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THE NATIONAL INSTITUTE OF GENETICS
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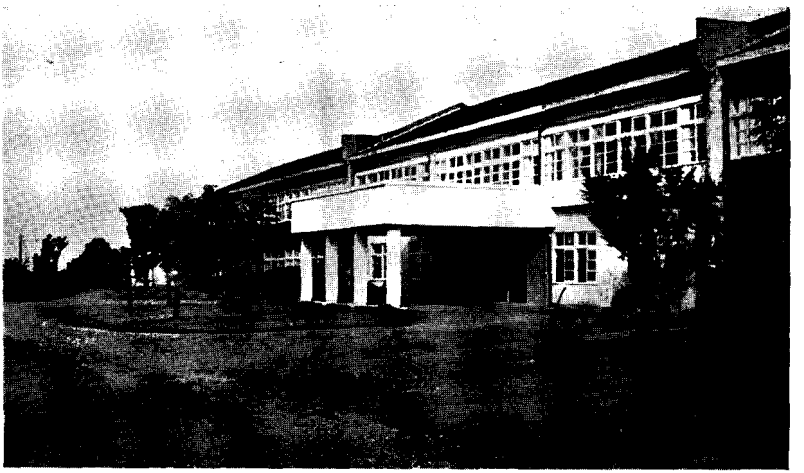
National Institute of Genetics

No. 5, 1954



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The Emperor examining a microscopic preparation

GENERAL STATEMENT

1954 was an eventful year for the Institute. Fortunately, all the main events were welcome ones. A new Department, that of Applied Genetics, was added to the preexisting four departments. This new department has two Laboratories, one of Applied Animal Genetics and the other of Applied Plant Genetics.

On June 1, the Institute celebrated its Fifth Anniversary, with the attendance of more than two hundred guests and about eighty members of the Institute. The Director made a report on the past history and present status of the Institute. Several guests spoke congratulatory remarks. After the ceremony, the guests were shown the buildings, equipment and experimental fields. Public lectures by the staff were given in Tokyo and Numazu in commemoration of this celebration.

On October 18, the Institute received a group of 48 visitors who were participants in the Congress of the Food and Agricultural Organization of the United Nations which was in session in Tokyo.

Next, on November 4, the Institute was honored by the visit of H. I. M. the Emperor. The Biologist Emperor spent five busy hours

in inspecting equipment and hearing reports on researches from the staff. He showed keen interest in these talks and demonstrations.

Hiko-Ichi OKA, D. Ag., Yukio YAMADA and Takatada KAWAHARA were newly appointed researchers during the current year, OKA as a laboratory head in the Department of Physiological Genetics, while YAMADA and KAWAHARA belong to the new Department of Applied Genetics. Katumi TANAKA, D. M. was appointed Research Associate; his specialty is human genetics.

SAKAI was promoted to the Head of the Department of Applied Genetics. MATSUMURA was sent abroad by the Government. He spent seven and a half months in the United States and Europe, visiting various genetics institutes and inspecting equipment and research projects, primarily those concerned with radiation genetics and polyploidy breeding. He also attended the Seventh Biological Research Conference held in April, 1954 at Brookhaven National Laboratory N. Y., U. S. A. and the Eighth International Botanical Congress held in July, 1954 at Paris. YOSIDA was awarded D. Sc. from the Hokkaido University. KIMURA took a leave of absence for another year to study mathematical genetics in the Department of Genetics of the University of Wisconsin. IINO also received a leave of absence for a year to study biochemical genetics in the same department of the University of Wisconsin.

The whole set of buildings and equipment for poultry breeding, as well as a residential house, which had belonged to the Whole-Japan Association for Poultry Genetics, were donated by the Association to the Institute for the use of the Department of Applied Genetics. The building for silkworm culture was extended to 81.71 tubo. The new section, 50 tubo in area, has some new arrangements which are rarely found in other sericultural houses in the country. A 30 tubo house for the temporary storage of crops from the experimental field was constructed. Two new residential houses were added to the group of such houses located in the detached campus.

The library has been steadily expanded by the acquirement of new books, periodicals and reprints. Many of these were given by scientific institutes or associations, or by individuals. Especially, Dr. GOLDSCHMIDT has continued to send in reprints and current numbers of various scientific journals which amounted to 1,079.

The mousery has acquired 15 new strains of mice from the

mouseries of Oxford University, Ohio State University and the National Institute of Preventive Hygiene in Tokyo. Various new strains of wheat, *Aegilops*, sugar beet, morning glory, *Iris* and radish were added to our collections.

Among the foreign visitors, besides those in the group of FAO congress participants, were: Dr. R. R. GATES of Harvard University; Mr. Khin MAUNG and Mr. San PE, Burmese rice breeders; Dr. R. A. SILOW and Dr. K. RAMIAH of FAO; Dr. L. N. H. LARTER, British plant breeder stationed at Singapore; Dr. N. PARTHASARATHY of the Central Rice Research Institute of India; and Dr. D. P. RAICHOUDHURY, Mr. C. L. VERNA and Mr. S. C. DAS, Indian silkworm geneticists and breeders.

The following grants were received during the current year by our staff. These grants have been of great aid to our research projects.

From the Fund for Grants-in-Aid of the Ministry of Education to Cooperative Investigations: to K. OGUMA and co-workers (including T. KOMAI, S. MAKINO, T. H. YOSIDA and K. TUTIKAWA), for: Cytological and genetical researches on tumors—¥670,000; S, MATSUMURA and co-workers, for: Researches on the physiology of crop plants under standardized temperature, humidity and day-light conditions—¥430,000: Y. TAKENAKA and co-workers, for: Genetics of morning glory—¥250,000.

From the Fund for Grants-in-Aid to Investigations in Applied Sciences: to Y. TANAKA, for: Research on artificial control of diapause in the wild silkworm, *Antheraea pernyi*—¥360,000; M. TSUJITA, for: Researches on the effect of environment in the manifestation of hereditary characters in the silkworm and its practical application to breeding—¥90,000.

From the Fund for Grants-in-Aid to Individual Workers: to M. TSUJITA, for: Electron-microscopical studies on the cytology of the silkworm—¥80,000; M. TSUJITA, for: Cyto-genetic studies on micro-organisms by means of ultra-thin sections—¥60,000; K. SAKAI, for: Population-genetic studies on the contamination of rice crop by mixture of "red rice"—¥90,000.

From the Fund for Grants-in-Aid to Young Research Workers, to K. GOTOH, for: Genetics on the mechanism of racial differentiation of the strain "Hosogara No 2" of barley—¥27,000; T. ENDÔ, for: Biochemical genetics of flower colors of *Viola tricolor*—¥25,000;

S. TSUDA, for: Studies on the propagation of bacteriophages—¥25,000; B. SAKAGUCHI, for: biochemical-genetical study on pterin metabolism in silkworm—¥27,000; S. NAWA, for: Chemistry of pterin occurring in insects and its genetics—¥20,000; T. ISHIHARA, for: Cytological and histological studies on the testis of male tortoiseshell cat—¥15,000; C. MATSUI, for: Genetic study of bacteriophages—¥20,000.

From the Fund for Grants-in-Aid for Promotion of Improvement of Agricultural Techniques of the Ministry of Agriculture and Forestry: to K. SAKAI and co-workers, for: Comparative studies between pedigree method and bulk method in plant breeding—¥95,000. (Taku KOMAI)

ABSTRACT OF DIARY FOR 1954

Jan. 14. Tenth meeting of the Board of Councillors.
Jan. 22. Twenty-third meeting of Misima Geneticists' Club.
Feb. 13. Board meeting of Association for the Propagation of the Knowledge of Genetics.
Feb. 22. Twenty-fourth meeting of Misima Geneticists' Club.
March 25. Twenty-fifth meeting of Misima Geneticists' Club.
April 23. Twenty-sixth meeting of Misima Geneticists' Club.
May 17. Symposium on Population Genetics.
May 24. Twenty-seventh meeting of Misima Geneticists' Club.
June 1. Ceremony of the Fifth Anniversary of the Institute.
June 9. General meeting of Whole Japan Association of Poultry Genetics.
June 22. Eleventh meeting of the Board of Councillors.
June 25. Twenty-eighth meeting of Misima Geneticists' Club.
Aug. 7. Public lectures in commemoration of the Fifth Anniversary in Numazu City Hall.
Sept. 20. Twenty-ninth meeting of Misima Geneticists' Club.
Oct. 8. Thirtieth meeting of Misima Geneticists' Club.
Nov. 4. H. I. M. the Emperor's visit.
Nov. 5. Public lectures in commemoration of the Fifth Anniversary in Yomiuri Hall in Tokyo.
Nov. 13. Meeting of Arrangements Committee for the International Genetics Symposia.
Nov. 19. Thirty-first meeting of Misima Geneticists' Club.
Dec. 20. Thirty-second meeting of Misima Geneticists' Club.

STAFF

Department and Laboratory Heads

Kan OGUMA, D. Ag., Director
Yoshimaro TANAKA, D. Ag., D. Sc., Head of Department of Morphological Genetics
Taku KOMAI, D. Sc., Head of Department of Physiological Genetics
Yô TAKENAKA, D. Sc., Head of Department of Cytological Genetics
Mitsuo TSUJITA, D. Ag., Head of Department of Biochemical Genetics
Kan-Ichi SAKAI, D. Ag., Head of Department of Applied Genetics
Seiji MATSUMURA, D. Ag.
Tosihide H. YOSIDA, D. Sc.
Kôzô HAYASHI, D. Sc.
Hiko-Ichi OKA, D. Ag.

Part-time Staff and Research Associates

Hitoshi KIHARA, D. Sc., Professor of Kyoto University
Sajirô MAKINO, D. Sc., Professor of Hokkaido University
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Hideo ETÔ, D. M., Assistant Professor of Tokyo University
Kazuo FURUSATO
Yoshinari KUWADA, D. Sc., Emeritus Professor of Kyoto University
Flora Alice LILIENFELD, Ph. D.
Yasunosuke OZAKI, D. M.
Katumi TANAKA, D. M.

Junior Investigators

Motô KIMURA, on leave of absence
Kanji GOTOH
Tôru ENDÔ
Akira MIYAZAWA
Kiyosi TUTIKAWA
Bungo SAKAGUCHI
Tetuo IINO, on leave of absence
Toshifumi TAIRA
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Saburô NAWA
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Masao MIYAZAWA, Head of Finance Section
Naomi MATSUBARA Hiroko NAKANO Junzô KADOWAKI
Clerks, Typist, Telephone operator, Chauffeur, Field laborers, Janitors, etc.—18

Misima Branch of Hatano Tabacco Experiment Station

Masao TANAKA, Head Seiji IMAI
Flora Alice LILIENFELD *Assistants*—4

Whole-Japan Association of Poultry Genetics

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Yoshimaro TANAKA, Vice President and Director of Researches

Association for Propagation of the Knowledge of Genetics

Kan OGUMA, President
Yô TAKENAKA, Managing Director
Seiji MATSUMURA, Managing Director

COUNCIL

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Kin-ichirô SAKAGUCHI, Professor of Tokyo University
Takeo SAKATA, President of T. Sakata & Company, Yokohama
Masatada TERADA, President of Jikei Medical College
Bungo WADA, Professor of Tokyo University

RESEARCH PROGRAM FOR 1954

DEPARTMENT OF MORPHOLOGICAL GENETICS

First Laboratory (TANAKA)

Studies on unstable genes in the silkworm (TANAKA)
Linkage tests in *Bombyx mori*, for making up chromosome maps (TANAKA)
Experiments on the control of diapause in *Antheraea pernyi* (TANAKA)
Genetical studies on the Japanese long-tailed fowl (TANAKA)

Second Laboratory (MATSUMURA)

Radiation genetics in wheat and barley (MATSUMURA and FUJII)
Studies on *Agropyron*, a genus related to *Triticum* (MATSUMURA and SAKAMOTO)
Nullisomic dwarfs in the offspring of pentaploid wheat hybrids (MATSUMURA)
Mutations in tobacco induced by X-ray (MATSUMURA, KIHARA and FUJII)
Relation between the induced mutation and the quality of radiation (MATSUMURA ond ETÔ)
Physiological and genetical studies of crops under control of temperature, moisture and day length (MATSUMURA *et al.*)
Improvement of crops by means of induced triploidy (MATSUMURA *et al.*)

Third Laboratory (KOMAI)

Genetics of human microcephaly (KOMAI, KISHIMOTO (Nagoya Univ.) and OZAKI)
Partial sex-linkage in man (K. TANAKA)

DEPARTMENT OF CYTOLOGICAL GENETICS

First Laboratory (YOSIDA)

Cytological study of tumors (YOSIDA and ISHIHARA)
Study on differentiation of sex-chromosomes (YOSIDA)
Genetical study on the tumor susceptibility of animals (YOSIDA and ISHIHARA)
Study on development of resistance of tumor cells to anti-cancer agents (YOSIDA and ISHIHARA)

Histological and cytological study of the testes of male tortoiseshell cats (ISHIHARA and YOSIDA)

Second Laboratory (TAKENAKA)

Origin of sex differentiation in higher plants (TAKENAKA)
Induction of abnormal mitosis and inhibition of growth by substances extracted from certain plants (TAKENAKA)
Cytogenetics of *Nicotiana* (TAKENAKA, FURUSATO and LILIENFELD)
Cytogenetics of *Citrus* (FURUSATO)
Genetic studies of *Pharbitis Nil* (TAKENAKA)
Collection and preservation of useful strains of plants (TAKENAKA)
Classification of Gramineae by karyotypes (TATEOKA)

Third Laboratory (TSUJITA)

Electron-microscopic studies on minute structures of cells of the silkworm (TSUJITA and TSUDA)
Electron-microscopic studies of microorganisms (TSUJITA and TSUDA)

DEPARTMENT OF PHYSIOLOGICAL GENETICS (KOMAI)

First Laboratory (KOMAI)

Population genetics of the lady-beetle, *Harmonia axyridis* (KOMAI)
Population genetics of the butterfly, *Neozephyrus taxila* (KOMAI)
Population genetics of the land-snail, *Bradybaena similaris* (KOMAI)
Population genetics of *Drosophila rufa* (TAIRA)
The *T* locus in *Mus musculus molossinus* (TUTIKAWA)
"*Alopecia periodica*" in mice (TUTIKAWA)
Tumor susceptibility in mice (TUTIKAWA)
Breeding and preservation of mouse and rat strains useful for medical investigations (OGUMA, MAKINO and TUTIKAWA)

Second Laboratory (OKA) and *Third Laboratory* (SAKAI)

Analysis of genes responsible for hybrid sterility in rice plants (OKA)
Analysis of genes controlling temperature respose in germination of rice plants (OKA)
Heritability of various characters and genetic correlations in rice plants (SAKAI and OKA)
Population-genetical studies on wild rice plants (OKA)
Physiological-genetical studies on cultivated plants under regulated

temperature, humidity and day-light conditions (MATSUMURA *et al.*)
Local differentiation of races in barley (GOTOH)

DEPARTMENT OF BIOCHEMICAL GENETICS (TSUJITA)

First Laboratory (TSUJITA)

Biochemical-genetical studies on pteridine compounds in insects (NAWA)
Embryological and genetical studies of the silkworm (TSUJITA and SAKAGUCHI)
Genetical and biochemical studies of some insects (TSUJITA and SAKAGUCHI)

Second Laboratary (HAYASHI)

Chemical analysis of coloring substances of various varieties of morning glory and their genetic behavior (HAYASHI and ABE)
Chemical composition of pigments in autumn leaves and alpine plants (HAYASHI and ABE)
Biochemical-genetical study on the colors of *Viola tricolor* (ENDÔ)
Bicchemical studies of color variation in flowers (HAYASHI)
Relation between flavonoid pigments and the corresponding genome in *Triticum* and allied species (ENDÔ)

Third Laboratory (HAYASHI)

Biochemical genetics of *Ustilago* (IINO)
Transduction and transformation of antigen type in *Salmonella* (IINO)

DEPARTMENT OF APPLIED GENETICS (SAKAI)

First Laboratory (TANAKA)

Breeding for high egg production in poultry (TANAKA and KAWAHARA)
Genetics of hereditary tremor found in Plymouth-Rock fowl (KAWAHARA)
Heritability and genetic correlation of economic characters in poultry (YAMADA)
On color changes of Plymouth-Rock fowl (TANAKA)
Variation in quantative characters in inbred animals (YAMADA)

Second Laboratory (SAKAI)

Studies on competition between individual plants of different genetic composition (SAKAI *et al.*)

Theoretical and experimental studies on selection in autogamous plants (SAKAI)
Genetics of "red leaves" in the tobacco plant (SAKAI and IYAMA)
Genetics of quantitative characters in eggplant (GOTOH)
On the hybrid between water-melon and *Citrullus colocynthis* (FURUSATO and MIYAZAWA)

Research Students and Research Subjects

Mizuho YOSHIDA: Genetics of silkworm
Setsuji KATAOKA: Cytogenetics of plants
Seiji TEZUKA: Cytogenetic studies on tobacco plants
Kôzô NAKAMURA: Cytogenetics of *Agropyrum*
Yasuo OTA: Cytogenetic studies on morphological characters of *Citrus*
Tarô AKIYAMA: Genetics and breeding of *Citrus*
Tôyô MITARAI: Fundamental morphological studies of animal cells
Toshio OMURA: Cytology of cancers
Kyôzô WATANABE: Cytogenetical studies on *Paramecium*
Chiaki MATSUI: Morphological studies on transformation of bacteriophages
Osamu YOSHIZAWA: Genetical studies on bacterial viruses
Shin-ya IYAMA: Population-genetics of upland rice
Yasuo SUZUKI: Population-genetics of crop plants

RESEARCHES CARRIED OUT IN 1954

A. HUMAN GENETICS

1. Genetics of Microcephaly

(by Taku KOMAI)

The study which had been in process for some years in cooperation with K. KISHIMOTO, M.D. of Nagoya University and Y. OZAKI, M.D., Research Associate of our Institute, was completed and sent to press during the current year. The material consisted of 93 male patients and 50 female patients. This was analyzed by using HALDANE's method (1938). Our original view that the great majority of these cases are probably due to a recessive autosomal gene has been sustained. The dominance of the normal gene over the gene for microcephaly is nearly always complete, but in exceptional cases the gene for abnormality manifests itself to a slight extent. The possible cause of the striking preponderance of males among the patients was looked for by making a distinction between the "primary" cases (probands) and "secondary" cases in the sibship. Thus, it has been disclosed that this sexual disparity among the patients is probably due in a large measure to a "social" cause in that male patients are more readily found outside their homes than female patients and become the "primary" cases.

Neither the birth order nor the mother's age seems to exert any influence on the production of a microcephalic child.

As previously reported, the incidence of the gene for microcephaly among the Japanese people is 0.0034-0.0063, and the rate of new mutation of this gene has been estimated as $(2.20\text{-}7.57)\times10^{-5}$.

Reference: KOMAI, T., KISHIMOTO, K. and OZAKI, Y. 1955. Genetic study of microcephaly based on Japanese material. Am. Jour. Human Genet. 7: 51-65.

2. Partial Sex-linkage in Man

(by Katumi TANAKA)

a) Estimation of the frequency of partially sex-linked genes. The frequency of an autosomal recessive gene may be given by the formula:

$$r=c(1-k)/(16k-15c-ck)\ldots\ldots\ldots\ldots\ldots\ldots(1),$$

where k and c are the rates of first cousin matings among the parents of the affected individuals and in the general population respectively. This formula is based upon the expectation that the probability of obtaining a homozygous child from a first-cousin mating is given by $(r/16+15r^2/16)$. Formula (1) is not applicable to partially sex-linked genes, since the probability is variable according to crossover values and the type of cousin mating.

The probability of getting an affected child in the mating in which the couple are the children of brothers (type 1), when a common grandparent of the cousins had the gene in question, is given by $d_1=pq(1+2p^2+2q^2)/8$, that in the mating of the children of sisters (type 2) by $d_2=(1+2p^2+2q^2)/32$, that in the mating of a son of a brother and a daughter of a sister (type 3) by $d_3=p(1+4q^2)/16$, and that in the mating of a son of a sister and a daughter of a brother (type 4) by $d_4=q(1+4p^2)/16$; where p denotes the crossover value of the gene and $q=1-p$.

The frequency of a partially sex-linked recessive gene may be estimated more accurately by means of the following formula:

$$r=\frac{(1-k)\sum_{i=1}^{4}d_ic_i}{(1-k)\sum_{i=1}^{4}d_ic_i+k-c}\ldots\ldots\ldots\ldots\ldots\ldots(2),$$

where c_1, c_2, c_3 and c_4 are the incidences of the four types of cousin marriages respectively in the general population. Actual instances of 466 cousin marriages collected from various parts of Japan are distributed in the proportions $c_1=18.9\%$, $c_2=30.0\%$, $c_3=23.2\%$ and $c_4=27.9\%$.

When these relations are taken into consideration, values 12–15% lower than those in NEEL *et al.*'s estimations are obtained as the frequency of the gene responsible for total color-blindness.

b) Unequal sex ratios among affected children of cousin and non-consanguineous marriages in partial sex-linkage. It is expected that for partially sex-linked traits there should be an excess of affected daughters over affected sons among the children of consanguineous marriages. However, no indication of such a tendency seems to have been found in the actual data.

The author has collected cases of congenital total color-blindness

from the Japanese literature, and also through personal communications with clinicians. The data are summarized in the table.

Relationship of parents	Number of affected children			
	Total	Males	Females	Sex-ratio
(a) First cousins	76	36	40	0.90
(b) Non-consanguineous	38	31	7	4.45

The sex ratio among affected children of cousin matings (group a) indicates only a slight excess of females, the difference being not significant. However, there is a remarkable excess of males among affected children of non-consanguineous matings (group b), the difference between groups (a) and (b) being significant at the 0.1% level. It appears thus that consanguineous matings produce relatively more affected females than non-consanguineous matings. More careful analysis has revealed that the excess of male patients among the offspring of non-consanguineous matings is only due to the tendency that more male patients than female patients are ready to visit hospitals in large cities, from which the great majority of the data are derived.

