NATIONAL INSTITUTE OF GENETICS

(JAPAN)

ANNUAL REPORT

No. 4.

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GENERAL STATEMENT

The scope of activity of the Institute was extended during the current year by the addition of a new department, that of Biochemical Genetics. This addition was intended to meet the increasing demand from the recent trend of genetics in general.

Most of the research programs from previous years were continued to this year. These include fundamental investigations on tobacco plants in cooperation with the members of the Misima Branch of the Hatano Tobacco Experiment Station of the Japan Monopoly Corporation, and genetic researches on egg production of poultry under the auspice of the Whole-Japan Association of Poultry Genetics. Some works were completed and published or are in press. Also, many new research programs were undertaken.

A new house for mouse and rat breeding was completed. This is constructed and equipped according to the plans of modern mouseries in Europe and America. It is 88.2 tsubo (350 square meters) in floor area, and contains four rooms for mouse breeding and two for rat breeding, besides a working laboratory, an assistants' room with a bedroom annex, a food kitchen and storage space. It has an automatically regulated heating system and a simple but effective device for ventilation. The cage racks are hanging and movable. This building can house from 8,000 to 9,000 animals. There are about 6500 mice and 500 rats now in it. Most of these animals are progeny of 15 strains of mice and 4 strains of rats which had been bred for several years in the Zoological Institute of Hokkaido University. Eleven new inbred strains of mice useful for medical and biological purposes were presented after the completion of the house by the kindness of Dr. W. E. HESTON of the National Cancer Institute at Bethesda, Md. and Prof. L. C. DUNN of Columbia University, New York.

An area of more than two acres was added to our experimental field by the removal of an old factory building which had stood close to the main building of the Institute.

The library has been expanded by the arrival of new books, periodicals and reprints. Dr. GOLDSCHMIDT has continued to send in reprints and current numbers of scientific journals which have amounted to 780.

To our *Drosophila* stocks were added 27 useful composite stocks of *D. melanogaster* kindly presented by Prof. H. J. MULLER of Indiana University.

K. HAYASHI and S. NAWA were appointed researchers in the new Department of Biochemical Genetics. M. TSUJITA, B. SAKAGUCHI, T. ENDO, T. IINO and S. TSUDA were transferred from the Department of Physiological Genetics to this new department. T. TAIRA was appointed a member of the Department of Physiological Genetics. M. KIMURA took a leave of absence for a year from July 1953 to study mathematical genetics in the Department of Genetics, Iowa State College. H. ETÔ, Assistant Professor of Tokyo University, and Y. OZAKI, Lecturer in The National Institute of Public Health, were appointed Associates of the Institute. M. TSUKAMOTO, who had been head of the Department of Administration since the founding of the Institute, was transferred to Sizuoka University. His ability and effort had contributed a great deal to the completion and expansion of the Institute. He was replaced by K. OTOFUJI.

T. KOMAI was invited to the International Congress of Scientists for Cultural Freedom held in July in Hamburg. He also attended the Fourteenth International Zoological Congress held in Copenhagen in August as delegate of the Japan Academy, and the Ninth International Congress of Genetics at Bellagio, North Italy, held in the same month. H. KIHARA and Y. SINOTÔ also attended this International Congress of Genetics, KIHARA as a vice-president. The latter also represented Japan at the Eleventh Committee Meeting of IUBS at Nice, France directly before the congress. SINOTÔ was nominated a member of the Permanent Committee of the International Congress of Genetics.

Y. HANDA, Assistant Professor of Wakayama Medical College, stayed one year at the Institute to prepare himself for the professorship in human genetics in the college. Three new research students were admitted during the year, working on special subjects under the direction of the staff.

The Genetics Society of Japan held its Twenty-fifth General Meeting on the 7th and 8th of November 1953. It was attended by more than 300 members.

Among the foreign visitors to the Institute during the current year were Dr. A. WOLSKY of Science Cooperation Office for South East Asia of UNESCO, Prof. M. W. YOUNG of Howard University, Washington, D.C., Dr. G. TAYLOR and Dr. J. MORTON of ABCC, Hiroshima, Dr. Chang of FAO, UNESCO, Drs. N. PARTHASARATHY, A. B. SARAN, M. B. VNARASINGARAO of the Indian Agricultural Research Institute, New Delhi, India, Prof. J. G. KIRKWOOD of Yale University, Prof. P. J. FLORY of Cornell University, and Dr. L. PRIGOGINN of Belgium.

