there is no dominance, no epistasis and no linkage between the genes concerned.  $D$  in these formulas stands for genetic variance due to additive effect of genes. The value of  $y$  in a perpetuating wild population will be (NEI, 1953)

$$y = \frac{2qx(1-x)}{1+q},$$

if the population has been unaffected by the pressure of natural selection with regard to the character under discussion.

Thus we get

$$\sigma_p^2 = x(1-x)(2-q)^2/(1+q)$$

and

$$\sigma_0^2 = qx(1-x)(4-q)/(1+q)$$

and

$$q = 2 \left( 1 - \frac{1}{\sqrt{1 + \sigma_0^2 / \sigma_p^2}} \right).$$

Seeds taken at random from the wild populations of *Setaria pumila* were grown in the experimental field and the harvested seeds were measured

with regard to their length and width. The result of analysis of variance of the length and width of seeds is presented in Table 1.

Table 1. Analysis of variance of length and width of seeds in the progeny of *Setaria pumila* and the estimates of  $\sigma_e^2$ ,  $\sigma_0^2$  and  $\sigma_p^2$ .

Source	d. f.	Mean square		Expectation of means square
		Seed length	Seed width	
Between parents	19	0.25163	0.18742	$\sigma_e^2 + 15\sigma_0^2 + 54.31\sigma_p^2$
Between offspring within parents	53	0.06772	0.05916	$\sigma_e^2 + 15\sigma_0^2$
Within offspring	1022	0.00429	0.00279	$\sigma_e^2$
$\sigma_e^2$		0.00429	0.00279	
$\sigma_0^2$		0.00423	0.00376	
$\sigma_p^2$		0.00339	0.00236	

The values of  $q$  for length and width of seed computed on the basis of estimated values of variance components were 67 and 76%, respectively. It should be noted in this connection that the correlation between length and width of seed was +0.6251 in the experimental material under discussion.

### 39. Genetical Studies on the Cherry-Red Leaf in Tobacco Plants

(By Kan-Ichi SAKAI and Shinya IYAMA)

In the last issue of this Annual Report, it was stated that the cherry-redness of tobacco-leaf was a heritable character in the Bright Yellow variety. In 1956, three lines from each of six line-groups were grown for the examination of cherry-red leaf character. The examination was also made for a number of  $F_1$  hybrids between lines. The first experiment was conducted by the randomized block method with two replications, each plot containing 12 plants. The second experiment was conducted by the same method with three replications, each plot containing 15 plants. Seven leaves from each plant were harvested and, after drying, they were classified into 5 classes ranging from 0, showing no sign of cherry-redness, to 4, showing the highest degree of occurrence. The class values were weighted so as to make the ratio of the between-plant variance over the within-plant variance maximum. On the basis of this weighting, the score  $X$  was constructed for individual plants. The formula for the year 1956

was as follows:

$$X = 0x_0 + 0.383x_1 + 0.707x_2 + 0.970x_3 + 1.000x_4,$$

where  $x_i$  stands for the number of leaves within plant individuals of the  $i$ -th class of cherry-redness.

The analysis of variance of the scores showed that the variation among groups of lines was highly significant while that among lines within groups was non-significant (Table 1).

Table 1. Analysis of variance of cherry-redness of harvested leaves of 18 lines belonging to 6 groups of the Bright Yellow variety of tobacco plant (1956).

Source	d. f.	Mean square	Expectation of m.s.
Between groups	5	0.2455**	$\sigma_e^2 + r\sigma_s^2 + rn\sigma_g^2$
Between lines within groups	12	0.0145	$\sigma_e^2 + r\sigma_s^2$
Replication	1	0.0880*	—
Error	17	0.0125	$\sigma_e^2$

\*, \*\* Significant at the 5% and the 1% level, respectively.

$r$ : Number of replications.

$n$ : Number of lines per group.

$\sigma_s^2$ : Variance due to strain differences within the same group.

$\sigma_g^2$ : Variance due to group differences.

$$h^2 = \frac{\sigma_g^2 + \sigma_s^2}{\sigma_g^2 + \sigma_s^2 + (\sigma_e^2/r)} = 0.863.$$

Table 2. Analysis of variance of the cherry-redness of dried leaves of 18 lines of the Bright Yellow variety of tobacco plants obtained in two successive years, 1955 and 1956.

Source	d. f.	Mean square
Between lines	17	0.1649**
Between years	1	0.2013**
Lines $\times$ years interaction	17	0.0149
Between plants within strain within year	54	0.0156

\*\* Significant at the 1% level.



Data of 1955 and 1956 were tested statistically; the result of the analysis of variance is presented in Table 2.

The table shows that yearly effect was significant for the occurrence of cherry-red leaves, though the interaction between years and strains was insignificant. It is concluded accordingly that lines of high occurrence of the cherry-red leaf character in one year seem to be high in another year and lines of low occurrence in one year low in another.

To find dominance relations, if any, of genes responsible for the cherry-red leaf character, two groups of crossings were made. In one group, the No. 1 line which was of low occurrence of cherry-redness served as a constant parent crossed with four different lines, and in the other group, the No. 13 line which was of high occurrence served as another constant parent crossed with the same four and one other line. Crossing was made reciprocally, and the occurrence of cherry-redness in two constant parents and five different parental lines as well as in reciprocal  $F_1$  hybrids are presented in Table 3.

Table 3. Occurrence of cherry-red leaf character in various  $F_1$  hybrids among different lines of the Bright Yellow variety.

Group	Crosses		Reciprocals		Average	Constant parent	Crossed line	Mid-parental value
	Mating	In-cidence	Mating	In-cidence				
(1)	(1) × (7)	0.3068	(7) × (1)	0.1833	0.2451	0.0832	0.4811	0.2822
	(1) × (15)	0.4152	(15) × (1)	0.1461	0.2807	0.0832	0.4513	0.2668
	(1) × (13)	0.1409	(13) × (1)	0.2113	0.1761	0.0832	0.2049	0.1441
	(1) × (22)	0.0490	(22) × (1)	0.0272	0.0381	0.0832	0.0467	0.0650
	Average	0.2280		0.1420	0.1850	0.0832	0.2960	
(13)	(13) × (1)	0.2957	(1) × (13)	0.3334	0.3146	0.2049	0.4811	0.3430
	(13) × (15)	0.3909	(15) × (13)	0.2705	0.3307	0.2049	0.4513	0.3281
	(13) × (1)	0.2113	(1) × (13)	0.1409	0.1761	0.2049	0.0832	0.1441
	(13) × (22)	0.0737	(22) × (13)	0.1846	0.1292	0.2049	0.0467	0.1258
	(13) × (29)	0.1362	(29) × (13)	0.1274	0.1318	0.2049	0.0392	0.1221
Average	0.2216		0.2114	0.2165	0.2049	0.2203		

The analysis of variance of data showed that statistically significant differences were found between different hybrid combinations and that, in the (1) group, there was a significant difference between reciprocals though no definite maternal effect was established. The data listed in Table 3 show that only a slight dominance effect, if any, was present.

40. *Change of Genetic Variance in Quantitative Characters Due to Change of Gene Frequency in Hybrid Rice Populations*

(By Hiko-Ichi OKA)

In populations of hybrids between distantly related varieties of rice, it is often found that the relative frequency of allelic genes is modified due to gametic and zygotic selection. It may then be surmised that polygenes for quantitative characters are also influenced, resulting in a change in genetic variances of those characters.

Let us consider an allele pair  $Aa$  which contribute increments  $d$ ,  $h$  and  $-d$  in  $AA$ -,  $Aa$ - and  $aa$ -plants respectively. Since selection for heterozygotes is not so important in autogamous plants, we may assume for the sake of simplicity that the only role of selection is to change the relative frequency of dominant and recessive homozygotes. Then, denoting the difference in frequency between  $AA$ - and  $aa$ -plants by  $x$ , the frequencies of  $AA$ -,  $Aa$ - and  $aa$ -plants in  $F_n$  will be written as

$$\frac{1}{2} - \frac{1}{2^n} \pm \frac{1}{2} x, \frac{1}{2^{n-1}} \quad \text{and} \quad \frac{1}{2} - \frac{1}{2^n} \mp \frac{1}{2} x.$$

Considering many similar genes with additive effects, the mean value of the population from the mid-parent point will be  $1/2^{n-1} Sh \pm Sdx$ , and the variance can be written as

$$\left(1 - \frac{1}{2^{n-1}}\right) Sd^2 - S(dx)^2 + \left(\frac{1}{2^{n-1}} - \frac{1}{4^{n-1}}\right) Sh^2 \pm \frac{1}{2^{n-2}} Sdhx.$$

Since the effect of selection becomes manifest in later generations where the effect of dominance is much reduced, the assumption that  $d$ ,  $h$  and  $x$  have no co-variation among one another may not lead to a serious error. Under this assumption,  $Sdhx$  can be assumed to be zero. Assuming further that  $d^2$  and  $x^2$  are not correlated,  $S(dx)^2$  can be regarded as equivalent to  $\bar{x}^2 Sd^2$ . Substituting  $D$ ,  $H$  and  $X$  for  $Sd^2$ ,  $Sh^2$  and  $\bar{x}^2$  respectively, and denoting the environmental variance by  $E$ , the variance can be written as

$$\left(1 - \frac{1}{2^{n-1}} - X\right) D + \left(\frac{1}{2^{n-1}} - \frac{1}{4^{n-1}}\right) H + E.$$

Thus, the decrease of genetic variance due to the change of gene frequency can be expressed simply by  $XD$ . From this, formulas for different hybrid populations may be derived as follows:

$$V_{F_2} = \frac{1}{2}(1-2X_{F_2})D + \frac{1}{4}H + E_1 = \frac{1}{2}D_{F_2} + \frac{1}{4}H + E_1$$

$$V_{F_3} = \frac{1}{2}D_{F_2} + \frac{1}{16}H + E_2$$

$$\bar{V}_{F_3} = \frac{1}{4}D + \frac{1}{8}H + E_1 = \frac{1}{4}\left(\frac{1}{1-2X_{F_2}}\right)D_{F_2} + \frac{1}{8}H + E_1$$

$$V_{F_4} = \frac{3}{4}\left(\frac{1-4/3X_{F_3}}{1-2X_{F_2}}\right)D_{F_2} + \frac{3}{64}H + E_2$$

$$\bar{V}_{F_4} = \frac{1}{8}\left(\frac{1}{1-2X_{F_2}}\right)D_{F_2} + \frac{1}{16}H + E_1$$

These formulas indicate that, if the genetic variance found in  $F_2$  is taken as the standard, the mean variances of families in  $F_3$  and later generations appear to be larger than the values commonly expected. The variances of family means may, however, appear to be smaller, since the values of  $X$ 's may have a tendency to increase markedly with the advance of generations.

Table 1. Observed and expected values of variances, with and without the effects of selection being considered (heading date).

Generation	Observed	Expected, Selection being	
		Considered	Not considered
$V_{F_4}$	35.63	40.13	54.83
$W_{F_3/F_4}$	44.68	40.17	54.87
$\bar{V}_{F_4}$	16.28	18.54	13.29
$V_{F_5}$	35.11	38.77	63.36
$W_{F_4/F_5}$	42.13	38.47	63.05
$\bar{V}_{F_5}$	12.64	10.93	8.30
$V_{F_6}$	25.15	29.94	67.57
$W_{F_5/F_6}$	33.69	29.41	67.04
$\bar{V}_{F_6}$	9.21	7.12	5.81
Sum of squares of deviations		121.11	4,659.80

The Pei-ku × Taichung No. 65 cross was propagated in bulk up to  $F_5$ , and about 40 lines were taken at random from each generation. They were arranged, together with plots belonging to  $F_2$  and the parental varieties, according to the randomized block design, and various agronomic characters

were measured on a single plant basis. The variance components,  $D$ ,  $H$  and  $E$ , of heading date were partitioned by the method described by Mather, using the data for  $F_2$  and  $F_3$ . The observed values of variances gave good fits to the expected ones. However, having put those values of  $D$ ,  $H$  and  $E$  into the formulas for  $F_4$  to  $F_6$ , it was found, in general, that, if the effect of selection was not considered, the observed values of mean variances of families were much larger than expected, while the observed values of variances of family means were smaller than expected. This finding suggested an effect of selection. Then, by using the formulas given above, the values of  $X$ 's for successive generations were estimated. It was found that  $X_{F_2}=0.186$ ,  $X_{F_3}=0.409$ ,  $X_{F_4}=0.543$  and  $X_{F_5}=0.682$ . As shown in Table 1, the observed values of variances gave good fits to the expected ones obtained by inserting these  $X$ 's into the formulas. The analysis of variance of the deviations between observed and expected values indicated that the effect of selection, or the change of gene frequency, was highly significant.