B. GENETICS AND CYTOLOGY OF SOME MAMMALS

3. *Further Studies on T Locus in the Japanese Wild Mouse, Mus musculus molossinus*

(by Kiyosi TUTIKAWA)

To examine whether the locus *T* in the Japanese wild mouse, ***Mus musculus molossinus***, is also mutable as in the other subspecies, ***Mus m. musculus***, wild mice caught in three localities were tested for the alleles at this locus.

The method of identifying *t*-alleles in ***molossinus*** populations was previously reported (Ann. Rep., No. 4: 13, 1953). Small samples of wild mice obtained from Misima, Niigata and Fukuoka were tested. All these samples contained some animals heterozygous for a *t*-type allele similar to that in ***musculus***.

Wild mice from Misima—The samples were from two sources: a male (9515) and a female (9613) caught in a rice-field at Kawaharagaya, Misima, and several males and females trapped in the barn in our Institute. Of the eighteen males and nine females tested,

Table 1. Results of testing mice of wild population Misima.

Wild parent	F_1			Wild parent	F_1		
	Normal	Brachy	Tailless		Normal	Brachy	Tailless
♂				♀			
m-129	10	13	—	*m*-185	8	5	—
m-1331	4	4	—	*m*-21029	9	11	—
m-3012	2	1	—	*m*-318	5	10	—
m-3611	2	3	—	*m*-42132	10	6	—
m-410	2	3	—	*m*-43122	2	3	—
m-42131	9	7	—	*m*-4463	1	2	—
m-42133	3	2	—	*m*-5323	2	6	—
m-42134	5	3	—	*m*-9613	1	—	—
m-4227	5	4	—	Totals from 8 ♀♀ +/+	38	43	—
m-425	7	2	2	*m*-4411	7	1	1
m-43121	12	9	—	Total from 1 ♀ +/*tm*	7	1	1
m-4321	3	4	—				
m-444	6	6	—				
m-4462	19	7	21				
m-8529	4	4	—				
Totals from 15 ♂♂ +/+	93	72	23				
m-306	4	—	6				
m-802	10	—	14				
m-9515	3	1	5				
Totals from 3 ♂♂ +*tm*?	17	1	25				

five males and one female produced some tailless offspring (Table 1). Five of these tailless mice produced by a male (4462) was tested against the tailless line (*T/t*), and gave Brachy offspring. Another wild male (425) produced 7 normals, 2 Brachys and 2 tailless. This male probably had no tailless gene but had a modifier which shortens the tail of *T*/+, since the six normal F_1 males, when tested against Brachy gave only normal and Brachy offspring. On the other hand, a F_1 tailless mouse produced by one female (4411) was crossed to the known tailless line, and to date has produced 4 normal and 16 tailless progeny. This result suggests that this F_1 tailless was perhaps

Table 2. Results of testing mice of wild population Niigata.

Wild parent	F_1			Wild parent	F_1		
	Normal	Brachy	Tailless		Normal	Brachy	Tailless
♂				♀			
n-61	13	14	—	*n*-62	1	2	—
n-6102	12	9	—	*n*-68	6	3	—
n-64	5	6	—	Totals from 2 ♀♀	7	5	—
n-69	5	4	—				
Totals from 4 ♂♂ +/+	35	33	—				
n-63	14	2	8				
n-65	7	—	10				
Totals from 2 ♂♂ $+/t^m$ or t^m/t^m	21	2	18				

T/t^m. It is probable that t^m represents a new allele occurring in *molossinus*. Detailed analysis of its genetic behavior is now in progress.

Wild mice from Niigata—The samples were caught in a barn at Wanô, Nishikambara-gun, Niigata Prefecture. The results of testing these wild mice are shown in Table 2. Two wild males were diagnosed as $+/t^m$ (or t^m/t^m). Their tailless offspring were probably T/t^m, since when crossed with tailless mice of the known balanced-lethal stock, they never yielded any Brachy young (12 normal and 15 tailless). The F_2 descendants from one F_1 male include 4 normal and 9 tailless. These results seem to indicate that the new t^m allele is viable, and gives normal tails when homozygous.

Wild mice from Fukuoka—The samples were sent us through the kindness of Dr. Yoshi-Kuni HIRAIWA and Mr. Satoru MINAMI of the Zoological Laboratory, Faculty of Agriculture, Kyushu University. One of the two males has to date produced 10 normal, 3 Brachy and 16 tailless offspring. The detailed analysis of these tailless mice is in progress.

4. *Test for Allelism of 'alopecia periodica' and 'furless' in the House Mouse (Mus musculus musculus)*

(by Kiyosi TUTIKAWA)

Alopecia periodica (Ann. Rep. No. 3: 9–10, 1953) is a new mutant which appeared spontaneously in 1951 in an inbred strain of mice (B 72) kept in this Institute. The mutant is characterized by periodical shedding of coat hair.

Recently, in 1954, a mutant with a similar phenotype *furless* was reported by Dr. E. L. GREEN of Ohio State University. Genetic examination was made by crossing the alopecia mice with the furless animals kindly supplied us by Dr. GREEN.

The six litters raised by matings of male furless (*fsfs*) with female alopecia (*apap*), consisted of 39 normal mice. The reciprocal crosses gave 32 normals (six litters). In the F_2 generation there were 183 normal, 57 alopecia, 59 furless, and 17 double recessive types. Tested against the 9:3:3:1 ratio there is satisfactory agreement between the observed and expected numbers ($\chi^2=0.624$, $n=3$). This clearly indicates that *ap* and *fs* are due to mutually independent autosomal genes which are neither allelic nor linked.

The alopecia mice are characterized by thin and lustrous skin; the furless mice may be distinguished from alopecia mice by having no vibrissae at birth. The double recessive type possesses both of these characteristics. Its growth, especially in the stage of the first coat appearance, is much retareded. In some severe cases, these mice weigh only about 1/3 as much as normal mice of the corresponding stage. The alopecia mice start shedding their coat hair at the age of from 13 to 15 days, and at the age of 20–24 days the depilation is usually complete except in some regions, especially around the muzzle. The furless mice begin to lose their first coat at about the age of 19 days, the depilation proceeds gradually backward and covers the entire body within ten days. The double recessive mice show a similarity to furless mice in the mode of depilation. Detailed observation on the interaction of *ap* and *fs* genes is now in progress.

5. *Histological and Cytological Studies of the Testes of Male Tortoiseshell Cats*

(by Takaaki ISHIHARA)

It is known that the male tortoiseshell cat is sterile. I have

studied the testes of five cats of this type. In no case has normal spermatogenesis been observed. The testes contained a few germ cells besides some SERTOLI's cells. Most of the spermatogonial cells showed signs of pycnotic degeneration, and they have never developed to the spermatocyte stage. In only one individual the testis contained a few 1st spermatocytes, but these showed some abnormality. In some there were some spermatogonial cells at the metaphase stage of mitotic division which was apparently more or less abnormal.

C. CYTOLOGY AND GENETICS OF TUMORS

6. *A Simple Squash Technique for Observation of Chromosome Structures in Normal Somatic and Malignant Tumor Cells*

(by Tosihide H. YOSIDA)

Recently, particular attention has been given to cytological study as an important aspect of cancer researches. In many recently reported experiments the usual squash technique and staining with acetic orcein, aceto-carmine or aceto-gentian violet are used. This technique does not show details in the structure of the chromosomes. The writer has developed a simple squash technique which gives excellent results in studies of chromosomes in tumor cells as well as in normal somatic cells. The technique is especially fitted for investigations of chromrsomes in ascites tumor cells. It consists of two simple procedures, pretreatment of material with hypotonic RINGER's solution and staining with acetic orcein.

(i) A small amount of ascites tumor or a piece of animal tissue is placed on a slide glass. (ii) Two or three drops of hyponic RINGER's solution are immediately applied. The concentration of RINGER's solution should vary according to the kind of tumor or tissue: in general, 10 to 20 per cent solution is good for many kinds of material. (iii) After treatment for 10 to 20 minutes, the solution is removed with blotting paper. (iv) The material is fixed and stained with acetic orcein. (v) After 5 to 10 minutes, a cover slip is placed over the material, light pressure is applied, and the slip is sealed with paraffin.

Photomicrographs of chromosomes in a male germ cell and tumor cells examined by using this technique are given in Figs. 1-5.

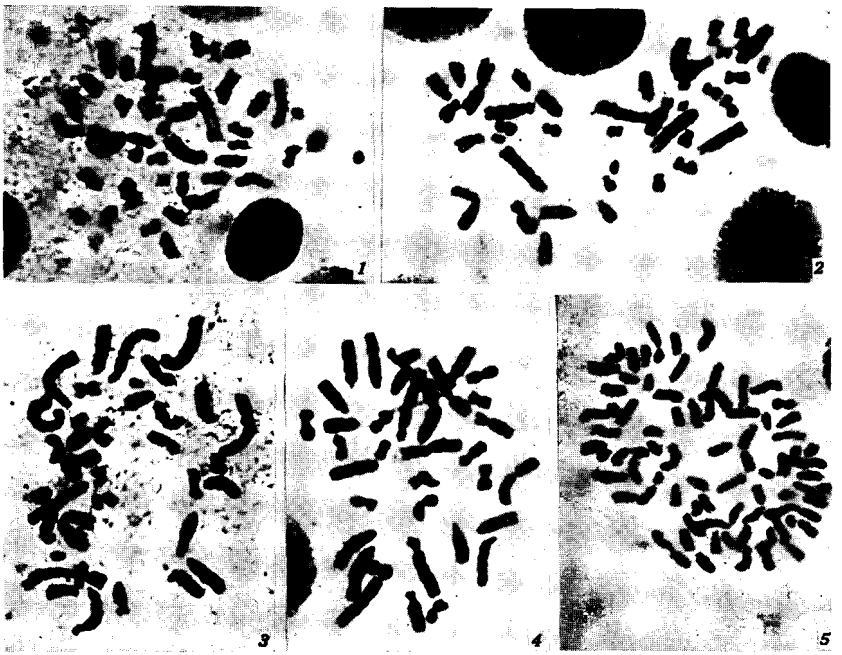


Fig. 1. Chromosomes of a spermatogonial cell of the rat. Fig. 2. Chromosomes of a YOSHIDA sarcoma cell. Fig. 3. Chromosomes of a tumor cell of the MTK-sarcoma II. Fig. 4. Chromosomes of a Hirosaki sarcoma cell. Fig. 5. Chromosomes of a tumor cell of the EHRLICH ascites carcinoma in the mouse.

7. *Chromosome Constitutions in Male Germ Cells of the Rat*

(by Tosihide H. YOSIDA)

Investigations of chromosomes in male germ cells of the rat (*Rattus norvegicus*) have been carried out by several workers (ALLEN '18, PINCUS '21, PAINTER '28, MINOUCHI '28, OGUMA '35, MAKINO '42, '52). They all record rat chromosomes as having terminal centromeres. GUENIN ('48) analyzed the chromosomes in germ cells of the newborn rat and found nine pairs of metacentric chromosomes characterized by unequal arms. Recently, MAKINO and HSU ('54) analyzed the chromosomes in the embryonic somatic cells of the rat by means of tissue-culture technique, and demonstrated that eight autosomal

Figs. 1–4. Chromosomes of male germ cells of the rat. Figs. 3, 4. Serial alignments of the chromosomes. Fig. 3 corresponds with Fig. 1. Fig. 4 corresponds with Fig. 2.

pairs (Nos. 1, 4, 8, 11, 12, 15, 16 and 19) and the X-element were two-armed.

The present author examined the chromosomes in the male germ cells of newborn rats with the new squash technique. The results obtained are as follows:

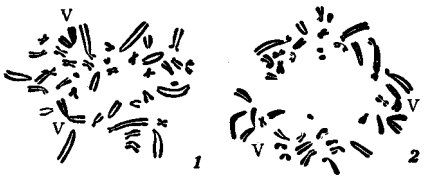
The number of the diploid chromosomes is 42, as recorded by the previous authors. The serial alignments of these chromosomes show 20 pairs of autosomes (pairs No. 1 to 20), and the X and Y elements (Figs. 1–4). Pairs No. 1, 2, 5, 10, 14, 17 and 20 are characterized by having subterminal constrictions, with the locus of the centromere differing in each pair. Pairs No. 3, 4, 6, 7, 8, 9, 11 and 15 have terminal centromeres. Pairs No. 12, 13, 16, 18 and 19 are characterized by having median or submedian centromeres. The elements X and Y are characterized by subterminal constrictions. It is a noteworthy fact that the splitting of the chromosomes, which could be observed by means of the author's technique, never occurs synchronously in all of the chromosomes. As shown in the figures, the splitting of some autosomes, as well as of the X-element, may be more or less delayed.

8. *Characteristics of V-shaped Chromosomes Occurring in Tumor Cells of the* YOSHIDA *Sarcoma*

(by Tosihide H. YOSIDA)

Karyological studies of the YOSHIDA sarcoma cells of the white rat (*Rattus norvegicus*) have been made by several investigators (MAKINO '51, '52; MAKINO & YOSIDA '49, '51, '52). They have found in these cells the characteristic V- or J-shaped chromosomes. MAKINO by careful observations has confirmed the presence of stem-cells of the tumor

which are characterized by subdiploid chromosome complex (about 40 in numbers). This complex consists of 22 to 24 rod-shaped elements and 16 to 18 V-shaped elements including one particularly large V-shaped element.



I have examined many tumor cells with the aid of the new squash technique, and found that the majority of the tumor cells of the

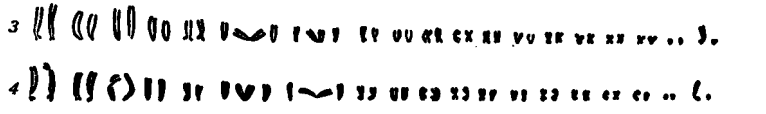


Fig. 1-4. Chromosomes of YOSHIDA sarcoma cells. Figs. 3, 4. Serial alignments of these chromosomes. Fig. 3 corresponds with Fig. 1. Fig. 4 corresponds with Fig. 2.

YOSHIDA sarcoma have 40 chromosomes including two large V-shaped elements. These two V-elements slightly differ in size from each other. The two arms in each element show a slight difference in size, moreover they often differ in the manner of splitting. From these observations it has been surmised that the two arms of the V-shaped elements are non-homologous and that these chromosomes have originated by terminal fusion of the non-homologous, rod-shaped chromosomes indicated as Nos. 6 and 7, and Nos. 8 and 9 in Figs. 1-4. All the other chromosomes exactly corresponed in their morphological characteristics to those in the normal somatic cells.

9. *Nature of V-shaped Chromosomes in Tumor Cells of MTK-Sarcomata II and III*

(by Tosihide H. YOSIDA)

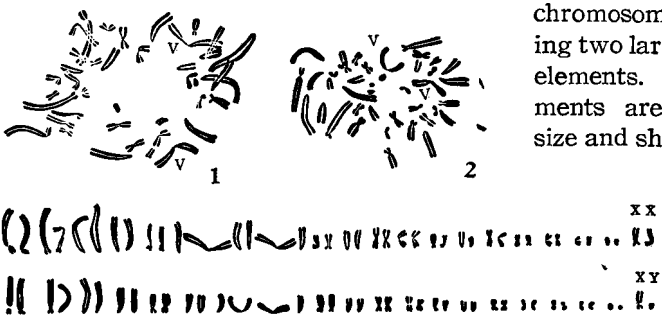
The MTK-sarcomata II and III represent two different strains of a transplantable ascites tumor which were induced by the application of azo dyes (TANAKA and KANÔ 1951, 1952, UMETANI 1954). According to MAKINO and KANÔ (1953), the tumor cells of the MTK-sarcoma II have a well-balanced complex of subdiploid chromosomes, 40 or thereabouts. A morphological analysis of the chromosomes, has revealed that the chromosome complex consists of two distinct

groups, one containing rod-shaped chromosomes and the other comprising V- and J-shaped elements. Among the latter group, the solitary large V-shaped chromosome is very prominent.

The chromosomes of tumor cells of MTK-sarcoma III have been observed by UMETANI (1954). According to him the chromosome number of MTK-sarcoma III varies widely from 26 to 82, with 40 as the mode. This tumor is characterized by having two strains of tumor stem-cells, of which one is characterized by having a prominent V-shaped element, while this element is missing in the cells of the other strain.

The present author investigated the chromosomes of MTK-sarcomata II and III with the new squash technique, and obtained the following results.

i) MTK-sarcoma II: Close microscopical examination of many tumor cells has revealed that most of the tumor cells contain 40 chromosomes, including two large V-shaped elements. These elements are similar in size and shape to those



Figs. 1, 3. Chromosomes of a tumor cell of the MTK-sarcoma II. Figs. 2, 4. Chromosomes of a tumor cell of the MTK-sarcoma III. Figs. 3, 4. Serial alignments of the chromosomes. Fig. 3 corresponds with Fig. 1. Fig. 4 corresponds with Fig. 2.

found in the YOSHIDA sarcoma cells. By comparing the chromosome set in the normal somatic cells with that in the MTK-sarcoma II cell, it has been concluded that both of the two large V-shaped elements probably arose by terminal fusion of rod-shaped chromosomes, one probably by fusion of No. 6 and No. 7, and the other by fusion of No. 8 and No. 9 (Figs. 1 and 3). This finding exactly corresponds with that made in the YOSHIDA sarcoma cells. A striking difference between the chromosome complexes of YOSHIDA sarcoma and MTK-sarcoma II may be noticed in the sex chromosomes. The former

sarcoma originated in the male rat, and consequently has X- and Y-chromosome sets, while the latter was derived from a female rat, and has two X-chromosomes.

ii) MTK-sarcoma III: The morphological characteristics of the chromosomes in this tumor nearly correspond with those of YOSHIDA sarcoma and MTK-sarcoma II. The number of chromosomes in many tumor cells was 40. The tumor cells usually include two large V-shaped elements. By comparing the chromosome sets of MTK-sarcoma III with those of the normal somatic cells, YOSHIDA sarcoma and MTK-sarcoma II, it has been revealed that one of these elements was probably derived from the terminal fusion of chromosomes No. 7 and No. 8, and the other from the fusion of the other member of No. 8 chromosomes and one No. 9 chromosome. This sarcoma includes the X- and Y- chromosomes. The remaining chromosomes exactly corresponded with those found in the normal soma, YOSHIDA sarcoma and the MTK-sarcoma II.

10. Karyological Characteristics of Tumor Cells of the Hirosaki Sarcoma

(by Tosihide H. YOSIDA)

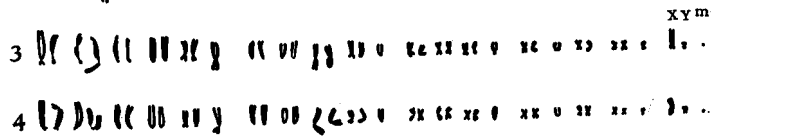
This sarcoma was established by Dr. USUBUCHI of the Hirosaki Medicai College, and seems to have been derived from a spontaneous epithelial tumor in a white rat (*Rattus norvegicus*). Observation of the chromosomes in this tumor have been carried out by MAKINO and KANÔ (1953), and KANÔ (1954). According to MAKINO and KANÔ (1953), the tumor cell possesses about 40 chromosomes, including certain numbers of rod-, V- and J-shaped elements of varying sizes. The cell is also characterized by having, without exception, prominent V-shaped chromosomes which vary in number from one to five.

Microscopical observations on many metaphase plates using the new squash technique have revealed that cell having 38 chromosomes occur most commonly. Among these chromosomes, there are usually four large V-shaped elements. Closer examination of these chromosomes has shown that all of them are of a composite nature, and seem to have originated by fusion of two original chromosomes in the following manner:

Composite chromosomes	Probable components
I	2a + 6a
II	6b + 17a
III	9a + 11a
IV	9b + 15a

One of these composite chromosomes was probably derived from the fusion of one No. 2 chromosome and one No. 6 chromosome; the second composite chromosome was probably derived from the fusion of the other member of the No. 6 chromosome pair and one No. 17 chromosome; the third composite chromosome, from the fusion of one No. 9 and one No. 11 chromosome; and the fourth composite, from the fusion of the other member of the No. 9 chromosome pair and one No. 15 chromosome (Figs. 1-4).

Another noteworthy fact about this sarcoma is that a very small dot-like element usually occurs in its chromosome complex. This element is similar in size



Figs. 1-4. Chromosomes of Hirosaki sarcoma cells. Figs. 3, 4. Serial alignments of the chromosomes. Fig. 3 corresponds with Fig. 1. Fig. 4 corresponds with Fig. 2.

and shape to the small knob of the No. 2 chromosome, and it is possible that the former originated by the detachment of the small knob of the latter.

11. A Further Study on the Transplantability of MY-mouse Sarcoma

(by Tosihide H. YOSIDA and Takaaki ISHIHARA)

The MY-mouse sarcoma is a kind of spindle cell sarcoma. It was developed in a So-strain mouse, which has not yet been established

as a pure inbred strain. It has been shown that the transplantability of this tumor shows considerable difference according to the strain of the host used (YOSIDA & ISHIHARA 1953). In our transplantation experiments, we found that the mice of D-strain showed a high transplantability. By selection of these mice we have succeeded in establishing a strain (D-103) showing a very high transplantability (92%). On the other hand, C3H strain mice commonly showed negative results regarding the transplantability of this tumor (YOSIDA & ISHIHARA 1953). The transplantability was examined in the F_1 hybrids between the two strains (D-103×C3H). In all of them the transplantation was successful. Accordingly, it was assumed that the transplantability of this tumor was controlled by some dominant genes (H-genes). To decide the number of these genes, the transplantability was examined in the backcross C3H×F_1; 25.72 per cent of the RF_1 hybrids showed a positive transplantability. Based on the above results, it has been surmised that about two dominant H-genes are concerned with the transplantability of the MY-mouse sarcoma.

It is noteworthy that the transplantability of this tumor to the S-strain has been recorded as high as 72.35 per cent (YOSIDA & ISHIHARA 1954). But the F_1 hybrid (S×C3H) usually shows negative results with respect to transplantation.