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 to Institutes of the Ministry of Education: to K. OGUMA, for: The project of breeding of mouse and rat strains for medical and biological purposes —¥ 5,530,000.

From the Fund for Grants-in-Aid to Cooperative Investigations: to T. KOMAI and co-workers (including K. SAKAI and M. KIMURA), for: Researches in population genetics—¥530,000.

Y. TANAKA and co-workers (including M. TSUJITA), for: Fundamental and applied genetics of the silkworm—¥440,000.

S. MATSUMURA and co-workers, for: Researches on the physiology of crop plants under standardized temperature, humidity and day-light conditions—¥380,000.

From the Fund for Grants-in-Aid to Investigations on Applied Sciences: to Y. TANAKA, for: Research on artificial control of diapause in the wild silkworm, *Antheraea pernyi*—¥450,000.

M. TSUJITA, for: Researches on the genetics and selection of abnormal eggs in the silkworm—¥150,000.

From the Fund for Grants-in-Aid to Individual Workers: to K. OGUMA and co-workers (including T. KOMAI and K. TUTIKAWA), for: Breeding and preservation of strains of rats and mice useful for medical research purposes—¥720,000.

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 T. ENDÔ, for: Genetics of flower colors—¥30,000.

S. TSUDA, for: Studies on the propagation 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 breeding of autogamous plants—¥100,000.

ABSTRACT OF DIARY FOR 1953

January 29. Eighth meeting of the Board of Councillors.

January 31. Fourteenth meeting of Misima Geneticists' Club.

March 18. Board meeting of Association for the Propagation of the Knowledge of Genetics.

March 28. Fifteenth meeting of Misima Geneticists' Club.

April 18. Sixteenth meeting of Misima Geneticists' Club.

May 20. Seventeenth meeting of Misima Genetists' Club.

June 18. Ninth meeting of the Board of Councillors.

June 27. Second general meeting of Whole-Japan Association of Poultry Genetics.

July 25. Eighteenth meeting of Misima Geneticists' Club.

August 21. Nineteenth meeting of Misima Geneticists' Club.

September 29. Twentieth meeting of Misima Geneticists' Club.

October 24. Twenty-first meeting of Misima Geneticists' Club.

November 6. Joint meeting of the National Committee of Genetic Researches and the National Committee of Researches in Animal and Plant Breeding of Japan Science Council. Committee meeting of the Genetics Society of Japan.

November 7, 8. Twenty-fifth general meeting of the Genetics Society of Japan. General meeting of the Society of Chromosome Research.

December 9. Meeting of Arrangements Committee for the International Genetics Symposium.

December 21. Twenty-second meeting of Misima Geneticists' Club.

STAFF

Department and Laboratory Heads

Kan OGUMA, D. Ag., Director
Yoshimaro TANAKA, D. Agr., 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. Agr., Head of Department of Biochemical Genetics
Kan-ichi SAKAI, D. Agr.
Seiji MATSUMURA, D. Agr.
Kôzô HAYASHI, D. Sc.
Tosihide H. YOSIDA

Part-time Staff and Research Associates

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

Junior Investigators

Motô KIMURA
Kanji GOTOH
Akira MIYAZAWA
Bungo SAKAGUCHI
Tôru ENDÔ
Kiyosi TUTIKAWA
Tetuo IINO
Toshifumi TAIRA
Saburô NAWA
Seizô TSUDA
Assistants—12

Department of Administration

Kan-ichi OTOFUJI, Head of Department
Sumiyoshi SUGIO, Head of General Business Section
Masao MIYAZAWA, Head of Finance Section
Naomi MATSUBARA
Hiroko NAKANO
Junzô KADOWAKI
Clerks, Typist, Telephone operators, Chauffeurs, Field laborers, Janitors, etc.—18

Misima Branch of Hatano Tobacco Experiment Station

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

Whole-Japan Association of Poultry Genetics

Kan OGUMA, President
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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Riichi KAWAKAMI, Head of Department of Biometry, National Institute of Public Health
Makita KOGURE, Professor of Tokyo College of Agriculture and Technology
Kiyoshi MASUI, Professor of Nagoya University