41. *A Method to Estimate the Effect of Linkages between Polygenes in Autogamous Plants.*

(By Hiko-Ichi OKA)

When two genes  $A-a$  and  $B-b$  are linked, the recombination value being  $p$ , the genetic variance due to those genes in  $F_n$  is given by

$$\left(1 - \frac{1}{2^{n-1}}\right)(d_a^2 + d_b^2) \pm 2 \left\{ \frac{1-2p}{1+2p} - \frac{(1-2p)^n}{2^{n-1}(1+2p)} \right\} d_a d_b \\ + \left( \frac{1}{2^{n-1}} - \frac{1}{4^{n-1}} \right) (h_a^2 + h_b^2) + 2 \left\{ \frac{(1-2p+2p^2)^{n-1}}{2^{n-1}} - \frac{1}{4^{n-1}} \right\} d_a d_b,$$

where  $d_a$ ,  $d_b$ ,  $h_a$  and  $h_b$  are additive ( $d$ ) and non-additive ( $h$ ) increments due to those genes. The above formula would be too complicated to be used for numerical calculation. An attempt to transform this formula into a simpler one is reported in this paper.

It has been demonstrated by Mather (1949) that the effect of linkage on a quantitative character becomes conspicuous only when the recombination value is around 0.25. Therefore, if we assume that  $p=0.25$  in the above formula, then the effect of linkage can be approximately shown by the relative value of  $d_a d_b$  to  $d_a^2 + d_b^2$ . If we consider many sets of such linked genes, letting  $S(d_a^2 + d_b^2) = D$ ,  $\frac{2Sd_a d_b}{D} = q$ ,  $S(h_a^2 + h_b^2) = H$  and  $\frac{2Sh_a h_b}{H} = r$ ,

we have the following expressions for the various hybrid populations.

$$V_{F_2} = \frac{1}{2} (1 \pm *0.5 q) D + \frac{1}{4} (1 + 0.25 r) H + E_1$$

$$V_{F_3} = \frac{1}{2} (1 \pm 0.5 q) D + \frac{1}{16} (1 + 0.25 r) H + E_2$$

$$\bar{V}_{F_3} = \frac{1}{4} (1 \pm 0.25 q) D + \frac{1}{8} (1 + 0.1563 r) H + E_1$$

$$V_{F_4} = \frac{3}{4} (1 \pm 0.4167 q) D + \frac{3}{64} \left( \frac{2}{3} + 0.5208 r \right) H + E_2$$

$$\bar{V}_{F_4} = \frac{1}{8} (1 \pm 0.125 q) D + \frac{1}{16} (1 + 0.0976 r) H + E_1$$

$$V_{F_5} = \frac{7}{8} (1 \pm 0.375 q) D + \frac{7}{256} \left( \frac{6}{7} + 0.2790 r \right) H + E_2$$

$$\bar{V}_{F_5} = \frac{1}{16} (1 \pm 0.0625 q) D + \frac{1}{32} (1 + 0.0610 r) H + E_1$$

\* + ...coupling; - ...repulsion.

Table 1. Observed and expected values of variances, with and without consideration of the effects of linkage and selection.  
(Plant height in logarithm)

Generation	Observed values	Expected values, considering		
		Linkage & Selection	Linkage only	None of the two
$\bar{V}_{F_3}$	.021,38	.021,43	.023,16	.015,09
$V_{F_4}$	.027,42	.022,13	.022,67	.013,87
$W_{F_3/F_4}$	.014,66	.019,96	.020,52	.011,55
$\bar{V}_{F_4}$	.022,00	.022,43	.017,41	.011,38
$V_{F_5}$	.033,06	.031,63	.029,49	.014,46
$W_{F_4/F_5}$	.027,07	.028,50	.026,36	.011,39
$\bar{V}_{F_5}$	.017,34	.015,91	.013,07	.009,53
$V_{F_6}$	.031,35	.030,59	.033,41	.014,64
$W_{F_5/F_6}$	.026,18	.026,96	.029,78	.011,10
$\bar{V}_{F_6}$	.010,51	.011,93	.010,46	.008,61
Sum of squares of deviations		.000,06	.000,13	.001,51

It is usually experienced in rice that plant height, panicle length, and other dimensions in length of various organs show a significant effect of linkages in repulsion phase. (Since these characters usually involve gene interaction, a logarithmic transformation of the scale seems necessary.)

In the Pei-ku  $\times$  Taichung No. 65 cross referred to in the foregoing paper, the partitioning of variance components with respect to these characters, based on the data for  $F_2$  and  $F_3$ , was carried out, and the expected values of variances in  $F_4$  to  $F_6$  were calculated from those values of variance components. The results are shown in Table 1.

As shown in the table, under the assumption of Mendelian segregation, the observed values were found to deviate widely from the expected values. If the effect of linkage was considered by the method mentioned above, the deviations were largely reduced. By considering also the effect of selection (or of the change of gene frequency), the deviations were further reduced. It then seems that the effect of linkage can be approximately measured by the present method.

#### 42. *Genotypic Correlations Among Five Economic Characters in Egg-plant.*

(By Kanji GOTOH)

This experiment is a part of a series of investigations on the inheritance of quantitative characters in eggplant.

45 randomly selected lines, each from  $F_3$  and  $F_4$  generations of a hybrid between two varieties, Kikunaga and Sendai-naga No. 1, were grown in experimental plots arranged according to the randomized block design with two replications. Plant height, stem diameter, period from seeding to flowering, shape index of fruit and fruit number were recorded on an individual plant basis.

Analyses of variance and covariance of the data obtained were conducted on a plot mean basis, and genotypic variances and covariances were estimated for each character and for all combinations of the characters.

One of the parents, the Kikunaga variety, belonging to the late maturing variety group, was of erect growth with thick high stem, while the other parent, the Sendai-naga No. 1 variety of the early maturing variety group, was of prostrate growth with slender branches and fruits. The latter variety produced far more fruits than the former.

Analysis of the data showed that the genotypic correlations were positive and high for the following combinations of characters: plant height-stem diameter (1.006 in  $F_3$  and 0.717 in  $F_4$ ); plant height-period from seeding

to flowering (0.624 in  $F_3$  and 0.410 in  $F_4$ ); stem diameter-period from seeding to flowering (0.889 in  $F_3$  and 0.882 in  $F_4$ ). These facts suggest that such agronomically important traits as described above may be strongly governed by closely linked genes or by genes exhibiting pleiotropic effects.

Genotypic correlations between shape index or fruit number per plant on the one hand and the other characters on the other hand were rather low and probably of little genetic importance.

The genotypic correlations obtained generally showed higher values than the corresponding phenotypic correlations.

43. *Genetic Variation in Heading Date in a Local Variety of Barley, "Aoikei No. 14"*.

(By Kanji ГОТОН)

The writer has pointed out before that the variation in heading data of the barley varieties, "Hosogara No. 2" and "Iwate Mensury No. 2", are mainly due to the genetic heterogeneity of the populations. Additional data obtained with another barley variety, "Aoikei No. 14", are described in this paper. The variety was obtained from the Aomori Agr. Exp. Sta.

Date of heading was recorded on a plant basis in 1954, and in 1955 200 derived lines were grown in a randomized block arrangement with two replications. Records of heading data were taken on a line basis.

According to the data obtained, the range of variation in the date of heading was 42 days (from March 29 to May 10) in 1954 and 38 days (from March 24 to May 1) in 1955. The correlation coefficient between parents and offspring was +0.904 and was statistically highly significant, the parent-offspring regression coefficient was as high as 0.785.

From a visual inspection it was found that "Aoikei No. 14" consisted of two distinct groups. One of them was an early maturing erect type and the other a prostrate type, though within each group variability was noticed in heading date.

This variety was also heterogeneous with regard to the presence or absence of hairs on the leaf sheath, 53.3% of plants being pubescent. No association was found between hairiness and maturity. The three barley varieties so far studied ripen simultaneously when grown in localities where they are adapted. However, they showed a wide variation in ripening time when grown in Misima. One of the important environmental factors causing such a variability may be the different length of the winter days in both localities.

## I. STUDIES ON THE TECHNIQUE OF BREEDING

## 44. Method of Estimation and Use of Genetic Correlation in Forest-Tree Breeding.

(By Kan-Ichi SAKAI)

One of the most difficult problems in forest-tree breeding lies in the fact that trees often need too many years for reaching maturity. The author introduces a method of estimating the value of the genetic correlation between a character that will be expressed after a tree grows up and some more or less related character measured in a young stage of the tree. It is assumed that the trees in a population produce seeds by random pollination.

Let the phenotype of a character  $A$ , e. g. trunk size, of a grown-up tree in a population be  $P_1$ , the phenotype of some more or less related character  $B$ , e. g. growth rate, of the tree's offspring in the nursery be  $P_2$ , and the phenotype of the character  $A'$ , corresponding to  $A$  of parent tree, which will be realized after the young trees grow up be  $P_3$ . Figure 1 illustrates the relation among those three characters.

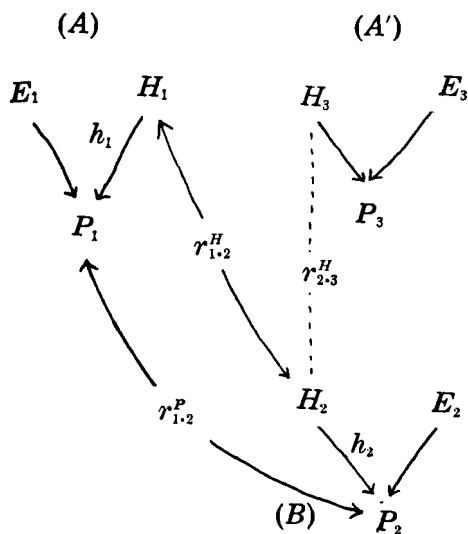


Figure 1. Path diagram illustrating phenotypic and genotypic relationships between mother trees and their offspring.

$H_i$  and  $E_i$  in the diagram stand respectively for genotype and environment taking part in the expression of the phenotype  $P_i$ .  $h_i$  stands for the square root of the heritability of the  $i$ -th character.  $r_{ij}^P$  and  $r_{ij}^H$  stand respectively for the phenotypic and genotypic correlation between the  $i$ -th and  $j$ -th characters

Then, we get

$$r_{1.2}^P = h_1 r_{1.2}^H h_2,$$

and

$$r_{2.3}^H = 2r_{1.2}^H,$$

because the parent plants are assumed to be pollinated at random in the population. Thus we come to have the equation,



$$r_{2.3}^H = \frac{2r_{1.2}^P}{h_1 h_2},$$

in which  $h_2$  is the square root of the heritability of the character of the young trees in the nursery and will be found by the method of analysis of variance. To find the value of  $h_1$  is impossible, but if we assume that the heritability of trunk size of mature trees be not much different from the heritability of growth rate of young trees, we can find the genetic correlation between  $P_2$  and  $P_3$ , making  $h_1 = h_2$ , as

$$r_{2.3}^H = \frac{2r_{1.2}^P}{h_2^2}.$$

Selection of mother-trees on the basis of characters of offspring will bring the genetic gain

$$\Delta G_3 = \Delta G_2 r_{2.3}^H \frac{\sigma_3^H}{\sigma_2^H} = i h_2^2 r_{2.3}^H \frac{\sigma_3^H}{\sigma_2^H},$$

where  $\Delta G_2$  and  $\Delta G_3$  stand respectively for the genetic gain in the character of trees after maturity and for that in the character of young trees by selection on the basis of young trees.  $\sigma_j^H$  is the square root of genetic variance of  $j$ -th character. The  $i$  is the selection differential. Though  $\sigma_3^H$  is impossible to find, the ratio  $(\sigma_3^H/\sigma_2^H)$  will take a constant value for a given population. If we assume that  $\sigma_3^H = \sigma_2^H$ , the approximate amount of genetic gain to be expected by the selection may be computed.