12. *Development of Resistance to Nitrogen Mustard N-oxide in the EHRLICH Ascites Carcinoma*

(by Takaaki ISHIHARA and Tosihide H. YOSIDA)

It is important from the medical and biological standpoints to investigate the mechanism of development of resistance against anticancer agents. In order to contribute something to this problem, we have experimented with the EHRLICH ascites carcinoma as tumor material, and nitrogen mustard N-oxide [methyl-*bis*-(β-chloro-ethyl)-amine-N-oxide] as anticancer agent.

The method was as follows: 5×10^6 of the EHRLICH ascites carcinoma cells were immersed in a solution (0.64 mg/ml) of nitrogen mustard N-oxide in vitro for one hour at 20°C. The treated tumor cells were injected into the intraperitoneal cavities of mice. When the transplantation showed a positive reaction, the tumor cells were again treated with the same solution in the same way as before; this procedure was repeated two or three times. As the result of this

treatment, we obtained two lines (SL-6 and RL-1) resistant against this chemical. Moreover, we were able to establish a resistant line (RL-2) from the untreated control tumor. By intraperitoneal injections of the chemical (0.32 mg/mouse), a marked reduction and disintegration of tumor cells occurred in the untreated control line, while in the resistant lines the reduction in the number of tumor cells was very small. After five successive treatments it appeared as if the tumor cells had developed a resistance to the lethal amount of this chemical, and SL-6 was established as the line resistant to the highest dosage.

To determine something of the nature of this resistance of tumor cells against the chemical, the following experiment was carried out. Some chemical such as ethoxy-nitrogen mustard N-oxide, urethane, colchicine, acriflavine or H_2O_2 was injected into the animal bearing a tumor, in which the resistance to nitrogen mustard N-oxide had been established. The tumor cells showed a sensitive reaction to such chemicals as urethane, colchicine, acriflavine and H_2O_2, while they were resistant to ethoxy-nitrogen mustard N-oxide. The tumor cells of the normal control line were very sensitive to all the chemicals used.

Based on the above experiment, it may be concluded that the resistance of tumor cells against nitrogen mustard N-oxide is independent of the resistance against the other chemicals.

13. Two Spontaneous Tumors Occurring in Inbred Rats

(by Tosihide H. YOSIDA and Takaaki ISHIHARA)

Both of these tumors spontaneously developed in the inbred rats of two pure strains W- and Wyne pink-eyed yellow reared in our laboratory. This report deals with the histological characters of these tumors and the results of some transplantation experiments.

i) Tumor in W-strain: A female of strain W-545 developed a peritoneal tumor in a lymph-gland of the mesentery. Histological observation revealed that this tumor was a reticulum cell sarcoma. It was transplanted into many rats, with negative results.

ii) Tumor in Wyne-pink eyed yellow rat: In this case the tumor developed on the left leg in a male. The histotogical study revealed it to be a kind of fibrosarcoma, which is characterized by short spindle cells. It was transplanted into six individuals of the strain in which it arose. All of them yielded positive results. By repeated transplantation experiments, this tumor was established as

a transplantable strain of fibrosarcoma.

The authors wish to express their gratitude to Prof. Kunio OOTA of the Cancer Institute of Tokyo for his important criticism of the histological characters of these tumors.

D. GENETICS OF POULTRY

14. A Sex-linked Nervous Disorder in the Domestic Fowl

(by Takatada KAWAHARA)

In a breeding stock hatched for replacement purposes in the spring of 1954, there were found some individuals afflicted with a nervous disorder characterized by a tremor of small amplitude of head and neck. The examination of the pedigree records has disclosed that all these individuals were derived from a single mating of a Barred Plymouth Rock sire SG_2–125 and his half-sib 307. This nervous disorder usually appears in chicks as young as one day old, but the appearance may be delayed to four weeks or more in some individuals. About half of the afflicted females are unable to stand up or walk when they reach the age of twenty weeks, and they eventually die. The other half reach sexual maturity, but often give poorer egg production than the normal pullets. As far as I know, five hereditary nervous disorders have been reported in the domestic fowl. Of these the "Shaker" described by SCOTT *et al.* (1950) seems to be very similar to the present case in symptoms of affliction. The two strains are, however, somewhat different in lethality and in histological characters of the nervous system. Two heterozygous and phenotypically normal males were mated with normal females and gave 21.1% afflicted females, the male offspring all being normal. The mating of F_1 afflicted females with the heterozygous males yielded 55.5% (females 24.4%, males 31.1%) afflicted chicks. The F_1 afflicted females mated with homozygous normal males produced no afflicted chicks. These breeding results indicate that this nervous disorder is due to a sex-linked recessive gene. Comparative histological examination of the tissues, particularly of the central nervous system, was made on the afflicted and normal chicks. The tissues were fixed in BOUIN's or MÜLLER's fluid, and stained with haematoxylin and eosin. In the cerebellum of the abnormal fowl, signs of degeneration of the PURKINJE cells, which are manifested first in the gyri region of cerebellum have

been observed. According to SCOTT *et al.*, in the "Shaker" breed the PURKINJE cells in the gyri regions degenerate later than those in other portions in the cerebellum.

15. Genetic Variation and Covariation in Economic Traits in Some Breeds of Chickens

(by Yukio YAMADA)

Estimations for heritability and genetic correlations were made from data of three years' records of some breeds of chickens, *i.e.* W. Leghorns, B. P. Rocks and R. I. Reds including 783 trapnested progeny which were sired by 57 males to 253 females. The birds studied were limited to those which had records for all traits to be analyzed in this study. The method used for estimation was based on combined sire and dam components of variance and covariance obtained from the pooled analysis within breed and year.

The traits investigated were sexual maturity, body weight and rate of egg production. Measurements of body weight were taken at the age of sexual maturity and at the age of about nine months. The rate of egg production in percent was measured for three periods, *i.e.* the winter rate from December 1 to the end of February, the spring rate from March 1 to the end of May and the total rate from the first egg to the end of May.

The heritability for sexual maturity has been estimated to be 0.480. This value is higher than the values so far obtained by other investigators, except the 0.522 reported by KING and HENDERSON (1954) assuming hatch effect. No maternal effect was found.

The estimate of heritability for body weight is 0.462, which agrees fairly well with the values reported by other investigators. The body weight at the first egg was estimated to have a heritability of 0.428.

Heritabilities for the rates of egg production have been found to be 0.313, 0.169 and 0.148 for winter, spring and total rate, respectively. WYATT (1954) reported the corresponding values to be 0.41, 0.38 and 0.51, respectively, on the basis of combined sire and dam components of variance, and also 0.11, 0.00, and 0.00, respectively, based on regression of offspring on parent. LERNER and CRUDEN (1948) reported the heritability of accumulative monthly egg production, in which an estimate of 0.145 was found for egg production in "production line" to the end of May; and WILSON (1948) obtained the value of 0.31 for production rate. Although the traits studied by them are not strictly

comparable with mine, the present estimates may be considered as fairly valid for production rate.

The genetic correlations involving sexual maturity, body weight and production rate have been estimated as follows: 0.003 for sexual maturity and mature body weight; -0.217 for sexual maturity and body weight at sexual maturity; -0.210 for sexual maturity and winter production rate; 0.173 for the winter production rate and body weight; 0.187 for winter rate and spring rate; 0.817 for spring rate and total rate; and 0.767 for body weight at sexual maturity and mature body weight.

These estimates of genetic correlations may be subject to rather large sampling errors, and further estimates based on the regression method are to be made. Further discussion and criticism will be attempted after additional information has been obtained.

E. GENETICS AND BIOCHEMISTRY OF SILKWORM AND OTHER INSECTS

16. "Small Egg", a Mutant in the Silkworm

(by Yoshimaro TANAKA)

"Small egg" is a recessive mutant which appeared in F_2 of the cross between a hereditary mosaic strain and a Chinese pure race, both of which strains had been kept under my own care for many years. The mutant gene is designated as *sm*.

The *sm* egg is smaller both in length and in breadth by about 20% than the normal egg of the same family. The *sm/sm* moths lay small eggs only, while +/*sm* females give batches of normal eggs only. All small eggs are unfertilized, hence we cannot breed a pure *sm/sm* strain.

1) Lethality of *sm*-gene: In the progeny produced by interbreeding +/*sm* individuals, the normal and small eggs segregate, but not exactly in a ratio of 3:1, the small egg type coming always short of expectation. There were, for example, 397 normal egg batches and 85 small egg batches among the total 482. From this and another sources, the lethality of *sm/sm* zygotes is calculated as about 35%. The lethal action seems to occur not in the embryonal, but in the larval and pupal stages, because no particularly large numbers of dead eggs are observed in segregating egg batches. This lethality must be taken into consideration when the phenotypic ratio is compared to the theoretical ratio.

2) Linkage of genes *sm* and *Ze*: The existence of linkage between *sm* and *Ze* (zebra marking in Chromosome III) was proved by the results of various matings, *i.e.* *Ze sm*/+ +♀×+ +/+ *sm*♂, *Ze* +/+ *sm* ♀×+ +/+ *sm* ♂, + *sm*/+ +♀×*Ze sm*/+ +♂ and + *sm*/+ + ♀×*Ze* +/+ *sm*♂.

3) Crossingover between *sm* and *Ze*: As is well known, crossingover takes place only in the male sex in *Bombyx*, so the crossingover values in the present case can be calculated exclusively from the data by the matings + *sm*/+ +♀× *Ze sm*/+ +♂ (repulsion phase) and + *sm*/+ +♀×*Ze* +/+ *sm* (coupling phase), *sm*/*sm* females being perfectly sterile.

The crossingover value obtained from the repulsion matings was 21.03%, while that calculated from coupling matings was 18.03%. The repulsion matings included as many as 5,974 batches (females) in total, while the coupling matings were only 893. For this and some other reasons, the figure 21.03% seems to be more reliable than the other.

17. Effect of Temperature in the Larval Stage on the Development of Multilunar and Multistar Markings

(by Mizuo YOSHIDA and Yoshimaro TANAKA)

A striking effect of incubation temperature on the size and number of brown spots composing the multilunar marking was reported by T. HIROBE (1951). Y. TANAKA (1954) observed similar results in the multilunnar as well as in multistar marking. The present experiment was undertaken to determine whether there is any influence of the rearing temperature on the development of the markings in question.

Three batches of multilunar strain and two batches of multistar strain were incubated at an intermediate temperature (20°C). Immediately before hatching, each egg-batch was divided into two halves, of which one was reared at a high temperature (25°C), and the other at a lower temperature (15°C). The markings in the fourth and fifth instars were carefully observed.

The result was distinctly negative; *i.e.* no trace of influence of the rearing temperature was found. We have come to the conclusion, therefore, that the distribution types of the above-mentioned markings, though they fully develop first in later instars, are determined before hatching, so as to be entirely immune to further environmental changes.

18. Genetical and Biochemical Studies on Yellow Lethal Silkworm Larvae. III. On the Property of Phenol Oxidase in this Mutant.

(by Mitsuo TSUJITA and Bungo SAKAGUCHI)

It is known that the embryos homozygous for the gene *lem*^{l} develop normally to the youngest larval stage and die immediately after the first moulting. We have been interested in the direct cause of this death, which is apparently due to imperfect differentiation of the mandibular cuticle and incomplete hardening of the cuticlar layer of the hypodermis. In a previous report, the maternal inheritance of this strain, and also biochemical studies of the larvae, with special reference to the difference in the nature of pterin and some other chemical substances contained in the normal and mutant larvae, were recorded. Further biochemical studies with the normal and the mutant strains were carried out, and the following results were obtained.

1) The phenol oxidase activity of the epidermal tissue in the larvae directly after the first moulting of the normal, *lem* and *lem*^{l} strains was measured by using a WARBURG manometer. A homogenate of the tissue in isotonic sucrose solution was prepared as the crude enzyme solution, and tyrosine or dopa was used as substrate. No difference in the activity of tyrosinase was detected among these strains, but the dopa oxidase activity in both the normal and *lem* larvae was stronger than that in the *lem*^{l} larvae.

2) The next question is whether or not the weak dopa oxidase activity in the epidermal tissue of the *lem*^{l} is due to a smaller quantity of the oxidase, or to the presence of some inhibitor of this enzyme. In order to ascertain this point, the following experiments were carried out. (i) The two crude enzyme solutions obtained by the above-mentioned procedure from the epidermal tissues of the + and *lem*, were mixed in the ratio 5:5, 7:3 or 3:7, and the dopa oxidase activity of these mixed enzyme solutions was measured by means of a WARBURG manometer. In these experiment, the enzyme activity is given by the mean of the activites of the two enzyme solutions. (ii) In order to make clear whether an inhibitor of dopa oxidase is present in the *lem*^{l} or not, a solution was extracted by phospate buffer solution (pH 6.4) from the homogenate of the first moulting larvae of the *lem*^{l}, and the solution was precipitated by saturated ammonium sulfate, and then the supernatant solution was filtered through WHATMAN No. 42 filter paper to remove the activity. The solution obtained by such a pro-

cedure was added to the crude enzyme solution extracted from the normal larvae, and the dopa oxidase activity was measured by the same procedure as that previously mentioned. The result shows that the enzyme activity was not inhibited by the addition of the solution. (iii) Therefore, it seems that the low activity of dopa oxidase in the epidermal tissue of *lem*^{l} is not due to the presence of an inhibitor, but that it is directly controlled by the gene *lem*^{l} itself.

3) Some + and *lem*^{l} larvae in the first moulting stage were homogenated and dividid into pigmental and residual fractions by the addition of an acidified methyl alcohol, and the metals contained in each of the fractions were traced by paper chromatography. Fe and Cu were found in the pigment fraction of the +, while Ni and Ti were detected in the fraction of the *lem*^{l}.

4) In order to know the effects of these metals on the dopa oxidase activity, metal ions of Cu, Fe, Ni and Ti were added to crude enzyme solution prepared from the larval epidermal tissue of the normal strain, and its enzyme activity was measured by the WARBURG manometer, by using dopa as substrate. The results have shown that Cu and Fe accelerate the enzyme activity, but Ni and Ti do not.

5) In order to ascertain the relation of xanthopterin-B obtained from the larval epidermal tissue to the dopa oxidase activity, an experiment was carried out by using as crude enzyme solution the homogenate prepared from the epidermal tissue of the normal strain. The dopa oxidase activity of the preparation, containing both xanthopterin-B and the crude enzyme solution, was measured by a manometer. No effect of xanthopterin-B on the dopa oxidase activity was found.

6) Based on these experimental results, the following hypothesis is proposed. The epidermal tissue of the *lem*^{l} can adsorb Ni and Ti, and the enzymes connected with these metals are closely related to the formation of xanthopterin-B. The tissue, however, shows only a slight tendency of adsorbing Cu which acts as an active center of tyrosinase, which in turn plays an important rôle in melanin formation, and this results in the production of a very small amount of melanin pigment.

19. *Comparative Studies of Yellow Lethal Lemon Larvae (lem^l) and Albino Lethal Larvae (al) of the Silkworm from the Viewpoint of Biochemical Genetics*

(by Mitsuo TSUJITA and Bungo SAKAGUCHI)

In both the *lem^l* and the *al* larvae, the mandibular cuticle does not develop properly, so that they cannot chew mulberry leaves, and starve to death directly after the first moulting. The lethal lemon larva contains in its epidermis a large amount of yellowish pigment which gives the body a yellowish color, while the lethal albino larva has a light brown color.

Comparative biochemical studies of these two lethal strains were carried out. The results may be summerized as follows:

1) Pterin. Extractions were obtained from the +, *lem*, *lem^l* and *al* larvae after the first moulting by N/2 ammonia, 80% methanol or water. Paper chromotagraphic analysis of the extracts was carried out for the detection of the pterin. Butanol acetic acid, 4% phenol or 3% Na-citrate were used as the developmental solvent of the analysis. Leucopterin, isoxanthopterin and riboflavin were detected in the *al* larvae, and xanthopterin-B in addition to these three substances was found in the *lem^l* larvae; the relative quantities of isoxanthopterin in these strains, have been shown to be $+ > al > lem^l$.

2) Comparison of phenol oxidase activity. By using a WARBURG manometer, the phenol oxidase activity in the epidermal tissue of the larvae directly after the first moulting of the four strains, +, *lem*, *lem^l* and *al*, was measured. The homogenate of the larval epidermal tissue (homogenized in isotonic sucrose solution) was used as crude enzyme solution. The four substrates, tyrosine, *p*-cresol, dopa and pyrocatechol were used. When tyrosine and *p*-cresol were used as substrates, no difference in the phenol oxidase activity could be detected among the three strains. However, when dopa and pyrocatechol were used as substrates, the enzyme activity in + and *lem* was found to be somewhat stronger than in *lem^l*. On the other hand, the phenol oxidase activity was weaker in *al* than in +, *lem* or *lem^l*, when all the four substrates mentioned above were simultaneously used. This tendency was especially clear in the case of the two substrates, dopa and pyrocatechol.

3) Comparison of cytochrome oxidase activity. The cytochrome oxidase activity in +, *lem^l*, and *al* larvae directly after the first moult-

ing was measured by SCHNEIDER and POTTER's procedure (1943). A homogenate of the tissue was prepared as the crude enzyme solution, and cytochrome c was used as the substrate. The oxidase activiiy was stronger in the normal strain than in *lem*^{l} or *al* larvae, but no difference was found between *lem*^{l} and *al* in this respect.

4) From these findings, the following conclusion may be deduced:

Although the lethal lemon and the lethal albino larvae show some phenotypical resemblance, they are different in their metabolic physiology. The gene *lem*^{l} is mainly responsible for the abnormal pterin formation, while the gene *al* has a smaller capacity of melanin formation. These physiological abnormalities have secondary effects upon other metabolic processes, and produce somewhat similar phenotypic expressions.

20. Biochemical Studies on the Hibernating Character in the Photoperiodism of a Wild Silkworm, Antheraea pernyi

(by Bungo SAKAGUCHI)

TANAKA ('41–'43, '50–'51) has reported that photoperiodism plays the most important role in determining the hibernating character of the Chinese tussar silkworm, *Antheraea pernyi* GUER. In order to make clear the biochemical background of this mechanism, the present experiments were undertaken. The results of the experiments may be summarized as follows:

1) Artificial control of the hibernating character has been attempted by injection of various chemical substances or by transplanting various organs into nonhibernating pupae. It has been found that blood, urine, brain or corpora allata of the pupae, or a mixed solution of substances of the citric acid cycle, Na-succinate or cytochrome c, are apparently effective in changing hibernating pupae to nonhibernating.

2) Fluorescent substances in the blood and urine of the hibernating and nonhibernating pupae were analyzed by paper chromatography. Greenish yellow, yellow and blue fluorescent substances were found in both of these pupae. In the nonhibernating pupae substances of intense purple and purplish blue color were detected in addition to the three substances mentioned above. Both these two substances seem to have a photo-decomposible character.

3) The cytochrome oxidase activity of the epidermal muscle was

measured by the WARBURG manometer. The enzyme activity in nonhibernating pupae was markedly stronger than in the hibernating ones. The activity of the enzyme was accelerated by the injection of urine and fluorescent substances extracted from the urine of nonhibernating pupae.

4) Enzymatic activity belonging to the citric acid cycle and appearing in epidermal tissue of the hibernating and nonhibernating pupae was tested by the modificatory method of GREEN ('50). The activity of each enzyme taking part in the cycle of the nonhibernating pupae was greater than in the hibernating pupae.

5) It may be surmised from these findings that the activity of cytochrome oxidase of the hibernating pupae may be activated by fluorescent substances in the urine and blood of nonhibernating pupae, and in turn a group of enzymes belonging to the citric acid cycle are activated.

Some of the fluorescent substances in blood and urine of the nonhibernating pupae are known to be decomposed by light. This fact seems to explain at least in part the close relationship between the hibernating character and photo-periodicity.

21. Biochemical and Genetical Studies on Wild Silkworm. II. On the Nature of the Pigments in the Epidermal Tissues of the Chinese Tussar Silkworm, Antheraea pernyi

(By Bungo SAKAGUCHI)

The chemical nature of the pigments in the epidermal tissue of the Eri-silkworm has been reported by the author (SAKAGUCHI 1952). In this work, the pigments in epidermal tissue of the Chinese tussar silkworm, in Eri-silkworm and in the silkworm are compared from the biochemical and genetical view points.

Detection of carotenoid pigments: The pigments were extracted with a mixture of petroleum ether and benzene from the hypodermal tissue of full-grown larvae. Several kinds of pigment in the extracts were separated by partition chromatography through a column of alumina, and then yellow, orange-yellow, orange, purplish fluorescent substances and a few other pigments were isolated. The pigments, except the fluorescent substances, were easily oxidizable and showed a peculiar reaction against H_2SO_4, HCl or $ZnCl_2$. Moreover, the

adsorption band showed three peaks between 400 and 500 mμ. These findings indicate that the pigments are composed of xanthophyll and carotene, which are found also in epidermal tissue of the Eri-silkworm and in the yellow cocoon of the silkworm.

Detection of fluorescent substances: The fluorescent substances were extracted by solvents, acidified ethanol or hot water, from the epidermal tissue of the full-grown larvae, and the extracts were analyzed by paper chromatography. In the larval epidemral tissue of the Chinese tussar silkworm, the same leucopterin and isoxanthopterin as those found in the silkworm and the Eri-silkworm were detected.

Permeability of the larval epidermal cells: The larvae of the Chinese tussar silkworm, of the Eri-silkworm and of the silkworm were fed with oak leaves painted with various synthetic pigments. It has been found that toluidine blue, neutral red and methylene blue can be adsorbed in the epidermal cells, and the hypodermis takes these colors. The pigments adsorbed in the cells in the posterior part of the body of the Chinese tussar silkworm, vanished after some time.

Conclusion: It seems clear from the above experimental results that among the three species of silkworms there are some differences in the carotenoid pigments and fluorescent substances contained in their epidermal cells as well as in the permeability of the pigments through the hypodermis. The synthesis of fluorescent substances and permeability of carotenoid pigment are probably controlled by gene action. The facts found in this experiment suggest some interesting problems regarding the differences in these physiological properties among the three silkworm species.