Toshitarô MORINAGA, Head of Department of Physiology and Genetics of Agricultural Technique Institute
Warô NAKAHARA, Director of Cancer Research Institute
Masanori NAKAIZUMI, Professor of Tokyo University
Toshio SAITÔ, Governor of Sizuoka Prefecture
Yusuké SUMIKI, Professor of Tokyo University
Yûshi UCHIMURA, Professor of Tokyo University
Bungo WADA, Professor of Tokyo University

RESEARCH PROGRAM FOR 1953

Tanaka Laboratory

Unstable genes in the silkworm—TANAKA
Genetics of "retarded" strain of the silkworm—TANAKA
Genetics of malformations in the silkworm—TANAKA
Photoperiodic effect on diapause of the wild silkworm *Antheraea pernyi*, and the genetics of diapause—TANAKA and ONIMARU
Artificial mutation in the modifying genes of "multilunar" character—TANAKA

Matsumura Laboratory

Radio-genetic studies on wheat—MATSUMURA and FUJII
Studies on *Agropyrum*, a close relative of *Triticum*—MATSUMURA
Nullosomic plants found among the progeny of pentaploid hybrid wheat—MATSUMURA
Breeding of wheat strains resistant to rust—MATSUMURA and HIRATSUKA (Tokyo University of Education)
Studies on the relation between the quality of radiation and mutation—MATSUMURA and ETÔ (Tokyo Univ.)
Induced mutations in tobacco plants by X-ray irradiation—KIHARA, MATSUMURA and FUJII
Breeding of triploid sugar beet—MATSUMURA et al.

Yoshida Laboratory

Studies on sex chromosomes in insects—YOSIDA
Experimental cytology and developmental genetics of tumors—YOSIDA and ISHIHARA
Genetics of cancer susceptibility of mice and rats—YOSIDA and ISHIHARA
Genetics of cancer found in *Drosophila*—YOSIDA and ISHIHARA

Karyology of normal and tumor cells in man—YOSIDA and OMURA

Takenaka Laboratory

Origin of sex differentiation in higher plants—TAKENAKA

Cytogenetics of *Nicotiana*—TAKENAKA

Cytogenetical studies on the effect of mitosis-inhibiting substances —TAKENAKA

Cytogenetical studies on the effect of mutagens—TAKENAKA

Studies on sterility in *Citrus*—FURUSATO

Studies on polyembryony in plants—FURUSATO

Studies on the origin of polyploidy in *Citrus*—FURUSATO

Studies on triploid water-melon—FURUSATO and MIYAZAWA

Studies on triploid tobacco plants—FURUSATO and MIYAZAWA

Karyology of Gramineae—TAKENAKA and TATEOKA

Collection and preservation of useful varieties of plants—TAKENAKA

Komai Laboratory

Genetics of human microcephaly—KOMAI, KISHIMOTO (Nagoya Univ.) and OZAKI

Population genetics of the land-snail *Bradybaena*—KOMAI

Population genetics of some species of *Drosophila*—KOMAI and TAIRA

Genetics of blue sclerotics in man—KOMAI, OZAKI and KUNII

Studies on tumor susceptibility in mice—TUTIKAWA

Studies on lethal genes in rats and mice—TUTIKAWA

Studies on phenocopies in mice—TUTIKAWA

Studies on viability in mice—TUTIKAWA

Breeding and preservation of mouse and rat strains useful for medical investigations—MAKINO and TUTIKAWA

Sakai Laboratory

Competition between individual plants of different genetic constitutions—SAKAI et al.

Theoretical and experimental studies on selection in plant breeding—SAKAI

Genetics of fruit crops—SAKAI, GOTOH and SUZUKI

Genetics of quantitative characters in tobacco plants—SAKAI and IYAMA

Population genetics on land rice and "red rice"—SAKAI et al.