#### 45. *Application of the Coefficient of Relationship in the Breeding of Autogamous Plants*

(By Kan-Ichi SAKAI)

The coefficient of relationship may be useful in the choice of parental strains for hybridization in the breeding of autogamous plants. The relationship between parental strains and the pure-breeding lines derived from the cross of the parental strains is illustrated in the following table. In this case parents are assumed to be  $AABB$  and  $aabb$ . Accordingly, the relation between parents and offspring is on the average,

		Parent	
		$AABB$	$aabb$
Offspring	$AABB$	1	0
	$AAbb$	$\frac{1}{2}$	$\frac{1}{2}$
	$aaBB$	$\frac{1}{2}$	$\frac{1}{2}$
	$aabb$	0	1

$$R_{P_0} = \frac{1 + \frac{1}{2} + \frac{1}{2} + \frac{1}{2} + \frac{1}{2} + 1}{8} = \frac{1}{2}$$

Similarly, the relationship between two lines taken at random from the same cross will be on the average,

$$R_{00} = \frac{1}{2}.$$

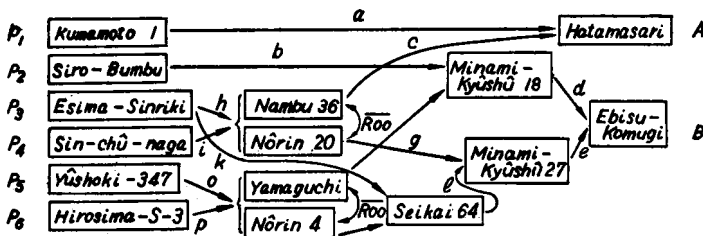
If two pure-breeding hybrid lines are selected from the same  $F_n$  line, the relationship between them will be

$$R_{00}(F_n) = 1 \times \left(1 - \frac{1}{2^{n-2}}\right) + \frac{1}{2} \left(\frac{1}{2^{n-2}}\right) = 1 - \frac{1}{2^{n-1}},$$

because the probability of a gene pair being heterozygous is  $1/2^{n-2}$  and the probability of being homozygous is  $1 - 1/2^{n-2}$ . For a new line selected from an already established variety by the method of so-called pure-line selection, the relationship between that line and its parent or the relationship between two selected lines cannot be determined exactly, but it will be in the range from 1 to  $\frac{1}{2}$ .

$$R_{P_0'} = R_{0_0'} = 1 \sim \frac{1}{2}.$$

On the basis of such relationships, we can compute the coefficient of relationship between two strains by looking into the pedigree. The following is an example showing the pedigree of two wheat varieties, *A* and *B*, explaining the method of computing the coefficient of relationship between them as well as between them and one of the parental varieties.



$$R_{AB} = c\bar{R}_{00}ge + chkle = \left(\frac{1}{2}\right)^4 + \left(\frac{1}{2}\right)^5 = 0.094$$

$$R_{P_3A} = ch = \left(\frac{1}{2}\right)^2 = 0.25$$

$$R_{P_3B} = egh + elk = 2 \times \left(\frac{1}{2}\right)^3 = 0.25.$$

The coefficients of relationship among parental materials will help the breeder to plan his crossing program.

## J. STUDIES ON COMPETITION AND MIGRATION

46. *Competition between Different Species and Species Hybrids in Nicotiana.*

(By Kan-Ichi SAKAI and Takashi NARISE)

Competitive ability of three species of *Nicotiana*, viz., *N. sylvestris*, *N. tomentosiformis* and *N. Debneyi*, and several species hybrids was tested using the Bright Yellow variety of *tabacum* as the standard. The hybrids were:  $F_1$  and amphidiploid hybrids between *tabacum* and *sylvestris*, a backcrossed progeny of the amphidiploid by *tabacum*, the  $F_1$  hybrid between *sylvestris* and *tomentosiformis*, and the amphidiploid hybrid between *Debneyi* and *tabacum*.

Pure-stand plots of Bright Yellow and mixed plots of that variety with different species or species hybrids were arranged according to the randomized block design with three replications. The mixture was made by alternate planting of plants of the test variety with those of the tested species or species hybrids. The distance between adjacent rows was 90 cm and the interhill spacing within each row was 45 cm.

Plant height, number of capsules, plant weight and number of leaves of the test variety Bright Yellow in pure-stand plots and mixtures were recorded on an individual plant basis.

Table 1. Plant weight (g) of the test variety Bright Yellow in pure-stand plot and in competitive plots with other species or species hybrids, on individual plant basis.

Competing species	Bright Yellow (control)	<i>sylvestris</i>	B.Y. $\times$ <i>sylv.</i>	B.Y. $\times$ <i>sylv.</i> Amphi-2X	B.Y. $\times$ <i>sylv.</i> Amphi-2X $\times$ B.Y.
Plant weight (g) of the Bright Yellow variety	404.9	327.3	296.3	313.9	273.8
Competing species	<i>Tomentosiformis</i>	<i>Sylv.</i> $\times$ <i>tomentosif.</i> $F_1$	<i>Debneyi</i>	<i>Debneyi</i> $\times$ B.Y. Amphi-2X	
Plant weight (g) of the Bright Yellow variety	358.8	409.2	451.2	446.5	

Analysis of variance of the data obtained showed that the competitive effect of different species hybrids on *tabacum* was statistically highly significant. Data on plant weight for Bright Yellow in pure-stand plot and mixtures are presented in Table 1 as an example.

It should be understood that species which caused Bright Yellow to have a smaller weight were stronger in competitive ability than those which allowed it to have a larger weight. Thus, the following order of species and species hybrids with regard to their competitive ability has been established:

- (1)  $\left( \begin{array}{c} \text{B. Y.} \times \text{sylv.} \\ \text{Amphi-2X} \end{array} \right) \times \text{B. Y.} > \left( \begin{array}{c} \text{B. Y.} \times \text{sylv.} \\ F_1 \end{array} \right) > \left( \begin{array}{c} \text{B. Y.} \times \text{sylv.} \\ \text{Amphi-2X} \end{array} \right) \\ > \text{sylv.} > \text{B. Y.}$
- (2)  $\text{Sylv.} > \text{Tomentosiformis} > \left( \begin{array}{c} \text{Sylv.} \times \text{tomentosif.} \\ F_1 \end{array} \right)$
- (3)  $\text{B. Y.} > \left( \begin{array}{c} \text{Debneyi} \times \text{B. Y.} \\ \text{Amphi-2X} \end{array} \right) \geq \text{Debneyi}$

The conclusion drawn from this experiment is in agreement with the previous findings that competitive ability of a hybrid in comparison with its parental species is dependent upon the genetic constitution of the parental species and that doubling of chromosomes in  $F_1$  producing an amphidiploid decreases the competitive ability.

#### 47. *Competitive Ability of Barley Varieties at Various Fertility Levels of the Soil.*

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

Competitive ability seems to be controlled by genes. It becomes then of great interest from plant breeding as well as from evolutionary stand-points to find if there is an interaction between the effect of those genes and some environmental conditions. OKA and SAKAI (1956: the last issue of the Annual Report of this Institute) described the results of their experiment on the effect of soil fertility on survival rates of two mixed rice varieties. The present report deals with the effect of various fertility levels of the soil on the expression of competitive ability of seven varieties of barley.

The seven varieties used are: Kagosima-Kobai-1, Sizuoka-Siro-6-rowed-1, Kuromugi-48, Sakigake, Kanto-Kawa-2, Suifu, and Siro-Chinko. Six fertility levels of the soil were prepared by applying the following doses

of the standard mixture: 0, 1/4, 1/2, 1, 2 and 4. Each variety was grown in a pure-stand plot and in a mixed plot with the Siro-Chinko variety which served as a test variety of the competitive ability of experimental plants. The plots were arranged according to the split-plot design with four replications, in which the main-plots differed with regard to soil fertility.

Plants in the plots with no fertilizer were damaged seriously by frost during the winter. Plants grown at the lower levels of fertility also showed some damages. Thus, the statistical analysis was made separately for each fertility level, the no-fertilizer plots being excluded from the analysis.

The analysis of variance of plant weight of the test variety "Siro-Chinko" is presented in Table 1.

Table 1. Analysis of variance of plant weight of the test variety at different levels of soil fertility.

Source	1/4		1/2		1		2		4	
	d.f.†	M.S.	d.f.†	M.S.	d.f.†	M.S.	d.f.†	M.S.	d.f.†	M.S.
Replication	2	148.1	2	23.3	3	180.5	3	59.6	3	480.2
Competition	6	69.4	6	61.4*	6	98.8	6	356.0**	6	653.5**
Error	11	44.8	10	15.2	17	59.7	17	48.2	17	125.4

† One or two missing plots are involved.

\*, \*\* Significant at the 5% and 1% level, respectively.

Table 2. Analysis of variance of plant weight, weight of ears and number of ears of the S-S-6-Rowed variety in pure-stand plots and in mixture with the test variety.

Source	d.f.	Mean square		
		Plant weight	Weight of ears	Number of ears
Replication	3	134.0	14.6	4.8
Fertility level	4	5520.7**	926.1**	384.2**
Competition (Pure: Mixed)	1	2748.5*	469.7**	77.4*
Fertility × Competition	4	319.3*	42.1	8.9*
Error	27	90.8	20.3	2.3

\*, \*\* Significant at the 5% and 1% levels, respectively.

The analysis of variance shows that the effect of competition tends to become more significant at the higher levels of soil fertility. Of the seven varieties, the variety Sizuoka-Siro-6-rowed appeared to be the strongest competitor, so the plant weight, and the weight and the number of ears per plant of this variety in pure-stand and in the mixture with the Siro-Chinko variety were analyzed statistically. The results of analysis of variance are presented in Table 2.

This table shows that the effect of competition as well as the effect of interaction between fertility and competition are statistically significant in all characters but one. Plant weight, ear weight and ear number of the Sizuoka-Siro-6-rowed variety in pure-stands and mixtures at five kinds of fertility levels are presented in Table 3.

Table 3. Plant weight and weight and number of ears per plant of the S-S-6-rowed variety in pure-stands and in the mixtures with the tester variety at 5 different levels of soil fertility.

Fertility level	1/4		1/2		1		2		4	
	Pure-stand	Mix-ture	Pure-stand	Mix-ture	Pure-stand	Mix-ture	Pure-stand	Mix-ture	Pure-stand	Mix-ture
Plant weight (g)	113.7	107.1	173.1	238.2	245.5	299.1	301.3	389.3	301.0	432.6
Weight of ears (g)	11.77	11.61	17.30	23.18	24.96	32.96	31.54	39.28	31.09	43.76
Number of ears	4.09	3.93	6.26	9.16	9.71	11.79	14.93	18.36	18.49	24.15

#### 48. *Correlation between Competitive Ability and Other Characters in Hybrid Populations of Rice*

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

The writers have been interested in the study of variation in competitive ability among varieties or strains derived from varietal hybrids of cultivated plants. This paper deals with correlations of competitive ability with other characters or gene differences in competitive ability found in hybrid populations of rice. F<sub>10</sub> strains of P. T. B. 10 (an Indian variety, Continental or Indica group) × Kinoshita-mochi (a Japanese variety, Temperate-Insular or Japonica group), and F<sub>9</sub> strains of Pei-ku (a Formosan native variety, Continental group) × Taichung No. 65 (a Horai variety of Formosa, Temperate-Insular group), were used as materials. Competitive ability was measured, in the experiment with the first cross, by the panicle number of the "tester" variety mix-planted with each strain, and,

in the experiment with the second cross, by the panicle number of each strain mix-planted with the "tester" variety; in both the cases, the difference in panicle number between the mix-planted and singly-planted plots was used as the measure of competitive ability. The field experiments were carried out in the Taiwan Provincial College of Agriculture in Taichung, Formosa.

The correlation coefficients found between the competitive ability and other various characters were as follows;

Correlation coefficients with competitive ability.

Characters	First Cross	Second Cross
Plant height	0.30*	-0.08
Panicle length	0.14	
Panicle number	0.16	-0.14
Seed number per plant	0.12	0.25*
No. of Days of growing period		0.17
Length/width ratio of grain	0.31	0.13
Weight for shedding	-0.18	0.02
Index-number of alkali-test	-0.30*	-0.16
Germination speed	0.14	0.07

\* Between the 5% and 1% levels of significance.

The correlation coefficients given above suggest that competitive ability is not correlated with any specific agronomic character. It appears that variation in competitive ability in hybrid populations may not bring about a change in quantitative characters, so far as the phenotypic measurement is concerned. However, characters which distinguish distantly related varieties generally showed a correlation with competitive ability; strains with characters similar to the varieties of the "Continental" group tended to show higher competitive abilities than those similar to the varieties of the "Insular" group. The corresponding tendency was also found between competitive ability and the degree of hybrid sterility with the parental varieties, when the strains were crossed with both parents. It was further found that strains with genes derived from a parental variety belonging to the Continental group, i. e. Ph (Phenol reaction), Rc (Red seed coat), and *c* (colorless apiculus), had higher competitive abilities than those with the genes allelic to them, derived from a parental variety belonging to the Insular group. This tendency was most marked in the seed coat coloration gene Rc,

49. *Experimental Studies on Migration Using "Population-Tubes" in a Strain of Drosophila melanogaster.*

(By Kan-Ichi SAKAI, Takashi NARISE, Yuichiro HIRAZUMI and Shinya IYAMA)

To inquire into the problem of migration in relation to competition in *Drosophila* flies, "population-tubes" were constructed. Each population tube had three radial branches protruding from its periphery, and a number of the tubes were connected with each other (See DIS 30: 166. 1956).

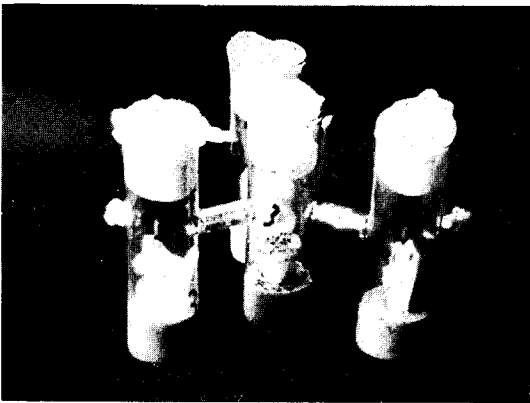


Fig. 1. Photograph showing a set of 4 population-tubes connected with each other by radial branches.