22. Pterins found in the Silkworm

(by Saburo NAWA)

It has been confirmed that the purple fluorescent snbstance detected in egg and larval epidermis in the normal strain of the silkworm is isoxanthopterin.[1,2]

The epidermis of the mutant lemon (*lem*) of the silkworm contains a yellow pigment which has been named xanthopterin-B[3]. We[4] have obtained 2-amino-4-hydroxypteridine-6-carboxylic acid through photodecomposition of this pigment. This shows beyond any doubt that xanthopterin-B is a derivative of 2-amino-4-hydroxypteridine.

These observations suggest that an abnormal metabolism of pteridine compounds exists in the mutant lemon.

It has been detected that the yellow pigment in the head of the mutant strains, *se* and *cl* of *Drosophila melanogaster*, is identical with xanthopterin-B found in *lem* of the silkworm. Recently, FORREST *et al.*[5] proposed the structure 2-amino-4-hydroxy-7, 8-dihydro-8-lactylpteridine-6-carboxylic acid for the yellow pigment.

1) HIRATA, Y. and NAWA, S. 1951. Compt. rend. soc. biol. 145: 651. 2) NAWA, S. *et al.* 1954. J. Biochem. 41: 657. 3) HIRATA, Y., NAKANISHI, K., and KIKKAWA, H. 1950. Bull. Chem. Soc. Japan 23: 76. 4) NAWA, S. and TAIRA, T. 1954. Proc. Japan Acad. 30: 632. 5) FORREST, H. S. and MITCHELL, H. K. 1954. J. Am. Chem. Soc. 76: 5658.

23. *The Relationship between Eye Pigment and Pterins of Drosophila melanogaster*[1]

(by Saburo NAWA and Toshifumi TAIRA)

The pterins found in *D. melanogaster* were investigated. The relative amount of isoxanthopterin contained in the whole body of the males of various mutants of *D. m.* separated by paper chromatography was measured by the fluorometric accessory set of a BECKMAN spectrophotometer.

The males of the age from 15 days after emergence were used as material. By the agreement in Rf value, fluorescence, ultraviolet absorption spectra and chemical nature, it has been confirmed that the fluorescent substances obtained from the material consist mainly of 2-amino-4, 7-dihydroxypteridine (isoxanthopterin) and the derivatives of 2-amino-4-hydroxypteridine. The relative amounts of isoxanthopterin in these mutants are as follows:—*v*, 54; *cn*, 48; wild type, 43; *pr*, 43; *dke*, 35; *st*, 35; *car*, 35; *cl*, 32; *ca*, 27; p^p, 17; *cm*, 12; w^a, 4; *rb*, 4; *bw*, *w* and *v*: *bw*, 0.

It has been found also that the yellow eye pigment contained in the *se* and *cl* mutants is a 2-amino-4-hydroxypteridine derivative and identical with xantopterin-B found in the mutant "*lemon*" of the silkworm.

The strains of *D. m.* without any red eye pigment, like *bw*, *w* and its alleles, have no pteridine compounds while the strains having red or yellow eye pigment, such as wild type, *cn*, *v*, *se* and *cl*, etc.,

contain pteridine derivatives in large quantities.

These data suggest that there is a close relationship between the red or yellow eye pigment and the pterins.

1) NAWA, S. and TAIRA, T. 1954. Proc. Japan Acad. 30: 632.

F. POPULATION GENETICS OF SOME INSECTS AND A LAND SNAIL

24. Polymorphism Found in Some Insects and in a Land Snail

(by Taku KOMAI)

a) The lady-beetle *Harmonia*. Some new population samples were obtained during the current year. The comparative study of a new sample from Suwa with older samples from the same locality has yielded some rather interesting results. The oldest sample from this locality dates back to 1912 while the newest one was obtained in the winter of 1954. Between these two extreme years, samples were taken in 1913, 1914, 1915, 1917, 1920, 1930, 1942, 1943 and 1950. It has been found by examining these samples, that a significant change in the composition of the population occurred during the period 1920–1950 which covers probably about 120 generations of the beetle. This change is perceived as a steady decrease of the red form (*succinea*) which is due to the recessive gene, and a concomitant increase of the darkest form (*conspicua*) which is due to the gene at the highest peak of dominance. The possibility of the effect of immigration from the adjacent colony has been ruled out by the examination of the ratio of the individuals provided with a ridge on the elytra. The presence or absence of this ridge is determined by a pair of mendelian genes, and the gene for presence is dominant over the gene for absence. The incidence of the gene in the colony shows a regular geographic gradient along the longitudinal axis of the Japanese island chain. It has been found that the incidence of the gene in the Suwa population has remained unchanged from 1920 through 1954.

This observation makes it almost certain that the regular change in the proportion of individuals with different elytral markings in the Suwa population was caused by selection. Based on this idea, the coefficient (s) of selection of the recessive form was calculated and the following results were obtained:

Period	*Number of generations*	*s*
1920—1930	40	0.00640
1930—1942, '43	50	0.00647
1942, '43—1950	30	0.00706

Thus, a rather uniform value 0.0064–0.00706 was found for *s*. From this valve of *s*, the rate of change in the incidence of the recessive gene Δq has been calculated, and the value 0.00093–0.00098 was obtained. It is to be noted that this value is too great, probably 100 times greater than what can be expected for any change due to natural mutation, and this last possibility is also excluded from the cause of this regular change in the composition of the population. My attempt to correlated this change to some regular change in the climatic condition in Suwa during the same period has been unfruitful so far, although the presence of such a correlation is most likely. At any rate, this finding seems to be interesting to the students of evolution in the sense that an actual instance of microevolutionary change in a natural population of an insect was witnessed during the life time of a man, and its process was followed, and its tempo and grade were numerically expressed in terms of gene incidence.

Reference: KOMAI, T. 1954. An actual instance of microevolution observed in an insect population. Proc. Japan Acad. 30: 970-975.

b) The lycaenid butterfly *Neozephyrus taxila*. The fifteenth population sample of this polymorphic butterfly was obtained from Nosé in Osaka Prefecture. This sample contained the four different types of females, *O* (uniform brown). *A* (with orange markings), *B* (with bluish suffusion) and *AB* (with both orange markings and bluish suffusion) in the following proportion:

Type	*O*	*A*	*B*	*AB*	*Total*
Number	106	31	99	26	262
%	40.46	11.83	37.79	9.92	100.00

$p=0.116$ $q=0.277$ $r=0.636$ $p+q+r=1.029$

$D=1-1.029=-0.029$ $\sigma D=0.0097$

If adjustment is made so as to make $p'+q'+r'=1$, then the values of p', q' and r' become, respectively, $p'=0.114$, $q'=0.273$ and

r'=0.613. From these values, the expected numbers of the four types may be obtained:

$$O'=98.452, \quad A'=40.034, \quad B'=107.210, \quad A'B'=16.308.$$

When each of these expected values is compared to the corresponding observed value, it is found that the observed value is higher for *O* and *AB* types and lower for *A* and *B* types than the expected value, and the difference between these values is significant. Thus, in this sample, as well as in several other samples previously examined, the double heterozygote *AB* and the recessive *O* types have a higher selective value than the single dominant types *A* and *B*, and this situation seems to be responsible for maintaining the polymorphism in a stable state.

Reference: KOMAI, T. 1953. Composition of wild populations in the lycaenid butterfly *Neozephyrus taxila*. Am. Nat. 87: 87-95.

c) The land-snail *Bradybaena*. More population samples were obtained from various localities in the country; thus the number of samples of natural populations of this polymorphic snail has been raised to 114. All these samples, except three only, contain the four types, *O* (yellow unbanded), *A* (yellow banded), *B* (brown unbanded) and *AB* (brown banded) in the proportion expected from the postulate that these types are determined by a set of triple-allelic genes, with no difference in selective value among them. In all the three exceptional samples, the observed proportions of *AB* and *O* types significantly exceed the corresponding expected proportions, while the reverse in the case with the *A* and *B* types.

Population samples were obtained by Prof. EMURA of Niigata University from several localities in Niigata, sometimes with a nearly 20-year interval. A few of these samples show a slight but significant temporal change in composition which took place during this interval. This change seems to be largely due to migration caused by a change of the topographic condition of the habitat which occurred during this time.

Reference: KOMAI, T. and EMURA, S. 1955. Genetic studies of the polymorphic land snail *Bradybaena similaris*. Evolution 9: (in press).

25. *Population Genetics on the Balanced Polymorphism in Drosophila rufa*

(by Toshifumi TAIRA)

The female of *D. rufa* in the *D. melanogaster* group is distinctly dimorphic. The "dark" type has a dark band on each abdominal segment, much like that in the male *melanogaster*. The "light" type has no such band, so that this type resembles the female *melanogaster*. The male is monoformic.

Such a polymorphism is rare in the Drosophilidae; so far it is known only in *D. montium*[1) 2)] and in *D. polymorpha*[3)] besides the present species.

Two natural populations, one in a village in Kôchi Prefecture and the other in Asakawa near Tokyo, have been examined monthly by using numerous traps. When the natural population is of the maximum size, the relative adaptive value of different genotypes has been found to be $D/d > D/D > d/d$.

On the other hand, in laboratory populations started at various initial proportions of each genotype, the relationship of the adaptive value in the equilibrium state is $D/d > d/d > D/D$.

The difference between the two populations seems to depend on the relative competitive ability in the larval stages of the heterozygote with the two homozygotes. In all cases, the heterozogote has some selective advantage over either homozygote. Such heterosis seems to be mainly responsible for the maintenance of balanced polymorphism.

1) WHEELER, M. R. 1949. Evolution 3: 268. 2) FREIRE-MAIA, N. 1949. *Ibid.* 3: 98. 3) DA CUNHA, A. B. 1949. *Ibid.* 3: 239; Nature (London) 171: 887 (1953).

G. POPULATION GENETICS OF RICE AND BARLEY

26. *Analysis of Genes for Hybrid Sterility in the Varieties of Cultivated Rice*

(by Hiko-Ichi OKA)

The sterility in hybrids between distant varieties of rice has been a problem of wide interest among workers on this plant, because such a study seems to afford them a key to classification of the varieties. The hybrid sterility in rice is characterized by the following

features: (1) Crossing between distant varieties is as easy as between nearly related ones; (2) The F_1 plants show no disturbance in chromosome pairing; (3) Micro- and macro-spores begin to deteriorate at a definite stage of development after the meiosis; (4) The degree of sterility, if measured by the percentage of good pollen, is not affected much by environmental conditions, and depends on the combination of parental varieties. The degree ranges from perfect fertility to nearly complete sterility. (5) The percentage of good pollen is generally similar to the percentage of seed setting; and (6) There is no significant difference between reciprocal crosses.

The F_2 of a semi-sterile hybrid generally shows a continuous variation in fertility. But when two related varieties, A and B, differing in fertility in the hybrid with C, are crossed in the manner $(A \times B) \times C$, there appears a discontinous variation, possibly because of the relative simplicity of genic difference between A and B. A few breeding examples are presented in the table.

Segregation of fertile and semi-sterile plants in some crosses of $(A \times B) \times C$ type.

Cross	% of good pollen																			Num. of plants
	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15			
563×(219×221)																				
Homozygote*	3	3 (9)	3				1	2 (6)	2	1										15
Heterozygote*	1	2 (6)	2	1				1		2 (11)	3	4	1							17
(108×143)×563		1	3	8	8 (37)	6	7	4	3	8 (25)	9	5	16	12 (42)	9	5				104
563×(108×143)					2	1 (7)	3	1		2 (9)	4	3	1	4 (14)	5	2	2			30

* for the glutinous gene

Based on this finding, genic analysis was conducted on the hybrid sterility. It has been revealed that the deterioration of gametes in hybrids is due to duplicate genes which act in the gamete as "development maintainers" (Gametic-Development, *GD*, genes). The segregatipn of fertile and semi-sterile plants, as well as of homo- and hetero-zygotes for the glutinous gene, in $563 \times (219 \times 221)$ shown in the above table, can be understood by assuming that the varieties 219, 221 and 563 are of such constitutions as "$+-x_1X_2$", "$gl-X_1X_2$"

and "$gl-X_1x_2$" respectively, where X_1 and X_2 are a set of duplicate genes for gametic development, the double recessive combination of which causes the deterioration of gametes carrying it. In this case, X_1 is linked with the glutinous gene *gl*. This linkage has been deduced from the fact that, as seen in the table, fertile plants were more numerous among the homozygotes for the glutinous gene, while semi-sterile plants were more numerous among the heterozygotes. Furthermore, as is inferrable from this linkage, glutinous pollen grains were much more numerous than non-glutinous ones in the semi-sterile heterozygotes.

The second cross shown in the above table can also be fully understood by assuming two sets of *GD* genes. In this case, however, it is seen that more semi-sterile plants were produced from $563\times(108\times 143)$ than from $(108\times143)\times563$. This behavior suggests that the pollen with a dominant combination of *GD* genes has lower fertilizing power, as the result of certation in favor of X_1x_2 or x_1X_2.

For testing the validity of these assumptions, the F_2 plants of "$A\times B$" were crossed witn "C", and in each progeny line the variation in good pollen percentage was examined. Moreover, the genotypes of the F_2 plants and their frequences were expected under the assumption of genes based on the "$(A\times B)\times C$" experiment. First, the probability that a given line carries the respective genotype was estimated from the data for each line. These values were summed up according to genotypes, and the sums of probabilities were compared with the expected frequencies of the genotypes. Next, the variation in fertility among all of these plants was compared with the expected frequency distribution of fertility, which was obtained by assuming that fertility variation due to various causes other than *GD* genes follows the normal distribution. The recombination values thus obtained from different sources were compared with each other. These tests have proved that all the data obtained from these experiments conform well with the hypothesis of *GD* genes.

27. Variation in Fertility due to Gametic-Development Genes

(by Hiko-Ichi OKA)

As mentioned in the former article, there are only one or two sets of "Gametic-Development" GD genes controlling the inheritance of sterility in some crosses of "$(A\times B)\times C$" type. But occasionally,

the outcome of such a cross shows a discontinuous variation with several peaks of frequency in the percentage of good pollen. In F_2 also, similar discontinous distribution may be found, though in most cases the variation appears to be continous. In these cases, the peaks are found at definite points of the frequency distribution, namely, 75-70%, 60-55%, 45-40% and so on, and the mode of distribution agrees well with that expected under the assumption of several sets of *GD* genes. This fact seems to indicate that the *GD* genes controlling fertility in a hybrid are rather numerous, and behave like polygenes.

Thus, the continuous variations usually seen in the F_2 and later generations may be explained by assuming certain sets of *GD* genes, though other genes modifying fertility may also exist. A biometric-genetical study is then needed for analyzing the variations in fertility. As an approach to such a study, the variation in fertility due to *GD* genes has been theoretically investigated.

In the case in which a set of *GD* genes comes into consideration, let the fertility in F_1 be r (=0.75), the frequency of semi-sterile plants in F_2 be $p(=\frac{2}{9-3s}$, where $1-s$ stands for the fertilizing capacity of double dominant pollen), and the propagation rate of semi-sterile plants be $1-s'$, the frequency of semi-sterile plants in F_n will be $p(pb)^{n-2}$, where b stands for $\frac{1-s'}{1-ps'}$. The frequency of fertile plants in F_n will then be $1-p(pb)^{n-2}$.

Considering k sets of *GD* genes, the frequency of fertile plants in F_n will be $\{1-p(pb)^{n-2}\}^k$. Then the mean fertility (M_{Fn}) and variance (V_{Fn}) may be written as follows:

$$M_{Fn}=\{1-(1-r)p(pb))^{n-2}\}^k$$

$$V_{Fn}=\{1-(1-r^2)p(pb)^{n-2}\}^k-(M_{Fn})^2$$

The computations based on these formulas of the frequencies of fertile plants, mean fertilities and variances in F_2 and later generations, have revealed that the hybrid sterility due to *GD* genes vanishes rapidly with inbreeding generations and may disappear almost entirely by F_4.

Analysis of variations in fertility with some actual data is still under way. The results so far obtained suggests that the variations in the progeny of semi-sterile hyprids are partly due the *GD* genes.

28. *Change of Segregation Ratio due to Gametic Development Genes*

(by Hiko-Ichi OKA)

When a set of *GD* genes, X_1 and X_2, operate in a hybrid, the segregation ratios of the genes linked with this X_1 or X_2 will be modified more or less. For instance, in a variety cross "$A-x_1X_2$" × "$a-X_1x_2$" (repulsion of A and x), if the recombination value between A and x_1 or a and X_1 is p, the ratio of the gametes A and a will become $1+p:2-p$, and the ratio of $AA:Aa:aa$ in F_2 will be changed as shown in the table below. ($1-s$ stands for the fertilizing capacity of double dominant pollen X_1X_2.)

Table showing the modified F_2 ratio of $AA:Aa:aa$ by the presence of a set of GD genes.

Repulsion: Coupling :	AA aa	Aa Aa	aa AA
Fertile	$(1+3p^2)-sp(1+p)$	$(2+6p-6p^2)-s(1+2p-2p^2)$	$(4-6p+3p^2)-s(2-p)(1-p)$
Semi-sterile	$2p(1-p)$	$2(1-2p+2p^2)$	$2p(1-p)$
Total	$(1+p)^2-sp(1+p)$	$2(1+p)(2-p)-s(1+2p-2p^2)$	$(2-p)^2-s(1-p)(2-p)$

In this table it is seen that the F_2 ratio may change from 1:2:1 to 1:4:4 (complete linkage, $s=0$), and the semi-sterile plants tend to be heterozygous for $A:a$. Then, the phenotypically deminant class, in which semi-sterile plants are included, will have a lower mean fertility value and larger fertility variance than the recessive class. If the frequencies of semi-sterile plants among plants with dominant and recessive phenotypes are x_D and x_R respectively, the differences between the two plant classes in mean fertility and fertility variance will become as follows:

Mean fertility difference	$-d(x_D-x_R)$
Fertility variance difference	$d^2(x_D-x_R)(1-x_D-x_R)$

($d=0.25$; the difference between fertile and semi-sterile plants in fertility). By use of these formulas, the values of x_D and x_R, and accordingly the recombination value of A and x, can be found from actual data.

The observed F_2 segregation ratios of various monogenic characters have been found to divert in the direction and to the extent theoretically expected. An example dealing with the apiculus coloration due to gene *C* is shown below.

The variatian in fertility among colored and colorless F_2 plants.

Cross (% of good pollen in F_1)	Color at apiculus	% of seed setting 100 90 80 70 60 50 40 30 20 10 0	No. of plants	Exp. No.	Mean	Variance
108×318	+	4 5 9 8 18 6 10 7 3 2 1	73	81.8	0.576	0.0544
(75%)	−	8 7 8 3 3 2 3 2	37	27.2	0.660	0.0470
1 ×325	+	6 12 13 9 21 21 8 4	94	117.0	0.485	0.0356
(60%)	−	9 18 17 6 6 3 2 1	62	39.0	0.520	0.0275

In these cases, by assuming one or two pairs of *GD* genes linked with $C:c$, the values of x_D and x_R can be estimated from the data. The frequency distributions for fertility computed from these values agreed well with the actual data of variations observed in both colored and colorless plant groups.

It has been noticed that in many crosses the glutinous gene, as well as the apiculus color gene (linked with the former), has a general tendency to decrease in frequency, while the phenol-reaction gene is relatively stable, when these are judged by their segregation ratio in F_2. For the glutinous gene, the tendency was found that the lower the F_1 fertility, the larger was the deviation of F_2 ratio from the 1:2:1 ratio.

29. Change of Gene Frequency in Hybrid Populations of Rice

(by Hiko-Ichi OKA)

Change of gene frequency is often seen in hybrid populations of rice, when they are propagated without any artificial selection. This may be due to various causes such as:

Gametic-Gevelopment (*GD*) genes: As mentioned in the previous article, if a pair of genes for a character, $A:a$, are tightly linked with a pair of *GD* genes, the ratio of $AA:Aa:aa$ in F_2 wil be $1:4-s:4-2s$, where $1-s$ stands for the fertilizing power of pollen having a double dominant combination of *GD* genes. Of these genotypes, *AA*, *aa* and about a half or $\frac{2-s}{4-s}$ of the *Aa* plants will be fertile, while the remaining half of the *Aa* plants will be semi-sterile. Let the frequencies of semi-sterile *Aa* plants, fertile *Aa*, *AA* and *aa* plants in F_2 be p, q_1, q_2 and q_3 respectively ($p+q_1+q_2+q_3=1$), and the propagation rate of the semi-sterile plants be u; the frequencies of *AA*, *Aa* and *aa* plants in F_n will be given by the following formulas:

$$AA: \frac{1}{2}q_1A+q_2(1+puA)-\left(\frac{1}{2}\right)^{n-1}q_1B\Big/1-p(1-u)A$$

$$Aa: \left(\frac{1}{2}\right)^{n-2}q_1(1+2puB)+p(pu)^{n-2}\Big/1-p(1-u)A$$

$$aa: \frac{1}{2}q_1A+q_3(1+puA)-\left(\frac{1}{2}\right)^{n-1}q_1B\Big/1-p(1-u)A$$

$$\left(A=\frac{1-(pu)^{n-2}}{1-pu};\ B=\frac{1-(2pu)^{n-2}}{1-2pu}\right)$$

When $s=0$, we have $p=\frac{2}{9}$, $q_1=\frac{2}{9}$, $q_2=\frac{4}{9}$ and $q_3=\frac{1}{9}$, and when $s=1$, we have $p=\frac{1}{3}$, $q_1=\frac{1}{6}$, $q_2=\frac{1}{3}$ and $q_3=\frac{1}{6}$ (*cf.* Table on p. 44). Putting these values into the above formulas, it is found that the ratio of $AA:aa$ in F_∞ is 2.5 : 1 ($s=0$) or 1.67 : 1 ($s=1$). The change will take place in a relatively few generations, because of the rapid elimination of the sterility due to *GD* genes.

However, such *GD* genes operating in the cross seem to be numerous in many cases. We should then consider a case where several *GD* gene sets are linked with *A* or *a*. In such cases, the exact computation is rather difficult. The following formulas, however, can be obtained under the assumption of the absence of recombination between those *GD* genes and $s=1$. Let the number of *GD* genes (or the number of chromosomes which produce sterility if recombined with $A:a$) be m, and the average recombination value between *A* or *a* and the *GD* genes be p, the frequency of *AA* or *aa* plants in F_n will be

$$\left(\frac{1}{2}-\frac{1}{2^n}\right)\pm\frac{3kP}{3-P}\left\{1-\left(\frac{P}{3}\right)^{n-1}\right\},$$

where $P=1-2p$ and $k=\frac{2^m-1}{4(2^m+1)}$.