Local differentiation of races in barley—GOTOH

Theoretical studies on population genetics—KIMURA
Genetics of right- and left-handedness in plant organs—KIMURA

Tsujita Laboratory

Developmental genetics in the silkworm—TSUJITA and SAKAGUCHI
Biochemical genetics on insect materials—TSUJITA, SAKAGUCHI and NAWA
Heredity and selection of abnormal eggs in the silkworm—TSUJITA and SAKAGUCHI
Studies on phenocopies in the silkworm—SAKAGUCHI
Biochemical genetics on lethal strains in the silkworm—SAKAGUCHI
Cyto-chemical studies on mitochondria—TSUJITA, SAKAGUCHI and TSUDA
Studies of the technique of ultra-thin sectioning in electron microscopy—TSUJITA and TSUDA
Electron-microscopical studies on cell inclusions—TSUJITA and TSUDA
Electron-microscopical studies on the propagation of bacteriophages—TSUJITA and TSUDA

Hayashi Laboratory

Studies on chemical composition of anthocyanin in races of *Canna*—HAYASHI
Chemical analysis of coloring substances of various varieties of morning glory and their genetic behavior—HAYASHI and ABÉ
Chemical composition of pigments in maples and alpine plants—HAYASHI and ABÉ
Biochemical genetics of *Ustilago*—IINO
Studies on the effect of chemical substances on mutation—IINO
Studies on the aquirement of metabolic capacity in fungi—IINO
Gene analysis of flower colors—ENDÔ
Analysis of environmental factors in the appearance of flower colors—ENDÔ

Research Students and Research Subjects

Kyôzô WATANABÉ: Genetics of protozoa
Yasuo SUZUKI: Population genetics of cultivated plants
Tarô AKIYAMA: Genetics and breeding of *Citrus*
Yoshihiko KAWAGUCHI: Biometry
Toshio OMURA: Cytology of cancers

Chiaki MATSUI: Morphology and variation in bacteriophages
Kôzô NAKAMURA: Cytogenetics of *Agropyrum*
Tôyô MITARASHI: Fundamental morphological studies of animal cells
Takatada KAWAHARA: Improvement of races of poultry
Yasuo OTA: Cytogenetic studies on morphological characters of *Citrus*
Shinya IYAMA: Population-genetics of land-rice

RESEARCHES CARRIED OUT IN 1953

A. HUMAN GENETICS

(Report by Taku KOMAI)

Genetics of Microcephaly

This study was completed during the current year, and the result was reported at the Ninth International Congress of Genetics held at Bellagio, North Italy, in August 1954. Altogether 143 cases of microcephaly consisting of 93 males and 50 females were collected by the cooporation of Prof. K. Kishimoto, M.D., of Nagoya University and Y. Ozaki, M.D., of the Institute of Pulblic Health. Of these, 64 (44.8 per cent) are progeny of first-cousin marriages. No authentic case of purely non-genetic microcephaly is included in this list. At least the great majority of these cases seem to be due to a recessive autosomal gene. The incidence of the gene among the Japanese population was estimated by means of the formula:

$$q=\frac{c\ (1-k)}{16k-15c-ck}\ ,$$

where q stands for the incidence, k the rate of first-cousin marriages among the parents of the affected individuals and c the rate of first-cousin marriages in the whole population. The value 0.0034~0.0063 was obtained for q, according to whether 0.04 or 0.07, or some other value between these two, is chosen for c. This value for the incidence of the gene in the Japanese population coincides well with 0.0043~0.0062, the corresponding value obtained for the Swedish population by Böök et al. by means of an entirely different method. The rate of mutation of the gene was estimated on the assumption of an equilibrium state of the incidence of the gene in the population by means of the formula:

$$m=(1-f)[\alpha q+(1-\alpha)q^2]\ ,$$

where m denotes the mutation rate, f the fertility of the abnormal individuals as compared with that of normal individuals, and α the mean coefficient of inbreeding. The value $(2.20\sim7.57)\times10^{-5}$ was obtained for m, depending on the values of c, α and q.