Using a set of four population-tubes as shown in Fig. 1, migration was studied with a laboratory strain of *Drosophila melanogaster*. A certain number of flies of the Samarkand strain were kept for one day in a tube, and then the tube was connected with three new tubes. One experiment dealt with the relation between the number of migrated flies and the period of time during which the migration was

allowed to take place. This experiment gave the following result.

It seems that mass migration has occurred in the first 6 hours if the number of flies in the original tube was more than 150, though the mig-

Table 1. Number of flies migrated in various periods of time after establishing connection with new tubes.

Initial no. of flies in the original tube	No. of replications	6hrs.	% of flies migrated to new tubes after					
			24	48	72	96	120	144
100-149	5	1.84	0.95	2.25	3.32	5.52	6.51	7.21
150-199	4	14.66	11.02	13.31	14.51	20.95	22.47	25.01
199-249	4	14.92	14.47	15.41	18.47	23.50	24.59	24.00
250-300	5	19.59	21.35	24.39	27.55	29.90	32.46	32.91



ration continued even after that time. Migration taking place after 24 hours through 144 hours was examined statistically. It was found that during this period the increase in the percentage of migrated flies took place approximately linearly and that there was no significant difference among the rates per day of migrating flies in four kinds of population densities. The average increase of migrated flies per day after 24 hours following the connection with new tubes was 2.25% of the population.

The other experiment was carried out to find out if the mass migration was dependent on the number of flies present in the original tube. In this experiment, the migrated flies were counted two days after the connection with new tubes. Data from this experiment are presented in Table 2.

Table 2. Relation between the percentage of migration and the number of flies in the original tube.

	Initial number of flies in the original tube					
	0-50	50-99	100-149	150-199	200-249	250-300
No. of replications	2	2	6	7	6	5
No. of migrating flies (%)	5.88	0.65	1.89	23.47	26.49	24.93

Table 2 indicates that mass migration of the Samarkand strain of *Drosophila melanogaster* occurred when the size of the original population exceeded 150. Thus, in the present experiment, two kinds of migration were found to occur: one, the so-called "mass migration", which occurs as the result of pressure of population density, and the other, the so-called "random migration", presumably the result of random movement of individual flies.

#### 50. Migration Studies in Several Wild Strains of *Drosophila melanogaster*.

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

Six wild strains of *Drosophila melanogaster* were collected from different localities in some parts of Japan. They were propagated as populations for two generations in the laboratory and were tested for their migrating

activity. In the original tube, 80, 120 and 160 flies were placed, and the number of migrated flies from the original tube was counted after 2 days. The experiment was replicated four times. Analysis of variance of data is presented in Table 1, which shows that local strains are different with regard to migrating activity.

Table 1. Analysis of variance of number of migrated flies for three densities of original population.

Source	d.f.	Mean square
Population density	2	308.98*
Local strains	5	489.31**
Density $\times$ strain	10	44.46
Replication	3	113.17
Error	51	67.48

\*, \*\* Significant at the 5% and the 1% level, respectively.

The percentage of migrated flies in six wild strains is presented in Table 2.

Table 2. Percentage of migrated flies from the original tubes at three levels of population density.

Strains	Migrating activity (%)			
	80 <sup>+) </sup>	120 <sup>+) </sup>	160 <sup>+) </sup>	Average
Tateba	39.4	40.9	42.8	41.03
Iguro	44.3	55.3	54.7	51.43
Kama	48.8	55.2	58.8	54.27
Te-sima	50.1	59.3	57.7	55.70
Isima	55.6	60.7	55.7	57.33
Katsunuma	55.0	55.8	64.8	58.53

<sup>+)</sup>  The number of flies placed at the start of the experiment in the original tube: Population density.

The migration in these wild strains was incomparably more active than in the inbred laboratory strain, Samarkand, in which the migration hardly occurred unless the number of flies in the original tube exceeded 150.

The next question to be answered was: Is there a threshold in the size of population below which the burst of migration rarely occurs? A series

of experiments each with 4 replications were again conducted making the size of population in the original tube at 10, 20, 40 and 60 flies. Analysis of variance of the data obtained in these experiments showed that the effect of population size as well as the effect of interaction between densities and strains were highly significant.

The migration percentage in relation to various sizes of population in the original tube is graphically illustrated in Fig. 1.

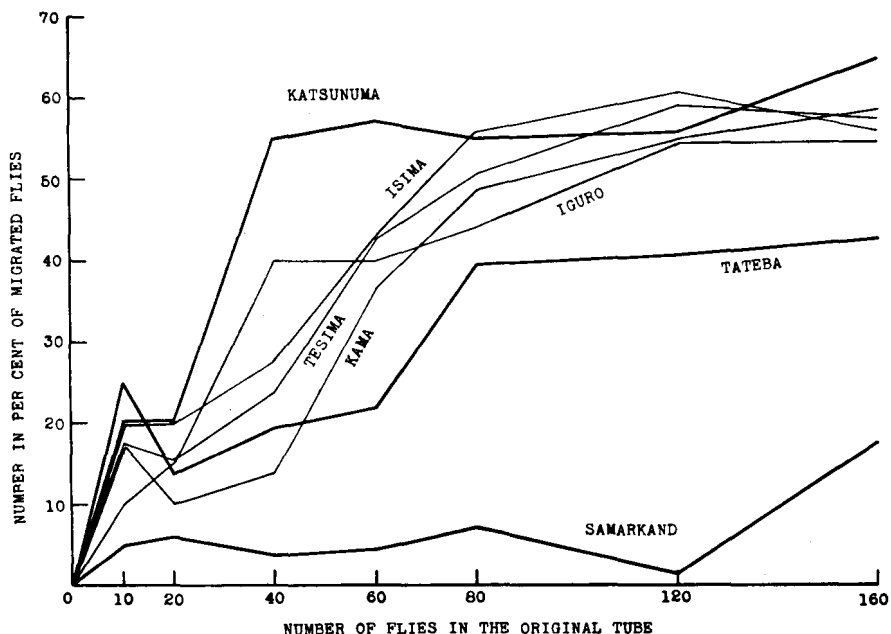


Fig. 1. Relation between migration percentage and size of population, in one laboratory stock, Samarkand, and six wild strains.

Fig. 1 shows that the thresholds in the size of population responsible for the burst of mass migration differ from strain to strain: the critical size of population was 60~80 in Tateba- and Kama-strain, 60 in Tesima- and Isima-strain, 40 in Iguro- and Katsunuma-strain.

## K. RADIATION GENETICS IN ANIMALS

### 51. *Effect of External Irradiation with $\beta$ -rays upon the Germ-Cells of the Silkworm*

(By Yataro TAZIMA)

When radioactive phosphorus is administered to the organism, a certain amount of it might become incorporated as a phosphorus constituent of chromosomes. The rate of occurrence of chromosome aberrations might be effectively increased as a consequence of the incessant attack of  $\beta$ -rays upon the chromosomes. Therefore, injection of radioactive phosphorus into the organism might be considered as an effective method for inducing mutations. However, it would be impossible to relate this effect to the amount of radiation, since the radioactive phosphorus content of the chromosomes is not constant because of metabolic activities which take place in the course of development. Another difficulty of this method lies in surveying the specific radiation effects at different developmental stages of germ-cells, since it is not possible to remove the radioactive phosphorus at a definite stage of development.

For these reasons, external irradiation with  $\beta$ -rays of germ-cells of silkworm was undertaken in order to estimate the relative sensitivity as well as mutability at different developmental stages for a definite radiation energy of a  $\beta$ -source.

An inbred strain N of Chinese bivoltine race was used in this study. This strain had the normal alleles for *pe* and *re*. Female and/or male germ-cells of N strain were irradiated by  $\beta$ -rays at different larval stages of the parents, i.e., fourth stadium, early fifth stadium and late fifth stadium. A small piece of filter paper which had been immersed in an aqueous solution of  $P^{32}$ -salt and desiccated was used as the  $\beta$ -source. This piece of filter paper was left pasted for a certain length of time on the dorsal side of the eighth abdominal segment of a larva, where the gonads are located.

Irradiated individuals were mated to red (*re*) or double recessives (*pe re*) after emergence, so that the mutation frequency could be estimated by a study of the frequency of uncoverings at the marked loci. The wild type, +, for *pe* (pink) and *re* (red) is black. Individuals which revealed themselves as such uncoverings may be regarded as having sustained point mutations, small deficiencies or gross structural changes. Though it may not be possible to identify to which type of mutations those changes belong, they may serve as indices to estimate the effect of radiation, i.e., mutability at specific loci. Sensitivity was estimated by the frequency of eggs that were left unfertilized or died early after fertilization.

Table 1. *pe re* (or *re*) ♀ × treated ♂

Year	Stage of parents treated	Duration of treatment in hrs.	Total emission from $\beta$ -source	Average number of eggs laid per mother	% of unfertilized and early dying eggs	Total number of fertilized eggs observed	Frequency of deficient types per $10^{10}$ MeV. of $\beta$ -source per single locus
1955	control	0 hrs.	0 MeV.	380.1(25)	3.6%**	9,167	0.00%
	IVth stadium	118.5	$1.331 \times 10^{10}$	244.3(24)	28.7	4,180	0.18
	early half of the Vth stadium	97	$1.744 \times 10^{10}$	334.0(21)	50.8	3,450	0.33
	late half of the Vth stadium	96	$1.440 \times 10^{10}$	408.9(20)	4.7	7,792	0.27
1956	early half of the Vth stadium	72	$8.738 \times 10^{10}$	162.9(18)	87.0	442	0.13
	" *	"	"	204.4(16)	92.9	332	0.00
	late half of the Vth stadium	79	$8.104 \times 10^{10}$	376.1(20)	21.2	6,100	0.10
	" *	"	"	286.1(11)	17.4	2,647	0.37

Table 2. treated + ♀ × *re* ♂

1956	early half of the Vth stadium	72	$8.738 \times 10^{10}$	138.9(22)	45.5	1,769	0.19
	late half of the Vth stadium	79	$8.104 \times 10^{10}$	602.2(25)	13.3	13,119	0.10

\* *re* ♀ × treated ♂

\*\* Lots with all eggs left unfertilized were excluded from calculation. Figures in parentheses indicate the number of lots observed.

### 1. $\beta$ -irradiation of the male.

The results are set forth in Table 1.

It is noteworthy that the average number of eggs per mother was very small in the groups irradiated at the fourth stadium and the frequency of unfertilized and early dying eggs was very high in groups irradiated at the first half of the fifth stadium, some of them being totally sterile. On the contrary, the effect was very small in the groups irradiated at the later half of the fifth stadium, giving almost the same frequency as the control.

In the fourth and early fifth stadia, the germ-cells remain mostly as spermatogonia. Meiosis starts after the insect enters into the fifth stadium and is completed only after the sixth day of the same stadium, provided that the fifth stadium had covered eight days. In the later half of the fifth stadium, most of the germ-cells are in spermatid and/or spermatozoan stage. It can, therefore, be said that spermatogonia and spermatocytes are probably very sensitive to radiation, while spermatids and spermatozoa are fairly resistant.

There was not much difference between the mutability of marked loci between spermatogonia, spermatocytes and spermatids.

### 2. $\beta$ -irradiation of the female.

The same trends as above was observed when the female germ-cells were irradiated (Table 2). The frequency of unfertilized eggs was remarkably high in the group irradiated in the early half of the fifth stadium, while it was very low in the later half. Also, with regard to the number of eggs laid per mother there was a marked difference between the early irradiated (Av. 138.9) and the late irradiated (Av. 602.2) groups.

The oogonial cells multiply rapidly in the ovary during the fourth and early fifth stadia. Development of oocytes starts at the later fifth stadium of the parent, and most of them enter into early prophase approximately at the stage of pupation.

It may be added here that in *Drosophila* spermatogonia are considered to be relatively more resistant to radiation than spermatocytes, in contrast to my results with *Bombyx*.

## 52. Genic Control of X-ray Sensitivity of Silkworm Germ-Cells

(By Yataro TAZIMA)

As was reported earlier, in the silkworm a conspicuous difference is known to exist in the relative sensitivity of germ-cells to X-rays according to the strains and to the stage of gametogenesis (TAZIMA and OHTA, 1952; TAZIMA, 1956). X-ray sensitivity of an "Aka-aka" was relatively higher

than that of *od* strain (TAZIMA, 1956). Since the former strain is characterized by *rb* gene and the latter by *od* gene, the author undertook an experiment to find out whether or not the X-ray sensitivity of both those strains was influenced by the two genes, *rb* and *od*.

After crossing the two strains ( $Z^{od}W$ ,  $+/+ \times Z^+Z^+$ ,  $rb/rb$  ♂)\*, four different combinations for both characters were segregated in  $F_2$  females, namely *od rb* ( $Z^{od}W$ ,  $rb/rb$ ), *od* ( $Z^{od}W$ ,  $+/+$ ;  $2Z^{od}W$ ,  $+/rb$ ),  $+rb$  ( $Z^+W$ ,  $rb/rb$ ) and  $++$  ( $Z^+W$ ,  $+/+$ ;  $2Z^+W$ ,  $+/rb$ ).