Difference in propagation rate: When *AA*, *Aa* and *aa* plants have propagation rates 1, $1-hs$ and $1-s$ respectively, their relative frequencies in F_n will be as follows:

$$AA: \frac{1-\left(\frac{1-hs}{2}\right)^{n-1}}{2(1+hs)} \quad Aa: \frac{(1-hs)^{n-2}}{2^{n-1}} \quad aa: \frac{(1-s)^{n-1}-\left(\frac{1-hs}{2}\right)^{n-1}}{2(1+hs-2s)}$$

Computation according to these formulas shows that the frequency of *AA* or *aa* plants is determined largely by the value of s, and the

effect of the value of hs is relatively slight. When $hs=0$, the frequency of aa plants in F_n may be written as

$$\frac{2^{n-1}(1-s)^{n-1}-1}{2^{n-1}(1-2s)+2^{n-1}(1-s)^{n-1}-2s}$$

In addition to the causes discussed above, there may be some other causes which would affect the genotypic make-up of hybrid populations, such as the effects of sterility genes other than the *GD* genes, certation and competition. These will be dealt with at some other time. The frequency of plants having the recessive phenotype due to the gene for apiculus coloration, phenol reaction, or glutinous endosperm was examined with hybrid populations of F_2 to F_7 of several crosses between distant varieties. The data obtained were analysed by using the above formulas, and the values of parameters such as k for the *GD* genes and s for propagation rate were estimated. The frequencies of a gene in different generations calculated from those values agreed well with the actual data.

30. *Restriction of Gene Recombination in Hybrid Populations of Rice*

(by Hiko-Ichi OKA)

When two independent pairs of genes, $A:a$ and $B:b$, are linked respectively with two pairs of *GD* genes of the same set ($X_1:x_1$ and $x_2:X_2$), gemetes possessing a recombination of the former genes in the hybrid, such as AB in "$A-x_1$ $b-X_2$" × "$a-X_1$ $B-x_2$", will be eliminated in every generation, since they carry *GD* genes in double recessive combination. In addition to this, the lowering of fertilizing capacity of the X_1X_2 pollen will reduce another recombination, ab. In this case, the F_2 phenotypic ratio will be $2AB:3-s\,Ab:3-s\,aB:1-s\,ab$, where $1-s$ stands for the fertilizing capacity of the X_1X_2 pollen. Thus, the segregation ratio may appear as if it were due to the linkage of A and B.

Let the recombination values between $A-x_1$ and $B-x_2$ be p and q respectively, the frequencies of AB, Ab, aB and ab in F_1 gametic series and in F_2 phenotype will be as follows:

F_2 gamete		Frequency in F_2 phenotype (when $1-s=0.5$)
AB	$\frac{1}{3}(p+q-pq)$	$\frac{1}{15}(4+6p+6q-4pq-p^2-q^2-p^2q-pq^2+3p^2q^2)$
Ab	$\frac{1}{3}(1-q+pq)$	$\frac{1}{15}(5-p-6q+4pq+q^2+p^2q+pq^2-3p^2q^2)$
aB	$\frac{1}{3}(1-p+pq)$	$\frac{1}{15}(5-6p-q+4pq+p^2+p^2q+pq^2-3p^2q^2)$
ab	$\frac{1}{3}(1-pq)$	$\frac{1}{15}(1+p+q-4pq-p^2q-pq^2+3p^2q^2)$

The genes for apiculus coloration ($C:c$) and for phenol-reaction ($Ph:ph$) in rice are independent of each other. They showed, however, in the F_2 of a semi-sterile hybrid, 414×325, a segregation ratio as given below; repulsion between these two genes will be suggested.

Apiculus : / Ph.-reaction :	*Colored* +	*Colored* −	*Colorless* +	*Colorless* −	*Total*
Observed Number:	96	49	69	13	227
Exp. Num. (9:3:3:1):	127.8	42.5	42.5	14.2	$X^2=25.54$ ($p<0.01$)
„ ($p=0.086, q=0.305$):	92.8	49.3	65.1	19.3	$X^2=2.94$ ($p>0.20$)

Since both of the genes $C:c$ and $Ph:ph$ have been found to be affected by *GD* genes, the values of p and q may be computed by the maximum likelihood method, using the above formulas, assuming the F_1 genotype to be $\frac{C-x_1}{c-X_1}\frac{ph-X_2}{Ph-x_2}$. The recombination values $p=0.086$ and $q=0.305$ were thus found. The expected numbers based on these recombination values showed good agreement with the observed numbers, as shown in the above table.

This experiment may serve as an example to justify the theory that genes linked with the *GD* genes of the same set behave as if they were linked.

31. Do Chromosomes in Tetraploid Hybrids between Distantly Related Varieties of Cultivated Rice Tend to Pair Selectively?

(by Hiko-Ichi OKA)

In tetraploid F_1 hybrids between distantly related varieties of cultivated rice, the fertility often becomes higher, and quadrivalent chromosomes become somewhat rarer, than in the parental tetraploid varieties (CUA, L. D. 1951: Proc. Jap. Acad. 27 (1), OKA, H. I. 1954: Jap.

Jour. Genet. 29 (3)). Thus the tetraploid hybrid behaves like an amphidiploid, and this behavior suggests the occurrence of selective pairing of chromosomes derived from the same parent. However, as far as the writer's experience with such cases goes, no significant correlation is found between fertility and the number of quadrivalent chromosomes. Furthermore, it has been found that the fertility at the tetraploid level varies markedly according to the diploid prototype, and it tends to be high in the plants showing vigorous growth, while the tetraploid F_1 plants are generally more vigorous than their parents. These facts seem to suggest that the improvement in fertility in F_1 is mainly due to hybrid vigor.

The experiments reported here were conducted in order to evaluate the relative probability of these two alternative hypotheses. First, with two tetraploid hybrids showing high fertility in F_1, a pedigree breeding experiment was continued up to the F_7, by selecting in every generation plants with high fertility. It was found in this experiment that in every generation segregation of plants with different fertilities occurred and the mean fertility tended to go down gradually with the repetition of inbreeding generations, and that high fertility was always correlated with vigorous growth.

Also the F_2 segregation ratios for several monogenic characters (phenol-reaction, apiculus coloration, seed coat coloration and glutinous endosperm) were observed, by use of several tetraploid hybrids between distant varieties. All the segregation ratios thus found were between the 35:1 (random chromosome segregation) and 20.8:1 (random chromatid segregation) ratios to be expected in an autotetraploid hybrid.

In F_2 plants of a cross in respect to glutinous endosperm, the number of glutinous homozygotes (*gl gl gl gl*) was significantly smaller than the expected number. However, the F_1 gametic ratio of $++$: $+gl$: $glgl$, computed from the F_2 genotypic ratio, was as follows:

	Observed Frequency	*Expected Frequency*	
		Chromosome segregation	*Chromatid segregation*
+ +	0.328	0.167	0.214
+ *gl*	0.551	0.666	0.572
gl gl	0.121	0.167	0.214

This gametic series shows that heterozygous gametes (+ *gl*), which are to be increased by selective pairing, were not more than

the expected frequency based on the assumption of random pairing. The excess in ++ and the deficiency in *glgl* were found to be due to a certation in favor of non-glutinous pollen, in the same manner as in diploid hybrids.

These experimental results contradict the hypothesis that tetraploid hybrids between distantly related varieties of cultivated rice behave like an amphidiploid.

32. *Genetic Analysis of Differentiation of Local Strains in the Barley "Hosogara No. 2"*

(by Kanji GOTOH)

a) Statistical differences among local strains.

"Hosogara No. 2" is an old commercial six-rowed barley variety. As was noted in a preliminary report (Ann. Rep. No. 4, p. 31), this variety includes some distinct local strains in respect to growth habit and some other characters.

In the present study, population samples of six local strains were statistically examined for several agronomic characters. In order to analyze the interaction between the strains and local environments, contrasting experiments were concurrently carried out at the Kitami Branch of Hokkaido Agr. Exp. Stat., Kitami, and at the National Institute of Genetics, Misima.

The results of comparative experiments conducted in the years 1952 and 1953, have demonstrated some statistically significant differences among the strains, and that the greater part of these differences are governed by genetic factors.

Based on the degree of phenotypic similarities and dissimilarities, the six strains were classified into three groups X(*A*, *C*); Y(*D*); and Z(*E*, *F*, *G*). It has been confirmed that the decline in vigor of plants raised from grains produced at Misima affects the grain yield. Furthermore, the interaction between strains and local conditions is highly significant relative to grain size. Among the four strains, *C*, *D*, *E* and *G*, *C* showed the greatest change in grain size in response to the difference in environment, while *D* showed the slightest change. The range of variation in the date of heading in *A* strain coming from Kitami, was 22 days in both years at Misima, and the parent-offspring correlation for this character was very high (0.763**). This wide range of variation in the date of heading within the *A* strain seems to be governed by polygenes. Polygenic variation was also found

within *A* and *D* strains for other characters, such as ear length and ear density.

In F_1 hybrids between the three strains, *C*, *E* and *G*, heterosis was observed. The F_1 hybrid between *G* and *C* showed great vigor. In respect to various performances, the differences between the northern (*A*, *C* and *D*) and southern (*E*, *F* and *G*) strains were fairly wide. This suggests a correlation between local conditions and strain formation. It is also highly probable that the original population of "Hosogara No. 2." included an abundance of genetic variability.

Reference: GOTOH, K. Genetic analysis of varietal differentiation in cereals. I. Statistical differences found among local strains of a barley variety "Hosogara No. 2." Jap. Jour. Genet., (in press).

b) Difference in growth habits among local strains.

In cultivated plants, the date of heading, photoperiodic response and growth habit seem to be closely connected with strain formation, since they are the characters which seem to have been subjected to natural selection under the local environment. Experiments were designed to detect the differences in growth habit among the local strains.

The spring seeding experiments were carried out in 1952 and 1953 on the experimental grounds of the Institute. The samples of each local strain were also grown in the air-conditioned greenhouse maintained at 20°C under a whole day illumination.

The greater part of the plants of the X group originating in Hokkaido showed a fairly pronounced winter habit. The *A–S* strain of the same group, however, proved to be of a pure spring-habit type. The *D* strain was of moderate winter habit, and the *Z* group originating in southern localities was homogeneous for its spring habit. Most of the X and Y group plants and a few plants of the *A–S* strain and of the Z group had long auricles on the first leaves. Most of the X group plants and none of the Z group plants had hairs on the leaf sheath. In the *D* strain, the development of hair was strongly affected by environmental conditions. The gene for short basal bristle (rachilla hair) occurred mainly in the X group, though in low frequency.

In the air-conditioned greenhouse under the whole-day illumination, all plants of *A–S* and Z types produced ears 33 days after seeding on the average, and the number of leaves on the stem was 6 in all plants. With regard to this response, both types behaved similarly. However, some differences were found in the breadth of the leaves between the *A–S* and Z types, and in the presence (*A–S*) or absence (Z) of hairs

on the leaf sheath.

On the whole, my observations apparently have confirmed that differentiation of the various growth habit types among this variety is correlated with adaptiveness of the genotypes to the various local environmental conditions, and these types were developed under the influence of natural selection.

Reference: GOTOH, K. Genetic analysis of varietal differentiation in cereals. II. Various growth habits in local strains of the barley variety "Hosogara No. 2." Jap. Jour. Genet., (in press).

c) Competitive ability of local strains. Experiments were designed to examine the relation between the competitive ability of the growth habit types and their differentiation. The results of the experiments conducted in 1952 and 1953 with the *A*, *A-S*, *C*, *D*, *E* and *G* strains are as follows:

The competitive ability of each local strain was tested by comparing the relative respose of the strains to competitive conditions with three tester varieties in each test. Under competitive conditions, the plants of the tested strains and the plants of the tester varieties were grown alternatively in rows. Three barley varieties, Aizu No. 7, Shinsakigake and Akashinriki were used as testers in 1952 and Aizu No. 7, Shizuoka-shiro-rokkaku No. 1 and Suihu in 1953. By previous experiments by SAKAI and GOTOH (1952, Ann. Rep. No. 3, p. 58), the relative competitive abilities of the tester varieties were determined. Based on the results of these two years, it was estimated that the competitive ability of "Hosogara No. 2" is fairly high, although the evaluation was slightly different between the two years, and a considerable variation in this character was found among the local strains in 1953.

E and *G* strains of the Z group show a higher competitive ability than those of the other strains when tested at Misima. According to the analysis of variance of the data in 1953, the term of interaction between testers and strains regarding grain yield was statistically significant at the 1 per-cent level. The order of the competitive ability of the strains was

$$E > C > D > A\text{-}S.$$

These results suggest that the differentiation of the Z group from the original "Hosogara No. 2" has been accelerated by its high competitive ability in the southern localities in Japan.

To examine this point more accurately, the following experiment was started in 1952. Equal numbers of seeds of each strain of the Z group and those of *C* were mixed (*e.g.* *C*+*E*, *C*+*G*, etc.), and these mixtures were grown at Misima and at Kitami.

The constitution of these mixtures showed some changes when examined after one year, and the proportions of the Z type in each mixture tended to increase at Misima and to decrease at Kitami, though to a slight extent.

The *D* strain, which showed the highest yielding capacity among the examined strains in both years, had rather poor competitive ability.

Reference: GOTOH, K. Genetic analysis of varietal differentiation in cereals. III. Competitive ability of local strains in the barley variety "Hosogara No. 2." Jap. Jour. Genet., (in press).

H. STUDIES ON COMPETITION

33. Effect of the Number of Competing and Non-competing Individuals on Competitional Increment

(by Kan-Ichi SAKAI)

Two experiments were conducted to investigate the effect of different numbers of competing plants on a plant placed in the center. In the first experiment, two varieties of barley, SZ and CK, differing in competitive ability, were used. In the second experiment, two upland rice varieties, one of the "Japonica" type and the other, "Red Rice" of the "Indica" type, were used. The "Red Rice" is often found in the field among the ordinary Japonica-type plants as a weed. In both of these two experiments, one variety was planted individually and surrounded by, (a) six plants of the same variety, (b) five of the same and one of the other, (c) four of the same and two of the other, and so on, till all six plants were of the other variety. The distance between any two plants in the combinations was fixed at 11.5 cm.

In the first experiment, the competing plants among the six surrounders were grouped together to form a "competing area" around the center, while in the second, the arrangement of competing and non-competing plants was at random.

The analysis of variance of the data showed that in the experiments, the effect of the number of surrounding competitors was significant and could be shown by a rectilinear regression line. It was then concluded that the competitional increment or decrement of a plant would

be proportional to the number of competing ones surrounding it, when the inter-plant spacing is constant.

34. *Polygenic Analysis of Quantitative Characters under Influence of Intergenotypic Competition*

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

The theory of polygenic inheritance so far developed by Mather has not taken into consideration the effect of intergenotypic competition. However, in some characters of plants such as culm number, the effect of competition between different genotypes is known to be so large that it should not be ignored in analyzing variance components in hybrid populations. It has been theoretically demonstrated, on the ground of a few premises, that the competitional variance in hybrid populations can be partitioned into the following four components:

C': Variance due to competitional increments between dominant and recessive homozygotes for genes causing competition (c),

G': Variance due to competitional increments between hetero- and homozygotes (g),

M': Covariance between c and d (Additive genetical increment),

N': Covariance between g and h (Non-additive genetical increment).

By using these components, formulas were constructed for F_2 and other generations and for back-cross generations of autogamous plants as follows:

$$V_{F_2}: \quad \frac{1}{2}D+\frac{1}{4}H+(C'+M')+\frac{1}{2}(G'+N')+E_1$$

$$V_{F_3}: \quad \frac{3}{4}D+\frac{3}{16}H+\frac{3}{2}(C'+M')+\frac{3}{8}(G'+N')+E_1$$

$$\bar{V}_{F_3}: \quad \frac{1}{4}D+\frac{1}{8}H+\frac{1}{2}(C'+M')+\frac{1}{4}(G'+N')+E_1$$

$$V_{F_4}: \quad \frac{7}{8}D+\frac{7}{64}H+\frac{7}{4}(C'+M')+\frac{7}{32}(G'+N')+E_1$$

$$\bar{V}_{F_4}: \quad \frac{1}{8}D+\frac{1}{16}H+\frac{1}{4}(C'+M')+\frac{1}{8}(G'+N')+E_1$$

$$W_{F_2/F_3}: \quad \frac{1}{2}D+\frac{1}{8}H \qquad +\frac{1}{2}M' \qquad +\frac{1}{8}N'$$

$$W_{F_3/F_4}: \quad \frac{3}{4}D+\frac{3}{32}H \qquad +\frac{3}{4}M' \qquad +\frac{3}{32}N'$$

$$W_{F_4/F_5}: \quad \frac{7}{8}D+\frac{7}{128}H \qquad +\frac{7}{8}M' \qquad +\frac{7}{128}N'$$

$$V_{B_1}+V_{B_2}: \quad \frac{1}{2}D+\frac{1}{2}H+(C'+M')+(G'+N')+2E_1$$

$$V_{B_1F_2}+V_{B_2F_2}: \quad D+\frac{3}{8}H+2(C'+M')+\frac{3}{4}(G'+N')+2E_2$$

(Variances among line means are not given here, as they contain no competitional variance component.)

In some cases, C' and M' can be grouped into C'' (Additive competitional variance), and G' and N' into G'' (Non-additive competitional variance). In some cases all of these competitional variance components can be lumped together.

In order to test the fitness of these formulas to actual data, the inheritance of panicle number in two intervarietal crosses of rice plant was analyzed. The results are given in Table 1.

Table 1. Showing the result of experiments of "Pei-ku" × "Taichung No. 65".

Methods	Estimated values of variance components						Deviation mean square
	D	H	C''	G''	E_1	E_2	
Mather's formulas	6.992 ±1.798	−3.344 ±11.199			16.153 ±1.276	1.016 ±1.473	4.614
New formulas	2.993 ±2.013	−4.576 ±28.571	5.076 ±1.181	0.563 ±14.638	13.474 ±0.714	3.184 ±1.114	1.246

As shown in Table 1, the mean square for deviations of the expected values from the observed values is apparently smaller when the formulas inclusive of competitional variances are used. The heritability values for panicle number, computed from the variance components, are also found to be comparable with the regression coefficient of progeny line means on parental measurements when the formulas inclusive of competitional variances are used. This is, however, not the case when the usual formulas of Mather are used, as shown in Table 2.

Table 2. Comparing the heritability values according to Mather's formulas and according to the new formulas.

Generation	Heritability value due to		Parent-progeny regression
	Mather's formulas	New formulas	
F_2	0.247	0.080	0.083
F_3	0.330	0.098	0.124
F_4	0.336	0.103	0.144
F_5	0.382	0.105	——

The same variance component analysis was conducted repeatedly with another cross, "Taichung No. 65" × "O-chiam", and similar results were obtained at each time. Thus the heritability values estimated from variance components can yield reasonable values only when the competitional variance is taken into consideration.

Heritability value		*Covariance*
Competitional variance		F_2/F_3
Considered	*Not considered*	
0.138	−0.153	0.145

These experimental results seem to serve as a verification of the theory of competition and formulas presented by the writers. They also demonstrate that in biometric-genetical studies of quantitative characters, the effect of competition should be considered, when the character under discussion is affected by it.

35. *How does a Mixed Population of Autogamous Plants Change in Response to Intra-population Competition?*

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

When different varieties of self-fertilized plants are mix-planted, the relative frequency of these varieties will change with generations as the result of differences in relative propagation rate and/or in competitive ability. Let us consider a mixed population of two varieties, A and B, where A has a lower propagation rate, but higher competitive ability than B. We may denote the propagation rate of A as x, taking that of B as unity. As to the competitional increment in the propagation rate, it is assumed as a premise that an A plant will, in mixture with B, acquire a certain increment from each of the B plants standing in proximity to it, while the B plant loses the same amount. Since a definite design of planting is followed in an experiment, the number of plants surrounding a given plant can be expressed by a constant k. The number of B (or A) plants surrounding an A (or B) plant will then be kb (or ka), where a and b stand for the relative frequency of A and B plants, respectively, in the given population. Then let the frequencies of A and B plants in the n-th generation after mixing be a_n and b_n, respectively; a_n and b_n may be written as follows:

$$a_n = a_{n-1}(x + kb_{n-1}p)/W_n ,$$

and $b_n=b_{n-1}(1-ka_{n-1}p)/W_n$,

where $W_n=a_{n-1}(x)+b_{n-1}$.

In the same manner, let the initial proportion of A and B plants be a_0 and b_0 respectively; the proportion in the n-th generation will be related to the change in each generation as follows:

$$a_n=a_0\prod_{i=0}^{n-1}(x+kb_ip)/\prod_{i=1}^{n}W_i,$$

and $$b_n=b_0\prod_{i=0}^{n-1}(1-ka_ip)/\prod_{i=1}^{n}W_i.$$

Considering m varieties (A, B,...M), the same computation leads us to the following formula:

$$j_n=j_{n-1}\{x_j+a_{n-1}(p_a-p_j)k+b_{n-1}(p_b-p_j)k+\cdots+m_{n-1}(p_m-p_j)k\}/W_n.$$

From this formula it is found that the population will reach an equilibrium when the relation $pk=1-x$ (viz., competitive superiority and propagation rate cancel each other) holds good for every kind of genotype in the given population.

Data for a mixed population of two rice varieties (consecutive mix-growing in Formosa, where the first and second crops alternate) were analyzed by using the above formulas. The below table shows the observed and expected frequencies of "Taichung No. 65" mixed with "O-chiam".

The proportion of "Taichung No. 65" mixed with "O-chiam" in mix-harvested seed.

Year	Crop	Initial Proportion of Taichung No. 65					
		0.05		0.50		0.95	
		Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
1952	1st	0.068	0.048	0.510	0.489	0.933	0.948
	2nd	0.010	0.009	0.206	0.221	0.900	0.877
1953	1st	0.007	0.008	0.133	0.213	0.896	0.872
	2nd	0.006	0.001	0.064	0.057	0.778	0.719

In this calculation, the relative propagation rates are denoted by $1-q$ and $1+q$ in place of x and 1, and the values of p' (pk; difference in competitive ability) and q in the first and second crops are estimated separately by the least square method. The values of p' and q obtained are as follows:

	$1-q$	p'
First crop	1.056	−0.155
Second crop	0.716	−0.511

These values indicate that the competitive ability as well as the relative propagation rate of Taichung No. 65 against O-chiam is much lower in the second crop which grows under higher temperatures, than in the first crop.

The expected frequencis in the above table have been obtained by substituting these values of p' and q into the formulas. They seem to agree well with the observed frequencies. It may then be said that the mixed population of self-fertilized plants behaves according to the rule given in these formulas.

36. *Competition Experiment with Diploid and Autotetraploid Races of Rice*

(by Kan-Ichi SAKAI and Yasuo SUZUKI)

Four varieties of rice and their autotetraploid races were tested to investigate their competitive ability. All the varieties used, Sekitori, Sensho, Sen-Ichi and Sin-Riki, were of the "Japonica" type. The split-plot design with four replications was used throughout the experiment. Planting was made individually in rows, the interval between the rows being 30 cm, and the interplant spacing 12 cm. The competitive ability is expressed by the difference between the mixed plot and pure stand plot in quantitative characters such as plant weight, culm number, panicle number, panicle weight and seed weight, taken on an individual plant basis.

Analysis of variance of the data showed that the effect of competition was highly significant in all of these characters. As an example, mean plant weights in the two chromosome races in pure stand and in mixture are given below.

Mean plant weight in diploid and autotetraploid races of four varieties of rice, each in pure stand and in the mixture with the other chromosome race of the same variety (in gm).

	Diploid		Autotetraploid	
Variety	*Pure*	*Mix*	*Pure*	*Mix*
Sekitori	30.45	38.73	22.62	21.45
Sensho	29.84	32.35	25.02	24.88
Sen-Ichi	27.85	34.32	19.75	15.15
Sin-Riki	35.55	45.57	21.11	19.27

The table shows that in all these four varieties the autotetraploid races had lower competitive ability than their diploid progenitors. This agrees well with the result of a similar experiment with barley varieties reported in the preceding issue of this Annual Report.

37. Competition Experiment with two Nicotiana Specics and their Allotetraploid.

(by Kan-Ichi SAKAI and Yasuo SUZUKI)

In order to estimate the competitive ability of allopolyploid plants, an experiment was conducted with *Nicotiana alata* (n=9), *N. plumbaginifolia* (n=10) and the allotetraploid synthesized from the two species, *N. diplumbalata* (n=19). Planting was made with the plant to be examined in the center, and six plants to compete with the central plant around the latter. The interspace between any two plants was fixed at 24 cm. The split-plot design was employed with three replications. Plant height, capsule number and flower number were measured on an individual plant basis. Analysis of variance of the data obtained showed that the effect of competition was highly significant for plant weight and for flower number.

Mean values of plant weight, capsule number and flower number in pure stand and in mixture are given in the following table.

Mean values of plant weight, capsule number and flower number of three Nicctiana species in pure-stand and in mixture with one of the other two species.

A: *N. alata* P: *N. plumbaginifolia* AP: *N. diplumbalata*

Characters		Competing species			Mean value
		A	P	AP	
Plant weight (gm)	A	78.9	49.4	53.9	56.4
	P	153.2	116.6	125.7	142.0
	AP	183.7	82.9	108.9	115.0
	Compet. effect	104.0	82.9	96.2	L.S.D. (5%) = 5.81
Capsule number	A	24.5	9.5	12.6	15.5
	P	65.9	40.7	48.6	51.7
	AP	30.6	20.4	17.8	22.9
	Compet. effect	40.3	23.5	26.3	

Flower number	A	4.8	1.1	0.7	2.2
	P	27.1	18.9	13.8	19.9
	AP	9.3	4.3	6.5	6.7
	Compet. effect	13.7	8.1	7.0	L. S. D. (5%)= 3.60

In the table it may be found that of the parental diploid species, *alata* is a weak competitor and *plumbaginifolia* is much stronger, while the synthesized *diplumbalata* behaved approximately the same as, or a little stronger than, the stronger parental species. It is suggested that allopolyploidy has an evolutional significance which is due to the superiority of such plants in competition.

I. RADIATION GENETICS OF WHEAT

38. Gene Mutations in Einkorn Wheat induced by X-rays

(by Seiji MATSUMURA and Tarô FUJII)

In order to study the relation between the frequency of gene mutations and the dosage or wave length of X-rays, dormant seeds of *Triticum monococcum* were exposed to X-rays at 180 KVP, 3mA, without a filter. The dosage ranged from 5,400 *r* to 13,500 *r* units. The results are shown in Table 1. The frequency of head progeny with induced mutations in the X_2-generation increases with X-ray dosage in a linear relation. There was some difference between the sensitivity of the varieties to X-rays.

Table 1. Relation between dosage or wave length of X-rays and frequency of induced mutations in T. monococcum.

Voltage (KVP)	Dosage (*r*)	% of head progeny in X_2 with mutants in			
		var. *flavescens*		var. *vulgare*	
Control	—	0/18	(0.00)	0/14	(0.00)
180	5,400	4/60	(6.67)	5/44	(11.36)
180	8,100	7/55	(12.73)	5/26	(19.23)
180	13,500	10/27	(37.04)	6/15	(40.00)
130	8,100	2/55	(3.64)	4/43	(9.31)
80	8,100	3/54	(5.56)	4/46	(8.69)

At the same dosage (8,100 *r*) and target distance, but with varying kilovoltage (80-180 KVP) and time of exposure, the mutation frequency increased with the decrease of wave length, though not in a linear relation (Tab. 1). In this case a very similar difference in sensitivity between the two varieties has also been confirmed. These findings on the relationship between the mutation rate and X-ray quality are largely in good accord with those in previous experiments which were primarily concerned with the frequency of chromosome aberrations. These facts can be explained on the basis of the difference in the distribution of ionization within the nuclei and the chromosomes: in hard X-ray irradiations, the majority of the resulting ionizations are more scattered than when soft X-rays are used.

About 70% of the mutants were chlorophyll abnormalities, of which about half were albina and the rest includes chlorina, virido-albina, basi-viridis, striata (white striped) and so on. Besides, there were: early, irregular ear, slender (narrow leaf), dwarf, shrunk, etc. These mutants behaved as simple Mendelian recessives. Virescent mutants with partly green leaves, virido-albina and basi-viridis, mostly died out during the winter in the field, but not in the green-house. Moreover, most of these mutants recovered completely and produced green leaves under the light of a fluorescent lamp.

As to the nature of the mutants, the same types were often observed in both varieties, *flavescens* and *vulgare*, showing a pronounced parallelism. But var. *flavescens* always showed a higher tolerance to X-rays than var. *vulgare*.

39. Chlorina Mutants in Einkorn Wheat induced by X-irradiation

(by Tarô FUJII)

Chlorina mutants appeared in the X_2-generation from dormant seeds of *Triticum monococcum* L. var. *flavescens* KÖRN. treated in 1951 with X-rays of 5,400 *r* dose, at 180 KVP, 3 mA tube current, 13 cm target distance, without filter. This type of mutation behaves as a simple recessive.

Chromosome conjugation of the mutants is always normal, and 7_{II} are formed. Nevertheless, the mutants are somewhat inferior to the normal in plant height and fertility; that is, the plant height is about 25 cm lower, the fertility is about 15% lower, and the time of heading and ripening is about 10 days later as compared with the normal plants.

The difference in chlorophyll content between the normal and mu-

tant plants was examined in the middle of May, 1954. The amount of chlorophyll was measured by extinction (E) at the wave length of 4230 Å with the BECKMAN-spectrophotometer. The amount of chlorophyll in the mutant was about half that in the normal plant. The chlorophyll components were separated into a and b by the paper-chromatographic method. The amounts of chlorophyll a and b in the mutants were also about half, as compared with those in the normal plant.

Next, the activity of cytochrome oxidase was measured with a WARBURG constant volume respirometer. The manometric rate 10, 30 and 60 minutes after treatment was 0.962, 2.743 and 5.214 μl. respectively in the normal plant, while it was 0.429, 1.413 and 2.609 μl. respectively in the mutants. Thus, the manometric rates of the mutants were about half those of the normals. These results were in good accord with those obtained in a previous experiment (1953).

The chlorophyll is presumably under the control of the activity of cytochrome oxidase. The close relation between the chlorophyll amount and the activity of cytochrome oxidase awaits further investigation. The differences seen in morphological characters and in viability between the normal and mutant plants are apparently due in large measure to the reduction of the activity of cytochrome oxidase.

Reference: FUJII, T. 1955. Mutations in Einkorn wheat induced by X-rays. I. Chlorina mutants. Proc. Japan Acad. 31 (2): 88-92.

J. CYTOLOGY AND GENETICS OF *NICOTIANA*

40. Cytogenetic Studies on the Genus Nicotiana, VI.

(by Yô TAKENAKA)

a) Reduction divisions in hybrids between *N. tabacum* and two other species.

The three species, *N. paniculata* (n=12), *N. tomentosiformis* (n=12) and *N. alata* (n=9), were crossed each with *N. tabacum*. The cross *N. paniculata*×*N. tabacum* and its reciprocal gave no seed, while a few seeds were obtained from the cross *N. tabacum*×*N. alata*, and abundant seeds were produced from the cross *N. tabacum*×*N. tomentosiformis*.

The hybrids obtained from the seeds of *N. tabacum*×*N. alata* were

polymorphic in external characters, in agreement with KOSTOFF's description (1941-43). The plants from the cross Pindorama (a variety of tobacco)×*N. alata* were generally intermediate with regard to the external characters of both parents, except for the length of stalk and leaf size which were diminutive, while the plants from Bright Yellow (another variety of tobacco)×*N. alata* resembled *N. tabacum* more than *N. alata*, but the size of the organs was intermediate between the two parents.

The external characters of the hybrid *N. tabacum*×*N. tomentosiformis* was in accord with the descriptions of KOSTOFF (1941-43) and many other previous investigators.

The above two hybrids showed considerable irregularities in the meiotic behaviour in the PMC's. Polysporous PMC's were often observed and the hybrid *N. tabacum*×*N. alata* was completely sterile, but the hybrid *N. tabacum*×*N. tomentosiformis* gave some seeds.

F_1 *N. tabacum* (n=24)×*N. alata* (n=9) showed 33 chromosomes in the root tip cells, the sum of the haploid chromosome numbers of the parents.

At the first metaphase in the PMC's of the plants, 1-6 bivalents were observed, and the most frequent configurations found were $3_{II}+17_I$, and $4_{II}+15_I$, although rarely trivalents occurred. According to KOSTOFF's description (1941-43), the hybrids, *N. tabacum*×*N. alata* and *N. tabacum*×*N. Sanderae* contained 5-9 bivalents and also some polyvalents.

In a previous paper, the writer reported that the hybrid *N. tabacum*×*N. sylvestris* had 0-9 trivalents with the mode at 3. In the hybrids *N. tomentosa*×*N. sylvestris*, GOODSPEED (1934) counted 0-7 bivalents with the mode at 2-3, and KOSTOFF (1941-43) found a similar meiotic pairing behaviour. In the hybrid, closely related to the former *N. tomentosiformis*×*N. sylvestris*, KOSTOFF (1941-33) observed somewhat fewer bivalents. These facts suggest that there are three pairs of homologous or partially homologous chromosomes between the *tomentosa* or *tomentosiformis* and the *sylveslris* genomes. Accordingly, the bivalents found in the F_1 hybrid *N. tabacum*×*N. alata* cannot be attributed simply to homologous relation between the two subgenomes of *N. tabacum* and the *alata* genome.

At first metaphase in the PMC's of F_1 *N. tabacum*×*N. tomentosiformis*, the most frequent configuration found was $12_{II}+12_I$ and the next, $11_{II}+1_{III}+11_I$. Besides these two main configurations, a few deviations from them were found.

This meiotic behaviour agrees with GOODSPEED's findings (1934) in the same hybrid. Since the studies on the hybrid *N. tabacum*×*N. tomentosa* by GOODSPEED and CLAUSEN (1928), *N. tomentosa* or *N. tomentosiformis* is recognized by many tobacco investigators to be one of the ancestors of *N. tabacum*.

b) Reduction divisions in several hybrids between *Nicotiana* species.

i) *N. sylvestris*×*N. otophora*. Concerning this hybrid or its reciprocal, no report has been published, so far as I know. In external morphology, F_1 *sylvestris*×*otophora* is more similar to *N. sylvestris* than to *N. otophora*, but the flower tube is markedly shorter than that of *N. sylvestris*.

At the first metaphase of the PMC's in F_1 *sylvestris*×*otophora*, 0–5 bivalents, mostly 2–3, were counted; also secondary associations between univalents were frequently observed. At the first and the second anaphase, a few chromosome bridges were found. At the tetrad stage, besides the four ordinary microspores, smaller microspores were occasionally detected. The hybrid was completely sterile.

The hybrids *N. tomentosa*×*N. sylvestris* and *N. tomentosiformis*×*N. sylvestris*, closely related to the hybrid *N. sylvestris*×*N. otophora*, have been studied cytologically, the former by KOSTOFF (1941–43) and also by GOODSPEED (1934), the latter by KOSTOFF (1941–43). The results of their investigations are very similar to those of my study. The number of pairing chromosomes, presumably reflecting the grade of homology between the *sylvestris* and the *tomentosa* group genomes, is about 2–3.

ii) *N. alata*×*N. Sanderae*. *N. Sanderae* is assumed to be a perpetually segregating hybrid between *N. alata* and *N. Forgetiana*. Its flower colour varies between white, dark red and purple. Genetic factors conditioning self-sterility are assumed to be the main cause for maintaining high heterozygosis in the forms of *N. Sanderae*. F_1 *alata*×*Sanderae* yielded various segregation forms.

The reduction division of the PMC's of F_1 *atata*×*Sanderae* proceeded quite regularly from the prophase to the tetrad stage, but, besides the chromosome configuration 9_{II} at the first metaphase, $8_{II}+2_I$ was occasionally found. This phenomenon suggests that a small portion of different chromatin or an inversion is present between the *alata* and *Sanderae* chromosome sets.

iii) *N. rustica*×*N. paniculata.* This hybrid has been very frequently produced, like the hybrid *N. tabacum*×*N. sylvestris*, and investigated by a great number of biologists since the premendelian period. The external characters of this hybrid agree with descriptions of previous investigators.

At the first metaphase of the PMC's, 2-12 univalents, mostly 7-10 with the mode at 9, were counted. This range of the univalent number suggests the presence of many PMC's containing 2-5 trivalents besides 7-10 bivalents, because polyvalents higher than trivalents seldom occurred. In fact, the typical configuration of the chromosome complement was $9_{II}+3_{III}+9_{I}$.

In the same hybrid, KOSTOFF and RADJABLY (1934) observed most frequently $12_{II}+12_{I}$ and sometimes $11_{II}+1_{III}+11_{I}$. Also GOODSPEED (1934) found very rarely $10_{II}+2_{III}+10_{I}$, besides the configurations mentioned above.

There are some differences between my observations and those made by KOSTOFF and RADJABLY and also by GOODSPEED. KOSTOFF (1941-43) counted 1-5 bivalents in the hybrid *N. paniculata*×*N. undulata*, while GOODSPEED (1934) observed a wider range, reaching 7 bivalents in the same hybrid. These findings suggest the presence of several semihomologous chromosomes between the *paniculata* and the *undulata* genomes. Accordingly, at the first metaphase of the PMC's of the hybrid *N. rustica*×*N. paniculata*, several trivalents seem to be naturally present. The discrepancies between the results obtained by previous investigators and myself in the cytological examinations of *N. rustica* and *N. paniculata* may be due to different varieties of these species used in the cross.

c) Reduction divisions in *N. Langsdorffii*. The haploid chromosome number of *N. Langsdorffii* is nine, as in *N. alata*, *N. Sanderae* and *N. bonariensis*. AVERY (1938) reports that this plant shows nine bivalents at the first metaphase of the PMC's; however, I have most often observed the configuration $7_{II}+1_{IV}$, occasionally 9_{II}, and rarely $7_{II}+1_{III}+1_{I}$ at the diakinesis and also at the first metaphase. The quadrivalent is N-shaped at the first metaphase. The two middle elements are the largest of all the chromosomes and the two terminal ones are about the same in size as the other chromosomes. At the first anaphase, the four elements of the quadrivalent are distributed equally between the two poles.

At the meiosis of haploid *N. Langsdorffii* KOSTOFF (1930, 1938, 1941)

observed one, two or three groups of two chromosomes in close proximity (secondary association) during the first metaphase, and occasionally one and rarely two bivalents during the first metaphase and early anaphase. Accordingly, he assumed that the basic chromosome number of the ancestral plant of the genus *Nicotiana* might have been six. My observations also suggest that the basic chromosome number of this genus is lower than 9.

41. Mutation in Tabacco Plants Induced by X-rays

(by Seiji MATSUMURA and Tarô FUJII)

In previous experiments (1951–1953), dormant seeds of Bright Yellow and Dixie Bright 101 were irradiated with hard X-rays at 180 KVP, 3 mA, without filter. The dosage was 15,000–50,000 *r*. From the results obtained, it has been surmised that the mutation rate is about 7% at 15,000 *r*, 20% at 30,000 *r* and 40% at 50,000 *r*, and also that the adequate dosage of X-rays for radiogenetical study of induced mutations in tobacco is 30,000 *r*. In 1954, dormant seeds of Dixie Bright 101 were exposed to the same dosage (30,000 *r*) of soft and hard X-rays with varying kilovoltage (20~180 KVP). The germination rate of treated seeds increased generally with the increase of kilovoltage, and the germination was more delayed and became more uneven at 20 and 50 KVP than at 180 KVP.

The mode of inheritance of many mutants obtained in the previous experiments at 180 KVP was investigated in the (X_3~X_5)-generations of both the varieties. Many recessive mutants were observed, such as early, pubescent, round, narrow, oblong, mottled, yellowish green, yellow-spotted leaves, yellow petiole, dwarf, lethal, etc. Several kinds of mutants, for instance early, pubescent and narrow leaves, appeared again by segregation even in the X_3- and X_4-generations. In the tobacco plant, relatively few chlorophyll mutants are found, compared with those in diploid wheat and barley. This may be due to the fact that the tobacco plant is tetraploid.

The "early" mutant of Bright Yellow appeared after 10,800 *r*-irradiation at 90 KVP in one of the earliest experiments (1950). This plant flowered about 2 weeks earlier, and was a little smaller than the normal plants. Its leaves, as well as those of the "pubescent" mutant of Dixie Bright 101 derived from 30,000 *r*-irradiation at 180 KVP in a previous experiment, seem to be of a good quality. Both mutants may prove to be useful for improvement of the tobacco breeds.

K. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF SOME PHANAEROGAMS

42. Hybridization Experiments with Citrullus vulgaris and Citrullus colocynthis

(by Kazuo FURUSATO and Akira MIYAZAWA)

In the course of our breeding work with seedless water melons, a cross between a water melon (*Citrullus vulgaris*) and Colocynthe (*Citrullus colocynthis*) was attempted with the following results:

1) When Colocynthe pollen was used on water melon, the parthenocarpic fruits grew to normal size and ripened well. No germinating seeds were produced. Most of the seeds remained immature, only a few attaining normal size. Therefore, Colocynthe pollen could be used for inducing parthenocarpy in water melon.

2) When water melon pollen was applied on Colocynthe, fruits of normal size were produced, and the seeds germinated well. Thus, the hybrid could be obtained only in this direction of the cross.

3) When Colocynthe (diploid) was crossed with tetraploid water melon, germinating triploid seeds were produced in abundance, in contradistinction with our experience that diploid water melon does not produce ripening or germinating seeds when tetraploid strains furnish the pollen. Those triploid seeds germinated well, and grew into triploid plants with seedless fruits with many Colocynthe characters: The pericarp was solid and preservable for a long time, but it was too bitter for human consumption. If it were possible to transfer from this cross to diploid water melon the ability to produce triploid progeny when pollinated by tetraploid strains, this would mean a remarkable progress in our seedless water melon breeding for two reasons: (i) It would become very easy to produce large amounts of triploid seeds, and (ii) the germination of the seeds so obtained would be much better, since their seed coat would be diploid in contrast to the triploid seeds obtained from pollinations of tetraploid plants with the pollen of diploids, whose seed coat is tetraploid, requiring tedious manipulations before sowing.

4) It is noteworthy that Colocynthe × water melon hybrids, both diploid and triploid, are vigorous, prolific, fast growing and to a certain extent disease resistant. The striped pattern of the exocarp and the texture of the endocarp of Colocynthe are dominant over the corresponding characters of water melon.

43. Karyotaxonomic studies in Poaceae, II

(by Tuguo TATEOKA)

In addition to the species described in the last report, the species listed below were examined cytologically during the current year. These findings seem to allow some phylogenetic conjectures about the genera *Brachypodium* and *Brachyelytrum*, and some cytotaxonomic understanding with respect to the genera *Digitaria*, *Setaria* and *Calamagrosits*.

Species	2n	Species	2n
Bromus secalinus	28	*Agrostis palustris*	42
B. mollis	28	*Calamagrostis Fauriei*	28
B. rigidus	56	*C. longiseta*	28
B. remotiflorus	14	*C. Pseudo-Phragmites*	28
B. catharticus	42	*C. sachalinensis*	56
B. tectorum	14	*C. purpurascens*	28
Agropyron semicostatum	28	*Polypogon fugax*	42
*A. Gmelini ?**	28	*Alopecurus aequalis* var. *amurensis*	14
*Elymus dahuricus ?**	42	*Arrhenatherum elatius* var. *bulbosum*	28
E. verginicus	28	*Trisetum bifidum*	28
E. sibiricus	42**	*T. sibiricum*	14
Lolium multiflorum	14	*Brachyelytrum japonicum*	22
L. perenne	14	*Stipa scribneri*	40
Brachypodium pinnatum	28	*S. spartea*	44
B. distachyon	30	*Achnatherum pekinense*	24
Festuca myuros	42	*Phaenosperma globosum*	24
F. arundinacea	42	*Pleioblastus Matsunoi*	48
F. elatior	14	*P. angustifolius*	48
F. extremiorientalis	28	*P.* sp. (*P. Simoni ?*)	48
Poa hayachinensis	42	*P.* sp. (*P. yoshidake ?*)	48
P. Matsumurae	28***	*P.* sp. (*P. communis ?*)	48
P. trivialis	28	*Pseudosase japonica*	48
P. sphondylodes	28	*Sinoarundinaria aurea*	48
Glyceria depauperata	20	*Sinobambusa tootsik*	48
Torreyochloa natans	14	*Sasamorpha mollis*	48

*) Plants collected in the Himalayas, the identification uncertain.