These four different types of females were X-rayed simultaneously with the same dose, in the same dish, to allow an accurate comparison of their respective X-ray sensitivities.

X-irradiation was given at the rate of 83.24 r per minute, in total doses of 1665, 3330 and 4994 r units. Irradiation was carried out 8 days and 18 hrs after mounting, that is on about five day old pupae. The treated females were mated to *re* or *pe re* males after emergence. Since all of the  $F_2$  segregants carried  $+^{re}$  gene,  $F_1$  individuals were expected to be black (+) except for the newly induced mutants at  $+^{re}$  locus. By counting the number of red eggs, the mutation frequency could be calculated.

The result is as follows.

Table 1. Irradiation of four kinds of segregating females in  $F_2$  from the cross *od* × *rb* (treated + female × untreated *re* male).

Phenotypes	Unfertilized and early dying eggs*			Uncoverings at the <i>re</i> locus		
	1660 r	3330 r	4994 r	1665 r	3330 r	4994 r
+	%	%	%	% **	%	%
+	4.2	7.5	14.2	0.120 ± 0.042	0.496 ± 0.129	0.846 ± 0.166
<i>od</i> +	8.7	15.0	11.8	0.173 ± 0.053	0.571 ± 0.146	1.118 ± 0.271
+ <i>rb</i>	8.3	11.8	26.8	0.308 ± 0.066	0.630 ± 0.135	1.142 ± 0.198
<i>od rb</i>	14.0	7.9	34.8	0.324 ± 0.090	0.524 ± 0.019	1.460 ± 0.242

\* Lots with all eggs left unfertilized were excluded from calculation.

\*\* Standard error.

With regard to unfertilized and early dying eggs, the difference between ++ and *od rb* it is clear, but it is not certain that such a difference exists between the + *rb* and the *od* +. In general, however, combinations having *rb* seem to be much more sensitive than the others. As for mutability, a difference is recognizable between ++ and *od rb*.

Although not very conclusive, these data appear to suggest that X-ray sensitivity of germ-cells is controlled to a certain extent by the *rb* and + alleles.

\* *od* is a sex-linked gene locating on Z chromosome.

53. *Chronic Gamma Irradiation of Mice at Different Developmental Stages and its Effects upon the Breeding Behavior*

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

A preliminary experiment was arranged for examination of the mutation rate by the multiple recessive method. Mice were subjected to chronic gamma irradiation of the entire body at different developmental stages and the breeding behavior of the treated mice was studied in terms of mating ability, litter size, sex ratio and abnormal progenies. All irradiations were made from the  $\gamma$ -ray source of  $\text{Co}^{60}$ , in a specially designed irradiation room, at a dose rate of 0.36 r per hour, 22 hours per day and 5 days per week. A total dose of 450 r was obtained in about 80 days. The CBA mice undergoing this treatment were kept in aluminum cages. On completion of the scheduled irradiations, all animals were mated with adult NH mice by placing each male with one or two females for ten days. During the period of three months animals were remated 3 to 6 times. The treated mice were divided on the basis of their developmental stages during irradiation into two groups, A and B. Group A consisted of animals receiving radiation from fertilization, through new birth, until the age of 160 days. Group B was composed entirely of adult animals. Seventeen males and five females of the first group, and five males and four females of the second group were studied. The results obtained are summarized below:

(1) Sterility: The percentage of sterility was high for all males in the first mating and decreased in the subsequent matings, as in the case of LAF 1 male mice exposed to a total dose of 1100 r at 8.8 r per day as reported by Lorenz et al (1954). The females of group B, on the contrary, were all fertile in the first mating and became sterile subsequently. Females of group A were all sterile and no recovery from sterility was observed within a three month period.

(2) Pregnancy with no litter: Nearly half of the females mated with irradiated males developed the typical changes of vaginal smears and delivered no litter in the first mating, and some of them behaved likewise in later matings. Presumably this was due to the death of all embryos in the uterus, suggesting the occurrence of clusters of dominant lethal mutations. As it was very difficult to diagnose pregnancies in the early stages, cases of pseudopregnancies could not be recognized and eliminated. For this study, females which had copulation plugs in their vaginal orifices or showed typical changes of the smear were all considered to be pregnant. It is possible that there were some cases of sterile sperms or oocytes among them. For the purpose of calculation of mean litter size, the num-



ber of pregnant females instead of that of litters born was used as a corrected estimate.

(3) Mean litter size: (Table 1) Quantitative comparison of the litter size from irradiated males suggested that group A corresponded roughly to a single X-ray dose of 300 r, and group B to that of 600 r. As for irradiated females group A was more heavily damaged than group B.

(4) Sex ratio: (Table 2 and 3) In the case of irradiated males of group A a statistically significant increase occurred in the proportion of males in their progeny. This increase in the frequency of males was gradually more pronounced from matings one month after the irradiation onwards.

(5) Abnormal progenies: Recessive mutations at three loci of double-

Table 1. Mean litter size.

Order of mating			1st	2nd	3rd	4th	5th	6th	Total	
Months after irradiation			0.4	0.9	1.4	1.9	2.4	2.9	No. of litters	mean litter size
Group A	male irradiated	a	4.6	6.2	5.7	5.8	6.7	4.6	42	5.66
		b	2.3	3.9	3.3	5.8	6.7	4.6	50	4.76
	female irradiated	a	0	0	0	0	0	0		
		b	0	0	0	0	0	0		
Group B	male irradiated	a	0	6.0	7.0	2.5	6.0	3.0	3	4.23
		b	0	6.0	2.3	2.5	6.0	3.0	13	2.69
	female irradiated	a	—	—	6.2	4.0	—	3.0	9	5.66
		b	—	—	6.2	2.0	—	3.0	10	5.10
Control non-irradiated									7	7.33

a) Litter size per born litter.

b) Litter size per pregnant.

Table 2. Sex ratio (males/females).

		No. of F <sub>1</sub> mice	Sex ratio	Statistical significance of the difference from 1:1 ratio (p=0.05)
Group-A	male irradiated	238	1.67	+
	female irradiated	0	—	
Group-B	male irradiated	35	1.91	—
	female irradiated	51	1.21	
Control		52	1.36	—

recessive NH mice were not observed in F<sub>1</sub>, which is only to be expected in view of the small size of the population (total 268 mice). Seven mice died within thirty days, and one of them was small and had a short and nicked tail.

The irradiated males and females appeared to differ in their breeding behavior. Females were more sensitive than males. The radiosensitivity of the reproductive organs in the embryonic and very young males appeared to be quite low compared with that of the adult males.

Table 3. Sex ratios of successive litters of the group A males.

Order of mating	1	2	3	4	5	6
Months after irradiation	0.4	0.9	1.4	1.9	2.4	2.9
Age at mating (in months)	2.4	2.9	3.4	3.9	4.4	4.9
No. of F <sub>1</sub> mice	14	31	40	41	67	23
Sex ratio	0.4	1.2	1.1	1.9	2.0	2.8

#### 54. *Radiosensitivity of Various Mice Strains*

(By Tsutomu SUGAHARA, Yoshihiko SUGIURA and Tetsuaki HASIMOTO)

The differential response of various strains of mice to a single X-ray dose was studied by observing the number of days of survival after a lethal dose. The survival curves showed a few characteristic peaks which corresponded to the modes of lethal effects. A relative small number of animals and a short observation period were found to be sufficient,

Table 1. Mean survival of various mice strains exposed to a single X-irradiation of 1,000 r.

Strain	Sex	No. of animals tested	Mean days survival (95% range)
CBA	♂	7	3.36±0.206
	♀	5	8.00±1.449
C 57 BL	♂	8	4.50±1.262
	♀	5	4.60±2.421
C 58	♂	12	7.17±1.915
	♀	7	7.55±0.872
SPS	♂	29	6.93±0.620
	♀	29	6.69±0.781
hy × C 57 BL-F <sub>2</sub>	♂	7	6.57±1.285
	♀	7	8.43±1.177

as compared with the number of animals and the period of observation used in the conventional measurement of LD 50 for 30 days.

The irradiations were made as follows: the mice were placed in hollow cylinders of acrylate resin which were fixed radially on a rotating disc. The animals were irradiated with a single dose of 1000 r delivered at the rate of 20.3 r/min. in air at the distance of 47 cm. from the target (160 KVP X-ray, 3 ma curr., 2 mm Al filter). The animals tested were six to twelve months old.

No correlation was found between length of survival and body weight at the time of irradiation. The frequency curve of survival days for both sexes had two peaks, at the fourth and at the eighth day, and was significantly different from the normal distribution. With reference to the sexes, for males the peak at the fourth day was higher than at the eighth day, and the reverse was true for the females. The differences in the mean number of survival days between males and females and between various strains were statistically significant, as shown in Table 1. In general, the females were more radioresistant than the males.

#### 55. *The Effects of X-Irradiation on Nitrogen Metabolism in Drosophila melanogaster*

(By Toshifumi TAIRA and Saburo NAWA)

Only a few studies have been reported on the effects of X-irradiation upon the metabolism of *Drosophila*. When the larvae of *Drosophila* are irradiated with either X- or  $\gamma$ -rays, melanotic tumors are often found in addition to other morphological abnormalities. Their incidence, however, differs among genotypes and varies according to environmental conditions. Both *sed* and *cn;ca* among the eye-color mutants seem to have rather a higher incidence of melanotic tumors than the others under the same environmental conditions.

After irradiation of second-instar larvae of *sed*, grown on normal medium, with X-rays (160 KVP, 3 mA) of 1,000 r, a delay in pupation was found though the treated larvae were not all killed. Those pupae showed a vacuole during metamorphosis, and no flies hatched from them. Larvae with melanotic tumors often could not go through normal pupation. Pre-pupae, therefore, were used as materials employed for spectro-photometrical measurements of the quantity of uric acid by means of degradation with uricase. The results are shown in Table 1.

The examination of melanotic tumors by roentgen-photography, revealed that the tumors showed a reduced X-ray absorption in comparison with

Table 1. Microdetermination of uric acid in prepupae of *sed* strain of *Drosophila melanogaster*.

Plots	Uric I	Acid II	( $\gamma$ /mg) III	Mean	Standard Deviation
Control (Non-irradiation)	4.16	4.04	3.78	4.0 $\pm$	0.13
Irradiated Without Melanotic Tumors	3.78	3.72	3.38	3.6 $\pm$	0.15
Irradiated With Melanotic Tumors	2.79	2.88	2.37	2.7 $\pm$	0.19

other parts of the body.

From these results, we may reach the following conclusions:

(a) The quantity of uric acid in *Drosophila* pupae decreases significantly after irradiation.

(b) The quantity of uric acid is apparently smaller in the pupae with melanotic tumors than in those without tumors.

(c) Since the decrease of uric acid suggests a decline of nitrogen metabolism, it may be said that irradiation causes an abnormal state in nitrogen metabolism which brings about melanotic tumors and finally death of the affected animals.

## L. RADIATION GENETICS IN PLANTS

### 56. Genetic Effects of Ionizing Radiation in Einkorn Wheat

(By Seiji MATSUMURA and Taro FUJII)

Dormant seeds of *Triticum monococcum* var. *flavescens* were exposed to X-rays,  $\gamma$ -rays and fast neutrons.

X-rays of different wave lengths at the same dosage (10 Kr) and intensity (82 r/min) were used with different filters; also the effect of  $\gamma$ -radiation by Co<sup>60</sup> was examined for comparison. The thickness of the filter was adjusted in inverse proportion to the wave length; that is, at 100 KVP a filter of 2 mm Al, and at 180 KVP one of 0.8 mm Cu+1.5 mm Al was inserted into MATSUDA's Type KXC-17 apparatus. At 50 KVP and 20 KVP, irradiation was applied by two other types, Modified Type KR-75 and Type TX-20 (Grenz-rays) without filter, respectively. The data are shown in Table 1.

There was no striking difference between hard and soft X-radiation, in

Table 1. Relation between wave length of X- or  $\gamma$ -rays and frequency of chromosome aberrations in *Triticum monococcum*.