**) Plants collected in the Himalayas, their chromosome number is different from that of other plants of the same species collected at Nagano-ken, Japan, previously reported (2n=28).

***) Plants collected on Mt. Mitsutôge in Yamanasi-ken, has a different chromosome number from that of the plant obtained at Mt. Yari in Nagano-ken previously reported (2n=70)

Semiarundinaria fastuosa	48	*C. pauciflorus*	34
Arundinaria sp. (*A. atamiana*?)	48	*Setaria chondrachne*	38
Sasa sp. (*S. nipponica*?)	48	*Digitaria chinensis*	18
Cleistogenes Hackelii	40	*D. adscendens*	54
Eragrostis megastachya	20	*D. violascens*	36
E. bulbillifera	40	*Oplismenus undulatifolius*	*ca.* 54
E. multicaulis	40	*Paspalum dilatatum*	50
E. pilosa	40	*P. Thunbergii*	40
E. poaeoides	40	*Pennisetum alopecuroides*	18
Eleusina indica	18	*Cymbopogon tortilis* var. *Goeringii*	20
Sporobolus japonicus	40	*Eccoilopus colutifer*	40
S. elongatus	36	*Microstegium vimineum* var. *polystachyum*	40
Muhlenbergia japonica	42	*Hemarthria japonica*	18
M. longistolon	42	*Andropogon brevifolius*	20
M. hakonensis	40	*Pseudopogonatherum quadrinerve*	40
Zoysia japonica	40	*Sorgum nitidum* var. *majus*	20
Z. macrostachya	40	*Phacelurus latifolius*	40
Cenchrus echinatus	68		

44. *Effects of Extracts from Two Poisonous Plants upon Living Plant Tissue*

(by Yô TAKENAKA)

a) Extract from *Gloriosa superba*. PARTHASARATHY (1941) first reported that the alkaloid gloriosine extracted from *Gloriosa superba* causes doubling of chromosomes. This finding was confirmed more recently by KUMMER (1953).

The alkaloid was extracted from fresh root stalks of this plant by the following method. Fresh root stalks (28,5 g) were macerated with 42 cc water, centrifuged, and the supernatant fluid was sterilized (100°C, 4 min.) and filtered.

The effect of this filtrate upon plant tissue was examined in the following way: (1) Tumor-test on growing root-tips soaked in aqueous solutions of the filtrate at various dilutions; (2) Dwarf-tumor test on seedlngs germinated from seeds soaked in the solution of the same extract; (3) C-mitosis test on growing root-tips dipped in the same solution.

The tumor tests on growing roo-tips were repeated twice in the spring of 1954 with *Allium scordoprasum* var. *viviparum* and *Vicia faba*. When the root-tips of *Allium* were treated with this filtrate

diluted to 1/2 concentration, they showed withering. When the concentration was made 1/4, tumors appeared, much as in the case of treatment with 0.04–0.1% colchicine solution. The largest tumors were formed by treatment with the filtrate diluted to one-eighth. With 0.01% colchicine solution, even small tumors are rarely formed.

In the experiment with root-tips of *Vicia faba*, the material was soaked in various concentrations of the filtrate. They showed withering in 1/4 concantration, grew small tumors in 1/8 concentration and large tumors in 1/16 concentration. After treatment with colchicine solution of the concentrations, 0.1, 0.04 and 0.01, small tumors, large tumors and insignificant tumors were obtained, respectively.

The dwarf-tumor tests in seedlings were carried out with a *Raphanus* species in the spring of 1954. The seeds of this species were soaked in solutions of different concentrations of the filtrate for 72 hours. The proportion of dwarfed seedlings carrying tumors caused by this extract reached about 70%.

The root-tips of *Allium scorodoprasum* var. *viviparum* treated for 6 hours with 1/4 conc. of the filtrate showed many cells with beautiful figures of C-pair chromosomes. Materials treated for 6 hours with 1/2 conc. of the filtrate and washed afterward also showed doubling in many cells when observed 18 hours after the treatment.

b) Extract from *Zephyranthes candida*. ERDMAN and EMMEL (1950) described *Zephyranthes atamasco* as a poisonous plant; cases of poisoning in horses, cattle and chickens have been reported. TYLOR, CARMICHAEL, MCKENNA and BURLAGE (1951) studied *Cooperia pedunculata*, a plant closely related to the former, and found that extracts of this plant inhibited tumor growth.

The author examined the toxicity of *Zephyranthes candida* in the spring of 1954. The extract from the bulbs of this plant was made by the following method. 26.2 grams of fresh bulbs were macerated with 20 cc water + 13 cc alcohol, filtered by suction and concentrated to 10 cc.

The effect of this extract upon plant tissues was examined in the following way: (1) Growth-inhibition-test on root-tips soaked in this extract; (2) Survival-test on growing root-tips and seeds soaked in this extract; (3) C-mitosis test on growing root-tips dipped in the same extract.

In the growth-inhibition test, growing root-tips together with bulbs of *Allium scorodoprasum* var. *viviparum* were soaked in the extract

for 2, 4, 6, 8 or 18 hours. After 8 days, the roots of the control plants were found to have grown about 3 cm in length, whereas the roots treated for 2 hours with this extract grew only about 1 cm, and in the remaining lots there was entirely no growth.

Survival-test on growing root-tips were carried out with the same plant. Roots treated with the extract for 1 or 2 hours were soaked in an aqueous soluion of neutral red after washing. In a few days, the treated roots had selectively absorbed so much neutral red that the solution became nearly colorless.

Survival-tests on the germination of seeds were performed with seeds of a *Raphanus* species. Seeds soaked for 24 hours in the extract and in aqueous solutions of 0.5% conc. of two kinds of maleic hydrazide, were sown in petri dishes after washing. The seeds treated by maleic hydrazides showed slightly delayed germination, and the growth of seedlings was obviously inhibited. The rate of germination, however, was about the same as that in the control. The seeds treated by the extract showed no sign of germination even after a week; germination took place only when the seeds were planted in the soil.

The seeds of the same species treated with the extract for 1, 2, 3 or 4 hours were sown in petri dishes. They were delayed in germintion as compared with the control; the degree of delay was nearly proportional to the duration of the treatment, and the rate of germination decreased in inverse proportion to the duration.

In the C-mitosis test, growing root-tips of *Allium scorodoprasum* var. *viviparum* treated with the same extract for 1, 2, 4, 8 or 24 hours were observed microscopically. Materials treated for one hour showed scarcely any difference from the control. In materials treated for two hours, many nuclei and nucleoli had a swollen appearance, and a few vacuoles were recognized in some nucleoli. Prophase chromosomes were somewhat puffed up, and occasionally showed two sister strands. Metaphase and anaphase chromosomes appeared shortened and plump. In materials treated for four hours, these changes were much more pronounced. Moreover, metaphase chromosomes showed signs of C-pair, and at the anaphase stage, various multipolar spindles were often observed. In the materials treated for 8 and 24 hours, all the changes described above were much more pronounced, and the separation of the centromeres in C-pair resembled those appearing after colchicine treatment.

45. *Analysis of Flavone Pigments in Triticum and Related Plants under Consideration of their Genome Constitution*

(by Tôru ENDÔ)

The relation between genome constitution and flavone ingredients was investigated by means of paper chromatography in eleven species of the subtribe Triticinae and one species of *Hordeum*. The plants and their genome constitutions are as follows; *Triticum vulgare* and *T. Spelta* (AABBDD), *T. Khapli*, *T. polonicum* and *T. dicoccum* (AABB), *T. monococcum* (AA), *Aegilops squarrosa* (DDDD), *Agropyrum glaucum* (BBEEFF), *A. elongatum* (BBEEEEFFFF), *Secale cereale* (RR), *Haynaldia villosa* (VV) and *Hordeum vulgare* (2n=14).

Methanol extracts of fresh leaves were analyzed by two-dimensional paper chromatography. The solvents used were (1) mixture of *o*-cresol/*iso*-propanol/water (5:1:4, v/v) and (2) 10% acetic acid. Lead subacetate was found to be most effective as the color reagent. After treatment with this color reagent, pigment spots in all chromatograms became either yellowish or brownish. The number of color spots per chromatogram ranged from 6 to 11; in addition, a few spots of fluorescent substances were observed under ultraviolet light. Although the chemical nature of the fluorescent substances is not yet clear, certain inter-relationships were found between the distribution of the spots and the genome constitution of the available species. Within the genus *Triticum*, the pigment spots produced by one species seem for the most part to correspond to those found in the others, and even to increase in number with the increasing participation of different genomes. Furthermore, spots produced by *Agropyrum* and *Aegilops* are partially similar to those of *Triticum*. *Secale* and *Haynaldia* showed almost identical figures of pigment spots, in particular as regards the two major pigments. However, they differed markedly from those of *Triticum*. *Hordeum* seemed to be quite different from all other examined genera.

46. *Paper-chromatographic Analysis of Anthocyanins Occurring in Several Varieties of Japanese Morning Glory (Pharbitis Nil) with Special Reference to the Role of the mg Allele*

(by Yukihide ABE)

In No. 4 of the Annual Report, a general survey of coronal anthocyanins occurring in several varieties of Japanese morning glory was presented. It was pointed out that the *mg* allele apparently plays the

main part in the hydroxylation of the side benzene nucleus in the anthocyanin molecule.

In order to find further evidence in support of this view, several experiments were carried out on eight strains having an *mg* or $+^{mg}$ gene (Table). The anthocyanin pigments were extracted with 1% methanolic hydrochloric acid, and the extracts were subjected to hydrolysis with hot 10% hydrochloric acid. Then the crude solutions of anthocyanidin were purified as usual and chromatographed with two kinds of solvent mixtures: acetic acid/36% hydrochloric acid/water (5:1:5, v/v) and *iso*-propyl alcohol / 5% hydrochloric acid (55:45, v/v). The results are summarized in the Table:

Anthocyanidins occurring in various genotypes of Pharbitis Nil.

Genotype concerning flower color	Corolla				Flower tube				Stem				Axis of seedling				Autumnal leaf			
	Epidermis[2]				Epidermis				Subepidermal tissue				Subepidermal tissue				Palisade parenchym			
		Pl	P	C[3]		Pl	P	C		Pl	P	C		Pl	P	C		Pl	P	C
$+^{mg}+^{pr}+^{ca}+^{c}+^{r}$[1]	B[5]	0	9	4[4]	C	0	5	5	C	0	1	9	C	0	8	2	C	0	+	###
$+^{mg}$ *pr* $+^{ca}+^{c}+^{r}$	P	0	6	4	C	0	5	5	C	0	3	7	C	0	6	4	C	0	0	###
mg $+^{pr}+^{ca}+^{c}+^{r}$	M	9	1	±	C	###	+	±	C	6	2	2	C	6	3	1	/		/	
mg pr $+^{ca}+^{c}+^{r}$	R	8	2	±	C	8	2	±	C	7	1	2	C	7	2	1	C	0	0	###
$+^{mg}$ *pr* $+^{ca}$ *c* $+^{r}$	M	0	0	0	W	0	0	0	C	0	2	8	C	0	6	4	C	0	+	###
mg pr $+^{ca}$ *c* $+^{r}$	W	0	0	0	W	0	0	0	C	6	2	2	C	6	3	1	C	+	##	+
$+^{mg}+^{pr}+^{ca}+^{c}$ *r*	W	0	###	+*	C	0	7	3	G	0	0	0	G	0	0	0	G	0	0	0
mg pr $+^{ca}+^{c}$ *r*	W	###	+	0*	Y	###	+	0*	G	0	0	0	G	0	0	0	G	0	0	0

* containing anthocyanin pigment probably in the form of colorless leucobase. 1) all homozygous. 2) showing localization of anthocyanins. 3) Pl: pelargonidin, P: peonidin, C: cyanidin. 4) Relative amount of anthocyanidins (in 10 parts). 5) B: blue, G: green, M: magenta, P: purple, R: red, W: white, Y: yellow, C: from red to purple.

As shown in the Table, pelargonidin does not occur in any tissue of the $+^{mg}$ plants, whereas it does occur in the *mg* plants. Moreover, the action of the *mg* allele appears not only in the corolla, but also in certain vegetative organs, in several strains which are capable of producing anthocyanins. In order to test this hypothesis, further experi-

ments are in progress, using the progeny of the inter-hybrids between the above strains.

47. Studies on Natural Anthocyanins

(by Kôzô HAYASHI and Yukihide ABE)

Brilliant and attractive flower colors produced by anthocyanin pigments were subjects of interest of classical plant genetics. When the genes controlling them were discovered, attempts were made to investigate the biochemical processes underlying pigment formation and color variation in relation to these genes. But, even today, a direct approach to these fundamental problems is virtually impossible, since we know too little about the complicated biosynthetic processes of anthocyanin production in living plant cells.

In order to contribute something to our knowledge of the nature of plant pigments, we have studied anthocyanins occurring in various plants. In 1954 the following investigations were carried out:

a) Red coloring matter in the leaves of *Perilla* varieties. The anthocyanin in the dark red leaves of *Perilla frutescens* var. *crispa* was shown by K. KONDO (1932) to be a pentose-glycoside of delphinidin combined with protocatechuic acid, which he termed 'perillanin'. C. KURODA and her co-worker (1935) arrived, however, at a different conclusion, holding that the leaf pigment of that plant is not perillanin, but an esterified anthocyanin composed of cyanin (cyanidin 3.5-diglucoside) and *p*-hydroxycinnamic acid. They have given this compound the name of 'shisonin'. Our careful experiments carried out by means of paperchromatography as well as by the usual method of chemical analysis have shown that the pigment component in question is shisonin, as pointed out by KURODA. Our studies have also disclosed that the red leaves of other garden varieties of the same species owe their color to shisonin.

Reference: HAYASHI, K. and ABE, Y. 1955. Studies on Anthocyanins, XXV. Paperchromatographic investigation on anthocyanins occurring in the leaves of *Perilla* varieties. Bot. Mag. (Tokyo), 68: 71.

b) Anthocyanin of purple-red flowers of *Lespedeza*. *Lespedeza*, one of the beauties among the autumn plants in Japan, includes several species. The deep purple-red flowers of *L. Thunbergii* seemed to be the best material for the preparation of the pigment component. After several unfruitful experiments, we were able to

isolate a coloring matter in a pure crystalline state. This consisted of deep chocolatebrown needless having a brilliant greenish metallic lustre. A series of chemical analysis have shown that the pigment is nothing else than malvin, *i.e.* 3.5-diglucoside of malvidin. The same anthocyanin occurs also in the flowers of several other species of *Lespedeza;* this was shown by paperchromatographic analysis. Finally, it is interesting to note that the snow-white flowers of *L. japonica* contain an appreciable amount of the pigment in a colorless form, which is readily converted into a malvidin derivative simply by treatment with warm hydrochloric acid. An extensive study concerning the mechanism of transformation of this colorless substance into malvin in living cells may throw some light upon the quite obscure biosynthetic process leading to the formation of anthocyanin pigments in general.

Reference: HAYASHI, K. NOGUCHI, T. u. ABE, Y. 1955. Studien über anthocyane, XXVI. Über den Farbstoff der Blüten von *Lespedeza Thunbergii.* Bot. Mag. (Tokyo), 68: 129.

c) Survey of anthocyanins responsible for the autumnal reddening of leaves in Japan. Fairly extensive investigations have been carried out on the reddening of autumn leaves of deciduous trees chiefly in the neighbourhood of Nikko. Leaves of about 70 kinds of plants were examined by the paper-chromatographic method. As has been previously suggested by foreign authors, the leaf anthocyanin consists, for the most part, of cyanidin monoglucoside, which is probably identical with chrysanthemin. This shows that in leaf tissues a pattern of biosynthetic reaction is preponderant, and that this reaction brings about the formation of a C_6-C_3-C_6 compound, in which the two terminal C_6-groups are substituted by a phloroglucinol- and a pyrocatechol-moiety, respectively. So far as the leaf anthocyanins are concerned, the intracellular processes giving rise to the formation of the above two moieties should provide an important clue for our biochemical approach to the basic problem of pigment production. A full account of our data will be published shortly.

L. CYTOLOGY AND GENETICS OF SOME LOWER ORGANISMS

48. *The Delayed Appearance of the O-Antigen Transformation in Salmonella*

(by Tetsuo IINO)

The change of the O-antigen of *S. anatum* from 10 to 15 is accom-

panied by its lysogenization for bacteriophage ε, and the phage itself is regarded as the transforming principle of this change (ISEKI and SAKAI, 1953). This phage ε also acts as a carrier of genetic elements on transduction, according to ZINDER and LEDERBERG's study (1952) on another strain of *Salmonella*. Accordingly, when the cells of *S. anatum* are transduced by the FA (filtratable agent) of *S. newington* which is lysogenic for ε, they change their O-antigen from 10 to 15. In general these changes can be detected simultaneously on the colonies which are developed on the plates spread with the treated cells of *S. anatum*. In the course of experiments designed to test the simultaneous induction of transduction and O-antigen transformation, it was found that the antigen transformation appeared later than the transduction, as is to be described below.

A maltose-broth culture (1 ml, 5×10^8 cells) of the streptomycin sensitive (S^s) strain A6-1 of *S. anatum* was mixed with an equal volume of cultural filtrate of the streptomycin resistant (S^r) strain 84 of *S. newington*. As a control, the same culture was mixed with the filtrate which had been heated at 100°C. They are incubated for 24 hours more, and S^r cells were screened by spreading the culture on media containing 5 mg/ml of streptomycin.

In three experiments, 122, 217 and 265 S^r colonies respectively were obtained from about 5×10^8 cells, but none from the control series. The lysogenicity and O-antigen type were examined on these S^r colonies using the turbidity test and the slide agglutination test. All of the S^r colonies obtained from the first two experiments showed lysogenicity and O-antigen 15, but some colonies obtained from the third experiment showed only the change to lysogenicity, the O-antigen 10 remaining unchanged. Thirty of these colonies were picked up and spread on broth agar successively at intervals of 48 hours, and the antigen type of their offspring was tested. It was found that 20 to 60% of the random colonies contained cells showing antigen type 15 in each spreading, and the cells, which had acquired the 15-type antigen, maintained this property through further generations. In the course of this change the colony should be agglutinated by broth anti<>10 serum and anti<>15 serum. As a matter of fact, no colony showed agglutination by both antisera. This fact is possibly due to phenotypic delay of antigen transformation, and is interesting, since it may provide a convenient material for analyzing the process of the expression of the antigen transformation.

49. *The Paths of the Reversion in the Methionine-requiring Strain of Ustilago maydis*

(by Tetsuo IINO)

The reversion of the methionine-requiring strain of *U. maydis* 4-25 (M_1^-) takes place through at least two distinct paths; $M_1^- \rightarrow$CR and $M_1^- \rightarrow$IR$\rightarrow$IR′ (Annual Report, No. 4, 1953). The estimation of the frequencies of each step is important in order to certify the distinction between the two processes. However, it is difficult to do this exactly, because of the simultaneous occurrence of these changes in the same culture, and of the similarity of the CR and IR′ cultures. An approximate estimation, however, is possible if the frequency of IR$\rightarrow$IR′ is not too large in comparison with the frequencies of other steps.

The rate of the change from CR to IR in the total reversion is variable according to experiments. It has been estimated that 25% on the average (ranging from 0 to 38%) of the reversional mutant, when tested immediately after screening from a single colony by the minimal agar plate method, is CR. As reported previously (Annual Report, No. 3, 1952), the mutation rate of M_1^- to methionine independant is 3×10^{-7}, so the mutation rates to CR and IR are estimated to be approximately 8×10^{-8} and 2×10^{-7} respectively.

For the estimation of the rate of the change IR$\rightarrow$IR′, the usual plate-screening method is inadequate, because the dense population on the plate suppresses the growth of IR′, and makes the comparison of growth rates between the two types impossible. Accordingly, the maximum frequency has been estimated from the number of cultures which contain IR′ among a series of successive minimal liquid cultures of IR, considering the selective advantage of IR′ over IR. The result indicates that the frequency of the mutation from IR to IR′ is lower than 5×10^{-5}. This value is low enough to eliminate the possibility of the appearence of IR′ in the single colony culture of M_1^-. In other words, all the reversional mutants obtained by the minimal plate screening method are regarded as one step mutants CR.

In parallel with these experiments, the possibility of the appearance of a conditional mutation in the course of reversion was examined by testing the nutritional requirments other than methionine of reverted strains which were obtained by spreading washed cells suspensions of strain 4-24 on vitamin-H_2O_2 treated peptone (methionine-free) media. All of the 75 mutants tested were found to be unaccompanied by any nutritional requirements other than methionine. This result indicates

that conditional mutation does not play important part in the reversion of the methionine requirement.