Dosage (Kr)	Voltage (KVP)	Germination rate (%)	Length of seed- lings* (cm)	Ferti- lity of spike in X <sub>1</sub> (%)	Chromo- some aberra- tion in X <sub>1</sub> (%)	Chloro- phyll muta- tion in X <sub>2</sub> (%)	Head proge- nies without germi- nation (%)
Control		92.00	17.44 (14.54)	60.45	0.00	0.0	0.0
10 (82 r/min)	20	82.00	15.85	44.20	12.50	6.3	3.0
" (82 " )	50	88.00	14.23	40.73	4.88	6.8	4.8
" (81.4 " )	100	60.00	10.20	32.33	10.81	8.3	7.7
" (81.2 " )	(with filter 2 Al) 180 (with filter 0.8 Cu+1.5 Al)	90.00	11.11	36.50	21.82	10.3	8.1
5 ( 8.3 " )	$\gamma$ -ray	92.00	13.93	62.95	1.67	4.8	1.2
10 (16.6 " )	"	38.00	12.73	38.72	5.56	2.2	0.0
15 (25 " )	"	50.00	8.96	32.18	6.25	0.0	5.1
10 Ah	Neutron (4-7 MeV) (10 <sup>9</sup> neutron/A. sec)	98.00	(14.25)	54.20	1.33	1.2	2.4
15 Ah	"	88.00	(14.53)	37.01	4.17	3.7	1.2
20 Ah	"	79.59	(13.84)	27.66	4.55	2.7	6.3

\* X- and  $\gamma$ -irradiated seeds were sown November 9th and the seedlings were measured 26 days after sowing. ( ) Sown December 12th and measured 26 days after sowing.

so far as the germination of seeds is concerned, but the growth of seedlings showed a slight delay with the decrease of wave length. The higher the dosage of  $\gamma$ -rays or neutrons, the lower was the germination rate of irradiated seeds, and the more delayed were the germination of seeds and growth of seedlings. It was shown, in terms of growth inhibition of the seedlings, that neutrons with a high specific ionization more uniformly affect the irradiated seeds than X- and  $\gamma$ -radiations with a low specific ionization.

The mean single-spike fertility of X-rayed plants generally decreased with the decrease of wave length. This relation is in good accord with that between the growth of seedlings and wave length. Also, the relation between the rate of induced sterility and wave length coincides roughly with the relation between the frequency of chromosome aberrations or chlorophyll mutations and wave length. But at 20 KVP the aberration frequency was unexpectedly high, while at 50 KVP it was too low.

It was also ascertained, as expected, that mean fertility decreased with decreasing germination rate accompanied by weaker growth of seedlings, and the chromosome aberrations increased in proportion to the dosage of  $\gamma$ -rays and neutrons. Concerning the frequency of gene mutations in  $X_2$ , the head progenies which did not germinate at all, must be added to the chlorophyll mutations.

57. *Effects of X- and  $\gamma$ -radiations upon Wheat Seedlings and Their Modification Due to Temperature or Polyploidy*

(By Seiji MATSUMURA, Taro FUJII and Sohei KONDÔ)

Dormant seeds of *Triticum monococcum* were subjected to X- and  $\gamma$ -ray treatments at the dosage 10 and 20 Kr. The germination rate of treated seeds and the growth of seedlings were compared for acute and chronic irradiation. In the former X- and  $\gamma$ -irradiation was applied either immediately before sowing or the irradiated seeds were kept for 30 days in storage and in the latter  $\gamma$ -irradiation lasted 54 days. In one experiment with acute irradiation one part of the treated seeds were kept at room temperature (about 20°C) and the remainder at low temperature (5°C) for 30 days.

There was no marked difference in germination rate between untreated and treated seeds at 10 Kr, while the germination rate was reduced to 1/2~2/3 at 20 Kr. In the case of 30 day storage,  $\gamma$ -rays inhibited the growth of seedlings more than X-rays, while the irradiation applied just before sowing showed the reverse relation. It was found further especially with  $\gamma$ -rays that low temperature was more effective in inhibiting growth than room temperature. At 10 Kr, the acute  $\gamma$ -irradiation was more effective in this respect than the chronic one. On the other hand, the reverse relation between acute and chronic  $\gamma$ -irradiation was observed.

To examine the relation between the sensitivity to ionizing radiation and polyploidy, dormant seeds of *Triticum monococcum* (2x), *T. durum* (4x) and *T. vulgare* (6x) were exposed to X- and  $\gamma$ -rays at the dosage 10-40 Kr. In general,  $\gamma$ -irradiation had a markedly stronger inhibiting effect upon seed germination and seedling growth than X-irradiation. 2x was most sensitive to X- and  $\gamma$ -rays and 6x was most resistant. There was unexpectedly no significant difference between 4x and 6x.

58. *Mutants in Tobacco Plants Induced by X-rays and Their Application*

(By Seiji MATSUMURA and Taro FUJII)

Dormant seeds of the variety Bright Yellow (in 1955) and its mutant "early" (in 1956) were exposed to the same dosage (30,000 *r*) of soft and hard X-rays with varying kilovoltages. The germination rate of treated seeds generally decreased with the increase of kilovoltage. Namely, it was 66.5, 47.8 and 25.5% at 20, 50 and 100 KVP respectively in 1956, while that of untreated seeds was 69.0%. Many recessive mutants were observed in the X<sub>2</sub>-generation of Bright Yellow, such as narrow, oblong leaves, dwarf, etc.

The mode of inheritance of many mutants obtained in the previous experiments was investigated in the X<sub>3</sub>-X<sub>6</sub> generations of Bright Yellow and Dixie Bright 101.

"Early" (No. 6) and "pubescent" (No. 12), mutants of Bright Yellow, appeared after a 10,800 *r*-irradiation at 90 KVP in one of the earliest experiments (1950). Their leaves, as well as those of "yellowish green" (No. 13) derived from 50,000 *r*-irradiation at 180 KVP (1951) of Bright

Table 1. Yield in Kg. and value in Yen of leaves of X-ray mutants of flue-cured tobacco. (1956)

Strains	% of dry matter	Yield in Kg. per Tan*	Value in Yen per Kg.	Average grade	Return per Tan* in Yen
Bright Yellow	17.6	134	303	3.78	40,469
6-2-1	17.2	129	276	4.32	35,528
6-2-2	17.4	135	344	3.10	46,330
6-3	16.5	153	294	3.92	45,158
12-2	17.9	132	339	3.17	44,903
13-1	15.4	133	257	4.55	34,061
13-2	15.8	180	277	4.21	49,994
19 (the same strain as No. 13)	15.9	176	256	4.56	45,172
20 (Dixie Bright)	17.0	170	222	5.14	37,753
23 (the same strain as No. 20)	16.9	178	268	4.35	47,741
21	16.8	153	241	4.91	36,860
22 (D. B. × X-rayed B. Y.)	18.2	159	303	3.77	48,386

\* Tan=ca. 0.1 ha.

Yellow, seem to be of good quality (Table 1). These mutants may prove to be useful for the improvement of tobacco and in 1957 comparative experiments with these mutant strains will be carried out on a large scale in various districts.

The "round leaf" mutants derived from 30,000 *r*-irradiation at 180 KVP of Dixie Bright 101 (Nos. 20 and 21) (1953), and the "broad leaf" mutants observed in the offspring of Dixie Bright pollinated with irradiated pollen (290 *r*, 50 KVP) of Bright Yellow (No. 22) (1953) showed a pronounced resistance against white fleck.

#### 59. *Studies on Chlorophyll Mutants in Diploid Wheat Induced by Radiation*

(By Taro FUJII)

Further studies on several mutant strains of *Triticum monococcum* var. *flavescens* induced by X-rays, were made.

The mutant strains "chlorina" (light green leaves) and "basi-*viridis* II" (base of leaves yellowish green) were crossed in 1955. All the F<sub>1</sub> plants from this cross were morphologically normal, and showed high germinating capacity and high seed fertility. In the F<sub>2</sub> generation, chlorina and basi-*viridis* II were found to be controlled independently by a recessive gene each, and a segregation according to the dihybrid ratio was observed. The chlorophyll content in both parents amounted to about 50% of that of the normal plants, but when basi-*viridis* II was illuminated by fluorescent lamps (about 4,000 luxes) in the dark phytotron (20°C), this mutant gradually recovered the green coloring and its chlorophyll content reached that of the normal plants, while in the chlorina mutant such a marked recovery of chlorophyll was not found. The chlorophyll content of the double-recessive plants grown in the field was about 20% of the normal and a high degree of mortality was observed. When these plants were grown in the phytotron, their leaves gradually became light green, and their chlorophyll content was restored to the chlorina level but further recovery did not occur. It is possible that the chlorina gene is epistatic over the basi-*viridis* II gene.

In 1956, crosses virido-*albina* × basi-*viridis* II (both parents have the ability to recover the chlorophyll content), virido-*albina* × chlorina, and chlorina × *striata* (neither has the ability to recover) were made. It was found from these experiments that these characters might be controlled by different genes, as all of the F<sub>1</sub> plants were normally green.

In 1955, X-rays were applied again to two mutant strains (chlorina and



slender) which had been induced by X irradiation. Results of these experiment are shown in Table 1. The percentage of chromosome aberra-

Table 1. Effects of X-irradiation on Einkorn wheat and its mutants.

Strain	Dosage	Germination rate in X <sub>1</sub> (%)	Average plant height in X <sub>1</sub> (cm)	% of chromosome aberration in X <sub>1</sub>	Average fertility in X <sub>1</sub> (%)	% of chlorophyll mutation in X <sub>2</sub>
Normal		94	111.1(100.0)	0	89.1(100.0)	0
	5 Kr	94	107.9( 97.1)	5.0	65.1( 73.1)	7.8
	10 Kr	88	104.5( 94.1)	0	36.7( 41.2)	7.9
	15 Kr	78	101.8(100.0)	3.1	27.5( 30.9)	0
Chlorina		80	94.8(100.0)	0	68.1(100.0)	0
	5 Kr	78	87.9( 92.7)	5.6	34.4( 50.5)	0
	10 Kr	66	89.3( 94.2)	—	20.1( 29.5)	0
	15 Kr	76	76.9( 81.1)	17.9	10.1( 14.8)	14.3
Slender		98	97.1(100.0)	0	47.8(100.0)	0
	5 Kr	96	106.5(109.7)	2.2	55.2(115.5)	3.6
	10 Kr	72	101.5(104.5)	8.3	49.7(103.9)	1.6
	15 Kr	76	93.1( 95.9)	9.1	25.0( 52.3)	6.3

tions in X<sub>1</sub> and that of chlorophyll mutations in X<sub>2</sub> were higher than in the normal plants. Further, the chlorina strain showed in X<sub>1</sub> more clearly than the normal plants a marked decrease in the average fertility with the increase of dosage. On the other hand, the slender mutant did not show these trends. This might be due to genotypic differences in the susceptibility to X-rays.

## M. GENETICS AND CYTOLOGY OF MICROORGANISMS AND VIRUSES

### 60. *Transductional Analysis of Phase Variation in Salmonella*

(By Tetsuo IINO and Joshua LEDERBERG\*)

The flagellar antigens of *Salmonella* can be divided into two groups, viz., phase-1: *a*, *b*, *c*, etc., and phase-2: 1.2, 1.5, *enx*, etc. Cultures of diphasic *salmonella* strains are composed of two types of cells. One type

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manifests an antigen in phase-1, and another in phase-2. The variation from one phase to another may occur at the frequencies of  $10^{-3}$  to  $10^{-5}$  per cell per division depending on the strains (STOCKER, 1949). This phenomenon is called phase variation. For the genetic analysis of the phenomenon, transduction was used to achieve recombination of antigenic factors.

Lysates obtained from *Salmonella typhimurium* TM2 culture, which has "i" antigen as phase-1 and "1.2" as phase-2 (This is described as *i:1.2*), were mixed with cultures of *S. abony* CDC-103 (*b:enx*). The mixtures were cultured on semi-solid media containing anti-*b* and anti-*enx* serums. The swarms developed from these cultures, which are expected to have flagellar antigen other than *b* and *enx* as a result of transduction, have antigenic composition *b:1.2* or *i:enx*, but never *i:b*, *1.2:enx* or *i:1.2*. This result indicates that the antigenic specificities of phase-1 and phase-2 are controlled by independent loci in each, which will be symbolized by  $H_1$  and  $H_2$ . Transductional experiments in reverse directions and between different strains proved the generality of this conclusion and led to the establishment of two series of multiple alleles of  $H_1$  and  $H_2$ . Therefore, flagellar antigen genotypes for each phase are  $H_1^a$ ,  $H_1^b$ ,  $H_1^c$ , etc. and  $H_2^{1.2}$ ,  $H_2^{1.5}$ ,  $H_2^{enx}$ , etc. respectively.

Next the same experiment is done with a single phase culture in which more than 90% of the cells are in one phase (Antigen of the predominant phase is described with under line). Then, different transductions are recovered in different frequencies depending on the combination of the phase of donor and recipient as follows:

Donor	<u><i>i:1.2</i></u>	<i>i:1.2</i>	<u><i>i:1.2</i></u>	<u><i>i:1.2</i></u>
Recipient	<u><i>b:enx</i></u>	<u><i>b:enx</i></u>	<u><i>b:enx</i></u>	<u><i>b:enx</i></u>
Transductions <u><i>i:enx</i></u>	39	3	23	3
recovered <u><i>b:1.2</i></u>	1	0	23	21

As it is clear from this table, frequent transductions of  $H_1^4$  are recovered when the recipient is phase-1 regardless of the phase of donor, whereas transductions of  $H_2^{1.2}$  are recovered only when donor is phase-2 on any recipient. The parallel results were obtained in the reverse transduction (transduction from *S. abony* to *S. typhimurium*). The few exceptions observed are assumed to be due to the cells of alternate phase which have been mixed at the proportion of lower than 10%. As  $H_1$  and  $H_2$  are transduced independently and the efficiencies of transduction do not differ markedly.  $H_1$ -transduction and  $H_2$ -transductions are produced in every combination of phases. However, anti-*b* and anti-*enx* serums were contained in the media for selection, and they suppressed the development

of transductions  $i:enx$  and  $b:1.2$ , which could not express transduced antigen types immediately. The difference of the result in different phase combinations may be explained by the non-recovery of such fractions of transductions. Consequently, it is concluded that  $H_1$  can be expressed only when transduced into phase-1 cells regardless of the phase of donor, whereas  $H_2$  can be expressed in any phase of recipient but only when donor is phase 2.

From these results, the following hypothesis is proposed: " $H_2$  can take two different states, active and inactive. Active  $H_2$  inhibits the phase-1 antigen production, specified by  $H_1$ , and controls the production of the specific phase-2 antigen. When  $H_2$  changes to the inactive state, which corresponds to the change from phase-2 to phase-1, the production of phase-2 antigen stops and alternatively the production of phase-1 antigen, specified by  $H_1$ , proceeds."

#### 61. *Electron Microscopy of Thin-Sectioned Nuclei in Paramecium*

(By Mitsuo TSUJITA, Kyozeo WATANABE and Seizo TSUDA)

The inner minute structure of the nuclei of *Paramecium* is not yet well known. Recently the structure of the nuclei in Ciliatae as revealed by electron microscopy has received considerable attention. BREITSCHNEIDER (1950), FINLEY (1951), SONNEBORN (1953), TSUJITA, WATANABE and TSUDA (1952, 1954) and EHRET and POWERS (1954, 1955) have studied the fine structure of the macronucleus by electron microscope.

The present paper deals with some additional information on the fine structure of the nuclei of *Paramecium caudatum* observed in ultra-thin sections. A clonal culture of *Paramecium caudatum*, which originated from one individual, was employed.

The macronucleus of *P. caudatum* is surrounded by a thin nuclear membrane. Inside numerous minute particles, spherical or oval in shape which sometimes look like beads appear connected by fine strands. These particles measure about 0.1–0.2  $\mu$  in diameter. The bead-like structures are distributed throughout the whole macronucleus. Spherical granules which measure 0.4  $\mu$  or more in diameter are scattered here and there. They are often observed in abundance at the periphery of the nucleus.

From methyl-green pyronin staining of the materials fixed by alcohol it is evident that these granules take pyronin dyes. From this fact it may be inferred that they are nucleoli.

Although we can easily observe the inner structure of the macronucleus, it is difficult to get a good view of the inner structure of the micronuc-

leus because the latter is much smaller. The micronucleus is also surrounded by a thin nuclear membrane which shows almost the same appearance as that of the macronucleus. As seen from the figure, in the right part of the section of the micronucleus appear entangled filamentous bodies, while in the other part globular particles connected by strands can be seen, some of the latter looking like thick fibers or spiral structures. The inner structure of the micronucleus somewhat resembles a prophase of cell division in higher organisms; however whether it is a true prophase is uncertain. To clarify this point further studies are required. In the micronucleus we cannot see nucleolar granules as observed in the macronucleus.

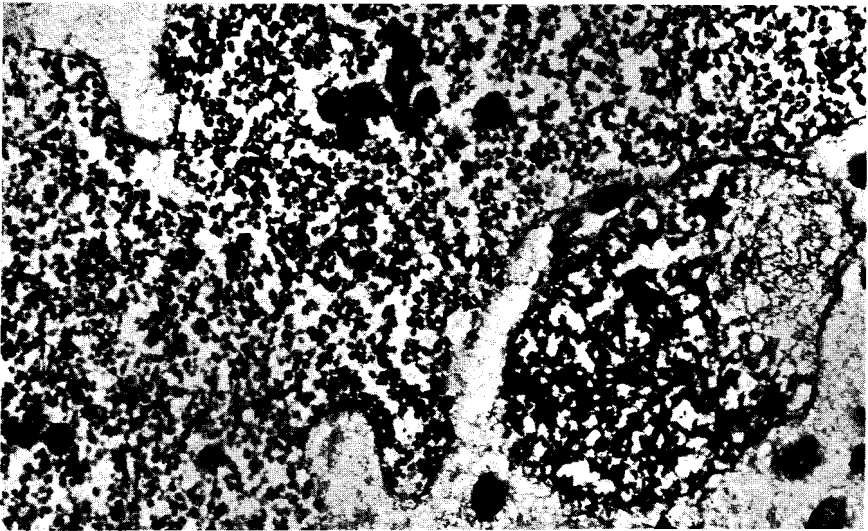


Fig. 1. Electron micrograph of a thin section of a macronucleus of *Paramecium*. In the concavity of a macronucleus, a micronucleus can be seen.  $\times 9,000$ .

There are several reports on the fine structure of the nucleus of *Paramecium*. BREITSCHNEIDER (1950) observed in the macronucleus of *P. caudatum* by electron microscopy many irregular spherical bodies connected by thin strands, and fewer larger bodies. He interpreted the former as chromosomes, and the latter as nucleoli. But no proof was offered of the correctness of this view. According to KIMBALL's cytological observations by phase contrast microscope, the macronucleus of *Paramecium* contains two types of bodies, namely spherical granules which are scattered more or less at random and many fine filaments almost completely filling out

the nucleus. SONNEBORN (1953) reported also that there are two kinds of granules in the macronucleus.

In our preparations many globular particles connected by fine strands or bead-like structures could be observed in the macronucleus. From the fact that these substances show positive reaction to Feulgen and that they stain with methylgreen by methylgreen-pironin-staining method, it may be said that these structures have some relation to chromosomes. The bead-like structures somewhat resemble spirals.

In our previous paper we have suggested that there may be some relation between the nucleoli of the macronucleus and the granules in the cytoplasm. Recently this point has been also discussed in detail by EHRET and POWERS (1955).

## N. MATHEMATICAL GENETICS

### 62. *Random Genetic Drift in a Tri-allelic Locus; Exact Solution with a Continuous Model*

(By Motoo KIMURA)

In the last report the author presented the exact solution of the process of random genetic drift in a locus with a pair of alleles. The details have been published in Proc. Nat. Acad. Sci. (Vol. 41). Also the asymptotic solution has been worked out for a multi-allelic locus by studying the process of change in the moments of distribution. The detailed results have been published in Evolution (Vol. 9). Recently the author succeeded in obtaining the exact solution for the case of three alleles based on a continuous model and the partial differential equation method.

Consider a random mating population of  $N$  breeding individuals and let  $A_1$ ,  $A_2$  and  $A_3$  be the three alleles whose respective frequencies in the population are  $x$ ,  $y$  and  $z$  ( $x+y+z=1$ ). If we denote by  $\phi(x, y|p, q; t)$  the probability density that the frequencies of  $A_1$  and  $A_2$  are respectively  $x \sim x+dx$  and  $y \sim y+dy$  in the  $t$ -th generation, given that their initial frequencies are  $p$  and  $q$  at  $t=0$ , then it is possible to show that  $\phi$  satisfies the following partial differential equation:

$$\frac{\partial \phi}{\partial t} = \frac{1}{4N} \frac{\partial^2}{\partial x^2} \{x(1-x)\phi\} - \frac{1}{2N} \frac{\partial^2}{\partial x \partial y} \{xy\phi\} + \frac{1}{4N} \frac{\partial^2}{\partial y^2} \{y(1-y)\phi\} .$$

$$(0 < x < x+y < 1)$$

The required solution satisfying the initial condition

$$\phi(x, y|p, q, 0) = \delta(x-p) \cdot \delta(x-q)$$

has been published in "Biometrics". The probability distribution of gene frequencies in the unfixed classes where all the three alleles coexist indicates that the distribution surface finally becomes flat and decreases in height at the rate of  $3/2N$  per generation as opposed to  $1/2N$  for a pair of alleles. (cf. Kimura, M. 1956. *Biometrics* 12:57-66.)

### 63. *Selection in a Finite Population (Case of Complete Dominance)*

(By Motoo KIMURA)

Studies of interaction between natural selection and random genetic drift should be important in evolutionary genetics. In the last report the present author discussed the process of genic selection (case of no dominance) in a finite population using the partial differential equation method and showed how the exact solution could be constructed in terms of the oblate spheroidal wave function. Very often, however, there is some dominance between alleles, and usually "complete dominance". In what follows new results obtained for the case of complete dominance will be reported.

Consider a random mating population of  $N$  breeding individuals. Suppose that the gene  $A$  is completely dominant over its allele  $A'$  and the dominants  $AA$  and  $AA'$  have selective advantage  $s$ , measured in MALTHUSIAN parameters, over the homozygous recessive ( $A'A'$ ). If we denote by  $\phi(x|p;t)$  the probability density that the frequency of  $A$  lies between  $x$  and  $x+dx$  in the  $t$ -th generation given that it is  $p$  at  $t=0$ , then  $\phi$  satisfies the following partial differential equation:

$$\frac{\partial \phi}{\partial t} = \frac{1}{4N} \frac{\partial^2}{\partial x^2} \{x(1-x)\phi\} - \frac{\partial}{\partial x} \{sx(1-x)^2\phi\}. \quad (0 < x < 1)$$

For a small value of  $Ns$  we can expand the eigenvalues into power series of  $Ns$ . The most important information is the smallest eigenvalue ( $\lambda_0$ ) given by the series

$$2N\lambda_0 = 1 - \frac{1}{5}c + \frac{199}{2 \cdot 5^3 \cdot 7}c^2 + \frac{17}{2 \cdot 5^5 \cdot 7}c^3 - \frac{23 \cdot 41 \cdot 29599}{2^3 \cdot 3^3 \cdot 5^6 \cdot 7^3 \cdot 11}c^4 \dots$$

where  $c = Ns$ . Here  $\lambda_0$  gives the ultimate rate of decrease of the probability that  $A$  and  $A'$  coexist in the population, namely the rate of decay. The most remarkable fact suggested by the present analysis is that as compared with the case of pure random drift, selection toward dominants ( $s > 0$ ) decreases the final rate of decay, while selection against dominants ( $s < 0$ ) increases it. The detailed study of this problem together with discussions of other topics will be published in *Ann. Math. Stat.*

64. *Probability Distribution of Gene Frequencies in Natural Populations*

(By Motoo KIMURA)

In 1938 WRIGHT published a formula for the probability distribution of gene frequencies at the steady state, which may be written in the following form.

$$\phi(x) = (C/V_{\delta_x}) \exp \left\{ 2 \int (M_{\delta_x}/V_{\delta_x}) dx \right\},$$

where  $x$  is the gene frequency, and  $M_{\delta_x}$  and  $V_{\delta_x}$  are respectively the mean and the variance of the rate of change in  $x$  per generation. As far as one locus with a pair of alleles is concerned the formula is quite general and it may be regarded as one of the most important formulas in population genetics.

In the evolution of species, however, genic interactions may be quite important and hence extension of WRIGHT's formula to cover multi-variate cases should be required.

Let us consider a bivariate case with two random variables  $x$  and  $y$  on which no constraints are imposed. Thus  $x$  and  $y$  may represent the frequencies of any two alleles in the case of a single locus with three alleles or the frequencies of any two non-allelic genes in the case of two loci each with a pair of alleles. We shall designate by  $\phi(x, y)$  the probability density of  $x$  and  $y$  at the steady state, and by  $M_{\delta_x}$ ,  $M_{\delta_y}$ ,  $V_{\delta_x}$ ,  $V_{\delta_y}$  and  $W_{\delta_x \delta_y}$  the means, the variances and the covariance of  $\delta x$  and  $\delta y$ . Then we obtain the following theorem: If the simultaneous linear equations in  $\phi_x$  and  $\phi_y$

$$\begin{cases} V_{\delta_x} \phi_x + W_{\delta_x \delta_y} \phi_y = 2M_{\delta_x} - \frac{\partial}{\partial x} V_{\delta_x} - \frac{\partial}{\partial y} W_{\delta_x \delta_y} \\ W_{\delta_x \delta_y} \phi_x + V_{\delta_y} \phi_y = 2M_{\delta_y} - \frac{\partial}{\partial x} W_{\delta_x \delta_y} - \frac{\partial}{\partial y} V_{\delta_y} \end{cases}$$

have a unique and non-trivial set of solutions  $(\phi_x, \phi_y)$  and if

$$\phi_x dx + \phi_y dy$$

is an exact differential, which we write as  $d\psi$ , then the steady state distribution is given by  $\phi(x, y) = Ce^\psi$ , where  $C$  is a constant determined by the condition  $\iint \phi dx dy = 1$  (terminal classes excluded).

The theorem can be extended to cover multivariate cases in general. As an application of the theorem or of its extended form, it is possible to give a rigorous proof of the simultaneous distribution of gene frequencies obtained by WRIGHT (1949):

$$\phi(x_1, x_2, \dots, x_k) = C \bar{a}^{2N} \prod_{i=1}^k x_i^{4Nm x_i - 1},$$

in which  $m$  is the rate of migration and  $\bar{x}_i$  is the frequency of the  $i$ -th allele in the migrants.