50. A Cytoplasmic Polyhedral Virus Occurring in the Silkworm

(by Mitsuo TSUJITA)

It is well known that the virus of the polyhedral disease of the silkworm invades the nucleus of epidermal cells, adipose tissue cells, tracheal epidermal cells or haemocytes (blood cells), and produces polyhedral crystals, ISHIMORI (1934) first found polyhedra occurring in the cytoplasm of the mid-gut epithelial cells. His observation has been confirmed by LOTMAR (1941), TSUJITA (1949), XEROS (1952), and SMITH and XEROS (1953). In order to know whether or not the virus occurring in the cytoplasm is different from that multiplying in the nucleus of the epidermal and some other tissue cells, I had been looking for larvae with mid-gut polyhedrosis. Recently I found material of this kind, and carried out microscopical and electron microscopical observation and experiments with it. The results obtained may be summarized as follows:

i) The mid-gut polyhedrosis of the silkworm shows symptoms similar to that of flacherie disease, especially as regards the change appearing in the alimentary canal. The mid-gut, especially its middle and posterior portions, takes on a distinctly whitish colour.

ii) Numerous polyhedral bodies are produced in the cylindrical cells, but never in the goblet or interstitial cells of the epithelium. The polyhedra are produced only in the cytoplasm; the nucleus is entirely free from polyhedra or virus-like particles.

iii) The polyhedra are almost identical in shape with those produced in the nucleus.

iv) The sections of the polyhedra exhibit numerous holes or protuberances which are of about the same size as the spherical particles which appear on treatment with dilute alkali. The polyhedra develop from clusters of spherical particles included in a proteinous matrix. These particles probably represent viruses.

v) These are two types of the polyhedral virus infecting the silkworm, a rod-shaped one and a spherical one. The former usually invades the nucleus of the epidermis and some other tissues, while the latter seems to invade specifically the cytoplasm of the cylindrical cells of the mid-gut epithelium.

Reference: TSUJITA, M. 1955. Cytoplasmic polyhedral virus infecting the silkworm. Proc. Japan Acad. 31 (2): 93-98.

51. *Studies on the Mitochondrial Granules isolated from Paramecium caudatum*

(by Kyôzô WATANABE, Mitsuo TSUJITA and Bungo SAKAGUCHI)

The cytoplasmic constituents of *Paramecium caudatum* were separated into four fractions, T, Mw, Pw, and S_2, by the differential centrifuge method.

In our experiment with *caudatum*, it was difficult to isolate completly the mitochondrial granules from other large granules or smaller particles. However, according to our microscopical observations of each of the fractions, it is certain that the mitochondrial granules are mainly concentrated in the Mw fraction and the large part of the smaller particles are precipitated in the Pw fraction.

Reference: WATANABE, K., TSUJITA, M. and SAKAGUCHI, B. 1955. Studies on the mitochondrial granules isolated from *Paramecium caudatum*. Bull. Educ. Fac. Shizuoka Univ. 5: 153-160.

52. *Fine Structure of Mitochondria in Paramecium*

(by Mitsuo TSUJITA, Seizo TSUDA and Kyôzô WATANABE)

The present paper deals with the results of an electron-microscopical study on the fine structure of mitochondria in *Paramecium* carried out by means of ultra-thin sections.

The mitochondria lying under the pellicle and those scattered in the cytoplasm show the same structure. Each mitochondrium is covered with a membrane. In addition, there is a membranous fold or a plicate structure in the interior of each mitochondrium. This plicate structure does not always have the same appearance; it may be indistinct or it may exhibit a network. These varieties in internal structure seem to represent the different phases of their physiological function.

The mitochondria occurring in the ectoplasm seem to have some relation to the formation of trichocysts; also they may be somehow related to the energy source for ciliary movement.

Reference: TSUJITA, M., WATANABE, K. and TSUDA, S. 1955. Fine structure of mitochondria in *Paramecium*. Electron microscope 4: (in press).

53. *Studies on the Lysogenicity of Pseudomonas solanacearum*

(by M. TSUJITA, C. MATSUI, S. TSUDA and O. YOSHIZAWA)

Two of the seven lysogenic strains of *Pseudomonas solanacearum* E. F. SMITH isolated by N. OKABE, S-9 and T-c200, together with several indicator strains of the bacteria were used as materials, and the results obtained are summarized below:

a) Lysogenic strain T-c200. The lysogenicity of the strain T-c200 is a stable hereditary character, and its cell progeny consists wholly of lysogenic cells. The virus liberated from the strain is active against *P. solanacearum*, Strain S-9, S-IX, E-3, A-8 and S, and some of these indicator strains can be lysogenized.

During the logarithmic growth phase of strain T-c200 in potato-dextrose solution at 34°C, the increase of free virus is paralleled by the growth of bacteria, and the ratio of bacteria-free virus is nearly constant, being about 5×10^4/1 ml. It is assumed, therefore, that the bacteria which spontaneously produce mature viruses are a small fraction of the whole bacterial clone.

Strain T-c200 and its free virus are killed by irradiation with ultraviolet light, and the ratios of inactivation of bacteria and virus are found to be roughly the logarithmic function of the irradiation dose. The proportion of strain T-c200 which produces mature virus is not increased, but decreased, by irradiation with ultraviolet light; therefore, strain T-c200 seems to be a non-inducible lysogenic strain.

After ultraviolet irradiation, bacteria without lysogenicity can be obtained from the survivors of the former colonies. The bacteria, having lost their lysogenicity, are sensitive to their original virus, but can be relysogenized. Thus, lysogenic bacteria may be cured from their lysogenicity by ultraviolet irradiation. The loss of lysogenicity is never spontaneously reversed. Thus, this bacterial character must have undergone a genetical change.

As for the mechanism of the loss of lysogenicity by irradiation with ultraviolet light it is most probable that the prophage, *i.e.* the anlage of the phage, is inactivated.

Reference: TSUJITA, M., MATSUI, C., TSUDA, S. and YOSHIZAWA, O. 1954. Studies on the lysogenicity of *Pseudomonas solanacearum*. I. Lysogenic strain T-c200. Virus 4 (4): 308-312.

b) Response types shown by the T-13 bacteria after

exposure to S-9 phage. Cells of a T-13 culture which were exposed to S-9 temperate phages showed the following five responses.

(i) Reversibility. The cell is unchanged, and given rise to a colony of T-13 cells that may themselves give any of the 5 responses. (ii) Lysis (productivity). Virus multiplication occurs within the cell, resulting in lysis and the production of new free phages. (iii) Lysogenicity. The infected cell gives rise to a colony containing lysogenic cells. These cells spontaneously produce S-9 free phages, but cannot induce multiplication and maturation of phages by the irradiation of ultraviolet light. The cell is immune to S-9 free phage. (iv) Lethality. The cell is killed, and the absorbed phage is inactivated. (v) Resistance. The cell gives rise to a colony of non-lysogenic S-9 resistant cells.

The proportions of the cells showing various responses change according to the environmental conditions of the culture, such as temperature, or age of culture. When a culture kept for 18 hours at high temperature (34°C) is infected with S-9 phages, most of them show a lethal response, and the remainder exhibit productive, reversible, and lysogenic responses. On the contrary, when the cells are cultured for 73 hours at the same temperature, 90% of the infected cells show reversible response, and only part of them can produce mature S-9 phages.

Lysogenic cells can readily be produced at low temperature (25°C). When cells cultured for 18 hours at the low temperature are infected with the phages, about 60% of them show a lysogenic response, and the others reversible response, while no cell producing free phages is found. However, when the cells are cultured for 73 hours, almost all the infected cells show reversible response and only a small portion are lysogenized.

As mentioned above, by raising the temperature during culture, the acquirement of a lysogenic property by the cells in the clone can be suppressed completely or partially. It must be noted, however, that the temperature treatment has no effect on cells in an established lysogenic culture.

The cause of these differences in response of different cells, except the 5th response mentioned above, does not seem to lie in any genetic factor, but may be found in the initial physiological differences among the cells, or in differences created by infection.

c) A double lysogenic strain. By superinfection of S-9 lysogenic cells with T-c200 temperate phage which is closely related to the carried prophage, the following three lysogenic strains were obtained: (i) A strain producing S-9 phage only; this strain seems to have originated by reversible response. (ii) A new strain producing T-c200 phage only; this strain has probably been originated by the substitution of the T-c200 prophage for the superinfected S-9 prophage. (iii) A double lysogenic strain carrying both prophages, T-c200 and S-9; this strain has the capacity to produce two types of phages, S-9 and T-c200, and also a new-type phage. The production of this new-type phage from the doubly lysogenic strain should probably be attributed to the recombination of the host specificity characters of the parent phages, T-c200 and S-9, which are serologically related.

Referance: TSUJITA, M. and MATSUI, C. 1955. A double lysogenic strain of *Pseudomonas solanacearum*. Proc. Jap. Acad. 31(3): 180-185.

54. Studies on the Multiplication of Bacterial Virus affecting Streptomyces griseus

(by Seizo TSUDA)

There are several works by previous authors on bacterial viruses attacking *Streptomyces*. The bacteriophage infecting *Streptomyces griseus* was first described by BONNETT (1946). His statements were confirmed later by several other workers (SAUDEK and COLINGSWORTH 1947, WOODRUFF *et al.* 1947, LANGLYKKE *et al.* 1950, '51, TERADA *et al.* 1951, AISO and MABUCHI 1950, '51, '52, and others). These authors gave some information concerning the size and shape of some *Streptomyces* phages, but little is known of the behavior of the virus after infection.

This report deals with electron-microscopical studies on the multiplication of a bacterial virus, S-1 actinophage, infecting *Streptomyces griseus*. As seen in Figs. 1 and 2, the virus is uniformly tadpole-shaped.

The *S. griseus* culture was prepared under submerged conditions for 24 hours at 25°C. The phage had propagated until the plaque counts cxceeded 10^7 per ml. The activity of the phage was then examined by means of quantitative plaque counts.

A thin watery growth was frequently observed in submerged culture of the host cells infected by S-1 phage strain, while non-infected

host cells showed normal growth. Multiplication of actinophage in submerged culture of *S. griseus* at each stage was estimated by the plaque count method, though the lysis was not complete in this stage.

In the present experiments, the lytic change shown in Figs. 1 and 2 took place. Mature phages were liberated from the host cell, together with its cytoplasmic inclusions, after the period of multiplication of the phage. The host cell then underwent gradual, lytic change which was apparent first on the margin of the cell. When the multiplication of actinophage ceased, the nucleus showed a filamentous structure (Fig. 3). Many rod-shaped particles, approximately 30 mμ in width and 100 mμ in length, can be seen in Figs. 1, 2, and 3. It seems that these particles are concerned with actinophage development.

It has been assumed that the bacterial virus includes many gentical units, and that the multiplication of the virus within the host cell proceeds with some regularity.

55. *Electron-microscopical Studies of Ultra-thin Sections in Penicillium chrysogenum*

(by Seizo TSUDA)

The cytology of microorganisms involves a number of structures and events which are beyond the resolution limit of the liget micro-

scope. In order to make them accessible to investigation, the materials are cut into thin slices by the use of sectioning techniques recently developed for electron microscopy. Studies of this kind have been carried out by such workers as HILLIER *et al.*, ROBINOW, CHAPMAN *et al.*, SJÖSTRAND *et al.*, TSUJITA *et al.* and HIGASHI.

The present paper deals with some observations of the structure of vegetative mycelium of *Penicillium chrysogenum*, the producer of penicillin, which appears in ultra-thin sections.

The strain of *Penicillium chrysogenum* had been cultivated in the submerged condition at 28°C. for 3 days. As fixative, 0.5% osmium tetraoxide in phosphate buffer solution was used. A fixed mycelium was dehydrated by passing through the alcohol series, and imbedded in a mixture of 95 parts of *n*-butyl metacrylate and 5 parts of methyl metacrylate with 2% benzoyl peroxide as catalyst. The material in this plastic substance was cut into thin sections of about 0.1 μ by an ultra-microtome.

The cell wall of the mycelia is thick, and it has a tendency to become somewhat separated from the cytoplasm. The mycelium contains several nuclei which have a thin membrane. The resting nuclei in the electron micrograph appear round or ellipsoidal. The nuclei as well as cytoplasm show a loose filamentous inner structure.

A great amount of cytoplasmic mitochondria-like granules are scattered in the reticulated cytoplasm. The cytoplasmic granules are mostly spherical or oval. They are surrounded by a limiting membrane, and the inner surface of the inner layer of the limiting membrane exhibits parallel lamellae which seem to protrude into the interior of the granules. Detailed studies on cytoplasmic mitochondria-like granules in bacteria and fungi carried out by MUDD *et al.*, HARTMAN and LIU and many others revealed cytoplasmic granules as dense bodies possessing limiting membranes.

The observation of the process of nuclear dividsion is under way.

M. THEORETICAL STUDIES ON BREEDING

56. Some Considerations on the Problem of Secondary Selection in Self-fertilized Crop Plants

(by Kan-Ichi SAKAI)

Certain Indian plant breeders have recently expressed the view that in self-fertilized crop plants such as rice or wheat, "secondary selection" is effective for improving the yielding capacity, because

there should be some genotypic segregation within the varieties of such plants. Theoretical considerations of this problem follow:

(a) To what extent can a variety established by hybridization be homozygous? Assuming that the yielding capacity is controlled by many "polygenes", and mutation occurs with a certain frequency, the degree of homozygcsity in lines selected from a hybrid population will be formulized as follows:—Let the rates of mutation from A to A′ and from A′ to A both be m, the frequencies of AA, AA′ and A′A′ plants in the g-th segregating generation (D_g, H_g and R_g) will be,

$$D_g=2m(1-m)^2D_{g-1}+\frac{1}{4}H_{g-1}+m^2R_{g-1},$$

$$H_g=2m(1-m)D_{g-1}+\frac{1}{2}H_{g-1}+2m(1-m)R_{g-1},$$

and

$$R_g=m^2D_{g-1}+\frac{1}{4}H_{g-1}+(1-m)^2R_{g-1}.$$

From these formulas, the frequency of plants homozygous for N pairs of genes will be found to be

$$(D+R)_g\fallingdotseq\left\{\frac{1-\left(\frac{1}{2}-2m\right)^g}{1+4m}\right\}^N.$$

Numerical calculations with these formulas show that, for instance, when $m=10^{-5}$ and $N=50$, a line of F_{10} will be heterozygous for a gene with a probability higher than 18%.

(b) Mutation in pure lines: The following formula shows how a variety may become heterozygous as the result of mutation when it was a pure line in a given generation, assuming that mutation occurs in one direction only, with the frequency of m'.

The frequency of desirable genes in the g-th generation will then be

$$N_g=N_0(1-m')^g.$$

It will be found by using this formula that the loss of desirable genes occurs at an extremely low rate; for instance, when $m'=10^{-5}$, the proportion of genes to be lost after 20 to 50 generations is only 0.02 to 0.05.

It may be said in conclusion that "secondary selection" can be effective in varieties bred by hybridization methods, but not so effective in varieties established from native varieties by the pure line selection method. In this connection, the writer has proposed an improved system of stock seed propagation.

57. *Theoretical Basis for Discriminating the Most Desirable Hybrid Combinations in Early Generations*

(by Kan-Ichi SAKAI)

It has been suggested by several plant breeders that the potentiality of a hybrid population to segregate superior yielders in later generations can be predicted by an examination of the yield in the F_2 or F_3. But some others are skeptical about this idea. In this paper a theoretical approach will be made to this problem.

First, let us consider the simplest case in which a character to be selected is determined entirely by genes with additive effects and there is no environmental variation. In this case, the mean value of a hybrid population will be the additive genetic mean and the mean square will provide an estimate of the additive genetic variance. If individuals of such a population are distributed normally regarding the measurement x, with the mean at $\bar{x}$ and with variance σ^2, we can transform x to a variate u, with unit variance and the mean at zero, as $u=(x-\bar{x})/\sigma^2$. When a definite proportion of superior individuals, A, are selected, the selection differential $\bar{I}$ can be written as

$$\bar{I}=\frac{1}{A}\int_{u=u'}^{\infty} u\frac{1}{\sqrt{2\pi}}e^{-\frac{1}{2}u^2}du=\frac{z}{A}$$

where z is the ordinate of the unit normal curve at the deviate u'. Since z is determined by the value of A, the value of $\bar{I}$ can be found from the value of A. The value of I on the x scale can be found by multiplying $\bar{I}$ by σ. The average value of superior individuals in the i-th population may then be given by $\bar{Y}_i=\bar{x}_i+\sigma_i\bar{I}$.

Next, we turn our attention to a more complicated case in which intra-allelic interaction of genes causing heterosis and environmental variation interferes with the additive gene effect. In this case we have to subtract both the non-additive effect of genes and the effect of environmental variability from the observed values. The mean values of parents and hybrid populations may be written as follows:

$$\frac{1}{2}(\bar{P}_1+\bar{P}_2)=\mathrm{MP}$$

$$\bar{F}_1=\mathrm{MP}+h$$

$$\bar{F}_2=\mathrm{MP}+\frac{1}{2}h$$

$$\bar{F}_3=\mathrm{MP}+\frac{1}{4}h$$

(MP is the mid-parent value; h is the value of F_1 from MP.)

The value of MP found from these formulas may be regarded as an estimate of the additively genetic mean of the given hybrid population.

The additive genetic variance may be estimated by the method of partitioning variance components of Mather (1949). For practical purposes, observation of three hybrid populations, F_1, F_2 and F_3, and the parental lines will be recommended. By using those formulas, we can estimate the values of D, H and E, which are variance components attributable to additive gene effect, non-additive gene effect and environmental variation, respectively. From the value of D, we can estimate the value of $\bar{Y}$ for a given hybrid combination (i) as follows:

$$\bar{Y}_i = \mathrm{MP} + \bar{I}\sqrt{D_i} = \mathrm{MP}_i + \bar{I}\sqrt{V_{F_3} - 2V_{F_2} - V_{P(F_1)}}\,.$$

In a further complicated case in which intergenotypic competition influences the character, additional breeding of F_3 lines and F_4 bulk, and if possible, both back-cross populations, will be needed. Formulas for various hybrid populations, taking competitional variance into account are as follows:

$$V_{F_2} = \frac{1}{2}D + \frac{1}{4}H + C'' + \frac{1}{2}G'' + E$$

$$V_{F_3} = \frac{3}{4}D + \frac{3}{16}H + \frac{3}{2}C'' + \frac{3}{8}G'' + E$$

$$V_{\bar{F}_3} = \frac{1}{2}D + \frac{1}{16}H + \frac{E}{N}$$

$$V_{F_4} = \frac{7}{8}D + \frac{7}{64}H + \frac{7}{4}C'' + \frac{7}{32}G'' + E$$

$$V_{B_1} + V_{B_1} = \frac{1}{2}D + \frac{1}{2}H + C'' + G'' + 2E$$

$$V_P, V_{F_1} = E$$

(C'' and G'' stand for variance components due to additive and non-additive effects of competition, and N is the number of plants within line.)

The value of D will be found from these formulas and $\bar{Y}$ may be estimated as before.

Comparison of $\bar{Y}$ values among a number of hybrid populations will show which of them are more promising for the breeder than others.

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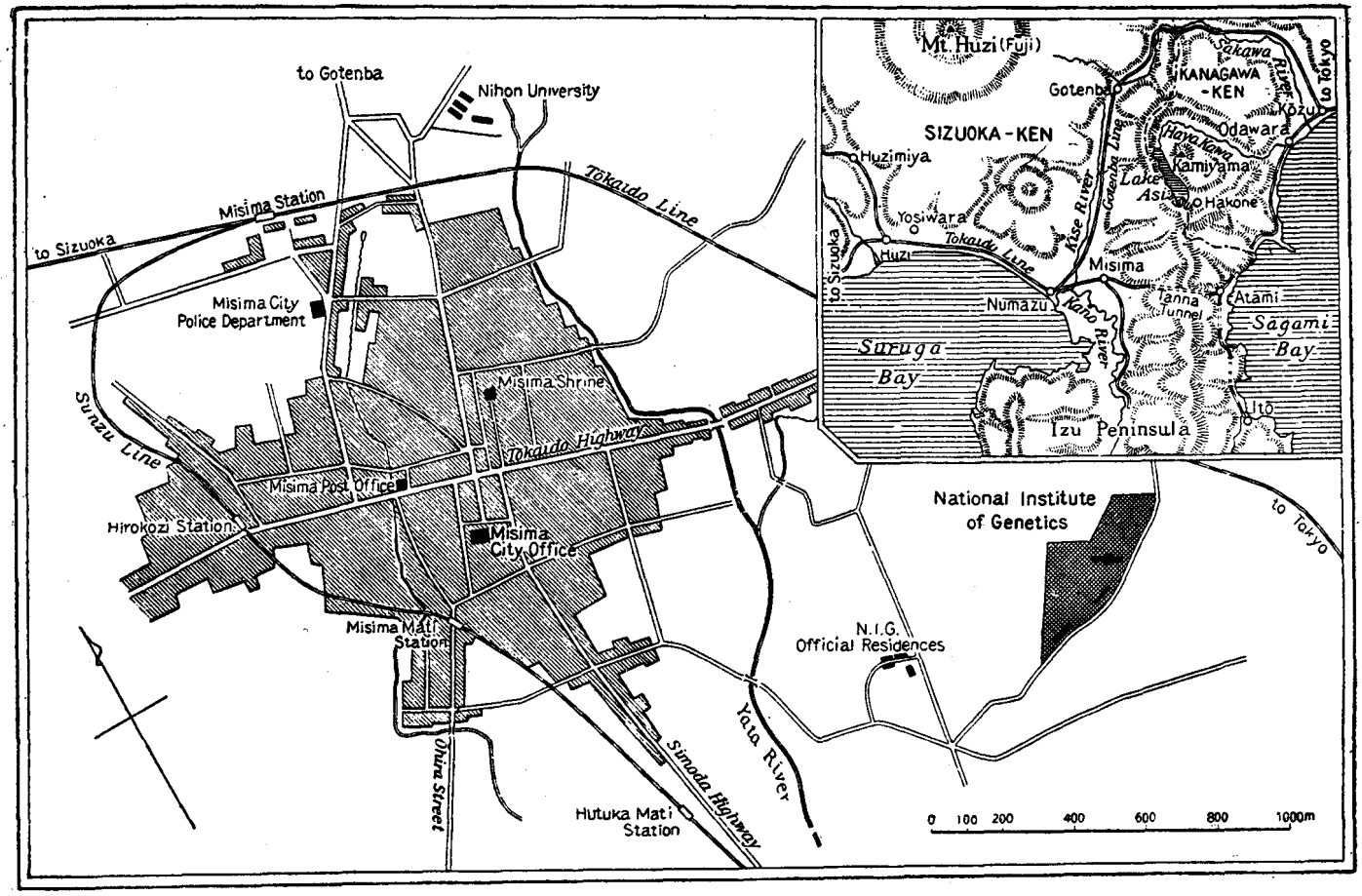
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