### 65. *The Rate of Change of Population Fitness by Natural Selection*

(By Motoo KIMURA)

R. A. FISHER's "fundamental theorem of natural selection" states that the rate of increase of the average fitness ( $\bar{a}$ ) of a population (measured in MALTHUSIAN parameters) is equal to its genic or additive genetic variance in fitness ( $V_g$ ) at that time, namely

$$\frac{d\bar{a}}{dt} = V_g.$$

Since the variance is a non-negative quantity, the fitness in relation to a fixed external environment always tends to increase. The formulation seems to be compatible with the general picture of evolution in the past and may be considered as a quantitative description of an essential feature of evolution.

However, in addition to increases in population fitness attributable to change in gene frequencies, the average fitness may be altered by such things as changes in the mating system when dominance or epistatic factors are involved, or changes in the relative fitness of individual genotypes.

Thus with one locus segregating we have

$$\frac{d\bar{a}}{dt} = V_g + \sum_{ij} P_{ij} \frac{da_{ij}}{dt} + \sum_{ij} P_{ij} d_{ij} \frac{d}{dt} \log \theta_{ij}$$

or more concisely

$$\dot{\bar{a}} = V_g + \bar{a} + \overline{\dot{\theta}_{ij} d_{ij}}, \quad (\dot{\theta} \equiv d \log \theta / dt)$$

where  $\theta_{ij} = P_{ij}/x_i x_j$ ,  $x_i$  is the relative frequency of the  $i$ -th allele  $A_i$ ,  $P_{ij}$  is the frequency of  $A_i A_j$  and  $d_{ij}$  is the dominance deviation of  $a_{ij}$  (fitness of  $A_i A_j$ ) from additivity.



With an arbitrary number of loci each with arbitrary number of alleles the corresponding formula is

$$\dot{\bar{a}} = V_{\sigma} + \bar{a} + \sum \bar{\varphi} \bar{\varepsilon} \quad (\bar{\varphi} \equiv d \log \varphi / dt)$$

where  $\varepsilon$  is the deviation from additivity due to dominance or epistasis and  $\varphi$  is defined as the ratio of the actual frequency of a genotype to the frequency expected from random combination between alleles (in the case of dominance) or between loci (in the case of epistasis). The summation is over all relevant loci.

Thus the rate of increase of population fitness by natural selection is expressed as a sum of the three terms: A term due to additive genetic variance, one due to average increase of individual fitness of genotypes and one due to the joint effect of genetic interaction and change in mating system.

66. *A Model of a Genetic System Which Leads to Closer Linkage by Natural Selection*

(By Motoo KIMURA)

At the 1955 Cold Spring Harbor Symposium (XX: Population Genetics), Dr. P. M. SHEPPARD proposed the following problem for mathematical analysis: Assume a locus with a pair of alleles, say  $A_1$  and  $A_2$ , kept in balanced polymorphism by heterozygote superiority in fitness. Another pair of alleles,  $B_1$  and  $B_2$ , are at another locus on the same chromosome, and interact with the genes in the first locus in such a way that  $A_1$  is advantageous in combination with  $B_1$  but is disadvantageous in combination with  $B_2$ , while the situation is reversed for the gene  $A_2$ . Then the second locus will remain polymorphic if linkage between the two loci is sufficiently close. Under this model, close linkage would maintain a larger fraction of the fitter genotypes than loose linkage and an inversion or other crossover reducing mechanism will be favoured by selection.

The present author carried out mathematical analysis of this problem and has succeeded in confirming SHEPPARD's view in quantitative terms.

(cf. KIMURA, M. 1956. *Evolution* 10: 278-287.)

## O. TECHNICAL NOTES

67. *Dosimetry of Gamma Rays with Glass*

(By Sohei KONDO)

It is well known that glass usually takes a brown or violet color when irradiated by ionizing radiations. According to the modern view, the coloration is, as in the case of alkali halides, ascribed to electrons trapped at negative ion vacancies or at other defects present inside the glass. For the present treatment, however, the nature of those centers is of no importance; we may assume that there are a certain number of colorable centers due to imperfections and that the electrons freed by irradiation can be bound to them to form colored centers.

Let us take  $N$  as the total number of the colorable centers per unit volume in glass,  $n$  as the number density of colored centers due to irradiation by  $r$  röntgen. Then, it may be assumed, as in the case of LEA's hit theory, that the increase in the colored centers,  $dn$ , due to an infinitesimal increase  $dr$ , is proportional to the number of uncolored centers:

$$dn/dr = a(N - n), \quad (1)$$

where  $a$  is a constant.

On the other hand, the light absorption coefficient of glass,  $\mu$ , is proportional to the concentration of colored centers  $n$ . Hence from (1) we obtain

$$\mu = \mu_{\infty}(1 - e^{-ar}), \quad (2)$$

where  $\mu_{\infty}$  is the saturation value of  $\mu$ . In the low dosage limit of  $r$ , equation (2) reduces to

$$\mu = \mu_{\infty} ar. \quad (\text{low dosage limit}) \quad (3)$$

This means that the color intensity of glass induced by irradiation is proportional to the dose of radiation so long as the dose is not very high. The relation (3) is well represented in Fig. 1. Furthermore, the remarkable homogeneity of glass is shown in Fig. 2 where the maximum (not the standard) deviation is given. Thus, we may conclude that even a small piece of glass can serve as a very accurate dosimeter.

There is, however, a defect, and that is the bleaching of the induced color occurring spontaneously even at room temperature, further accelerated by heat and visible light. Therefore, the linearity between the

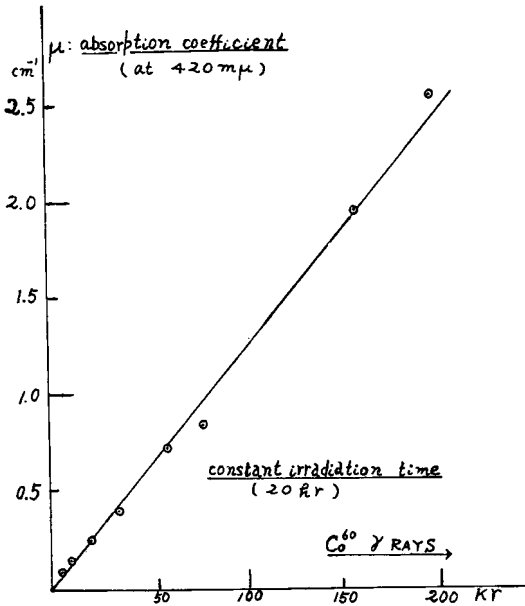


Fig. 1. An example of the linear increase in the induced absorption coefficient of glass with increasing doses of gamma rays delivered for the same duration of time.

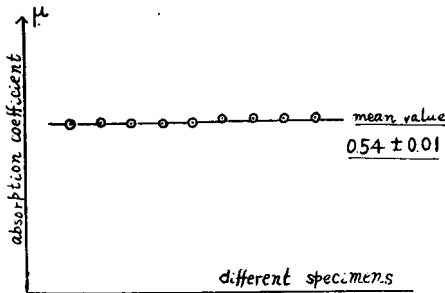


Fig. 2. Homogeneity of glass with respect to its change in the absorption coefficient due to gamma rays as seen from values for glass pieces sampled from the same glass plate.

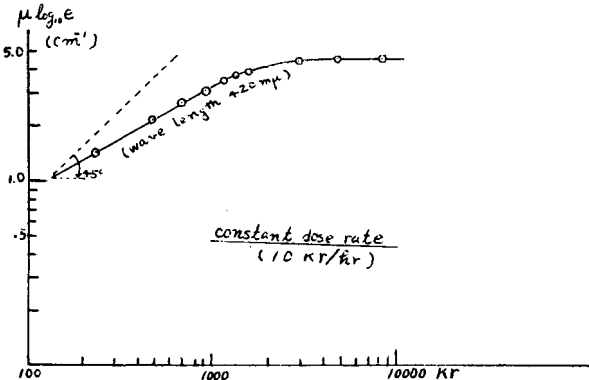


Fig. 3. A curve showing the non-linear increase in the absorption coefficient of glass with increasing doses of gamma rays delivered at a constant rate. (Compare with a linear increase line ----.)

color intensity and the radiation dose can be observed only under constant irradiation time but not under constant dose rate of irradiation (see Fig. 3 in log-log scale). This bleaching effect is now under investigation with a view to making possible the use of glass dosimeters, accurate up to one percent in measuring errors.

68. *Thermodynamical Fundamental Equation for Spherical Interface\**

(By Sohei KONDO)

The relation between the radius of the Gibbs dividing surface,  $a$ , and superficial density was investigated in detail and the generalized Kelvin relation was obtained. Consequently the fundamental equation for spherical interface is expressed by

$$dE = TdS + \mu dN - p_{\alpha} dV_{\alpha} - p_{\beta} dV_{\beta} + \gamma dA + (\partial\gamma/\partial a) A da ,$$

where the last ambiguous point of the conventional Gibbs treatment is eliminated. This method is being utilized in the thermodynamic treatment of surface tension ("Molecular theory of surface tension in liquids" written in collaboration with Prof. S. Ono, Tokyo University, for the Handbuch der Physik Vol. 10).

\* (cf. J. Chem. Phys. 25, 662-669, 1956).

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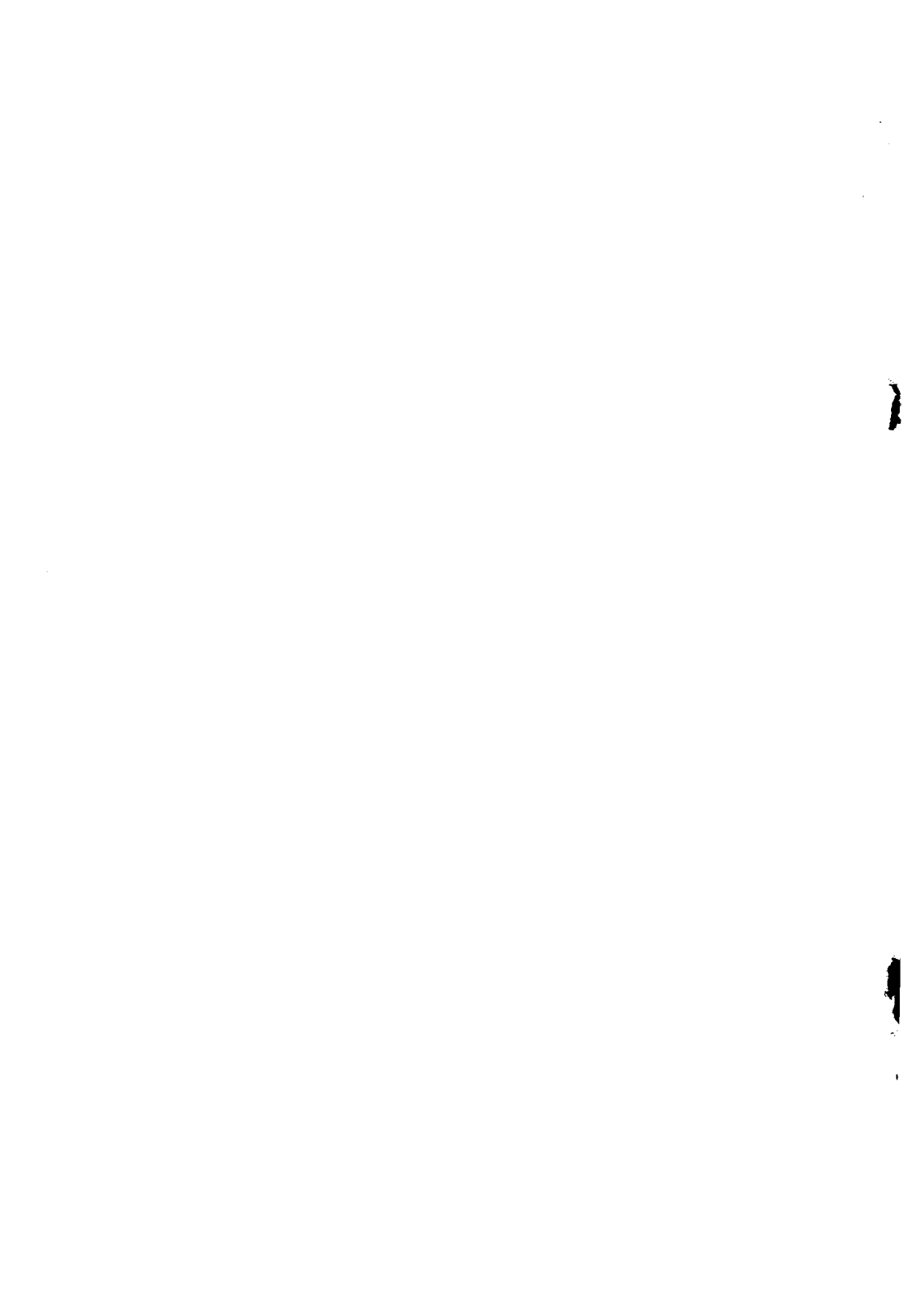
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