B. GENETICS OF SOME MAMMALS

1. The Tortoiseshell Male Cat and its Sterility

(Report by Taku KOMAI)

The Japanese common people have a traditional interest in the tortoiseshell male cat. Also, the incidence of the gene for orange coat color,

which is an essential component of the tortoiseshell pattern, is apparently two or three times higher in Japanese cats than in European cats. Thus, it is certain that we come across tortoiseshell male cats more often than our European colleagues, and we should have a better chance to find the key to the solution of this old puzzle of genetics. The writer has continued to collect materials of this type of male cats, and found more than fifty of them, together with records of their parents and litter mates. Special attention has been paid to a search for reports of the birth of more than one tortoiseshell male from the same mother. Since the writer's hypothesis concerning the origin of the tortoiseshell male and its sterility postulates a crossing over between the X and Y chromosomes in a paternal spermatocyte, such an occurrence would virtually invalidate the hypothesis. So far all available records conform to the hypothesis, and no authentic case of the birth of more than one tortoiseshell male from the same mother has been found. Intensive cytological study by T. Ishihara of the testes of tortoiseshell male cats is in progress.

2. *Studies on T locus in the Wild Japanese Mouse, Mus musculus molossinus*

(Report by Kiyosi TUTIKAWA)

To examine whether the locus *T*, which is known to be mutable in both domestic and wild *Mus musculus* (Dunn and Gluecksohn-Schoenheimer

Table 1. Results of test-crossing to a wild population by Brachy *T*/+

Wild parent	Offspring			Total
	Normal	Brachy	Tailless	
♀ 185	8	5	....	13
21029	9	11	....	20
318	1	4	....	5
42132	6	4	....	10
5323	1	4	....	5
43122	2	3	....	5
	27	31	....	58
♂ 42131	5	4	....	9
444	6	6	....	12
4321	3	4	....	7
42134	5	3	....	8
	19	17	....	36
♂ 425	7	2	2	11
4462	2	1	4	7
	9	3	6	18

'50, Dunn and Morgan '53), is also mutable in the Japanese mouse, ***M.m. molossinus***, some wild animals of the latter subspecies were crossed to animals of the *T* strain kindly provided by Prof. L. C. Dunn. The results are shown in Table 1. The six males and six females used were wild mice trapped in a barn in our Institute. Following Dunn's method, each mouse was crossed to a Brachy $T/+$ mouse derived from outcrossing with the original tailless line. All the six females of ***molossinus*** tested gave only normal and Brachy offspring (27 normals and 31 Brachys), while two of the six males produced some tailless animals besides normals and Brachys (9 normals, 3 Brachys and 6 tailless), and four produced only normals and Brachys (19 normals and 17 Brachys).

If this new tailless character is due to the presence of an allele (or rather pseudo-allele) of *t* which might be designated as t^m, then tailless (T/t) and normal (t^1/t^m or t^o/t^m) progeny should be expected from the test-cross of this tailless with the known tailless lines (T/t^1 or T/t^o). Such procedure is now in progress.

The average tail length of the F_1 Brachys in the reciprocal outcrosses was greater than in the Brachy ***musculus*** animals used for the test. This indicates the presence of a modifier or modifiers which inhibit the effect of the *T* gene to some extent.

Comparative studies of the number of vertebrae and other skeletal features of wild samples from different localities are also in progress.

C. CYTOLOGY AND GENETICS OF TUMORS

1. Karyological Study on the MY-mouse Carcinoma

(Report by Tosihide H. YOSIDA)

The MY-mouse carcinoma is a gland cell carcinoma originally developed in the D-strain mouse, established by the present author as a transplantable tumor (Yosida 1952). The present report deals with cytological observations of this tumor.

1. Abnormal nuclear divisions: It has been reported by many investigators of tumor cytology that mitotic abnormalities are most common phenomena in tumors. In the cells of the MY-mouse tumor also, various types of abnormal mitosis have been observed. Multinucleate cells and multipolar spindles are of common occurrence. Polyploid cells are frequently found. Atypical arrangement of chromosomes on the metaphase plate, abnormal swelling of chromosomes and c-mitotic chromosomes are also common. Displacement of chromosomes on the metaphase plate, and hollow metaphase plates are likewise noteworthy. Coalescence or irregular agglutination of chromosomes, which is usual feature of degenerating

cells, is also rather frequent.

2. Chromosome numbers of tumor cells: The number of chromosomes in these tumor cells fluctuated around 40, cells having 39 or 40 chromosomes being the commonnest (Table 1).

Table 1. Chromosome numbers in tumor cells of the MY-mouse carcinoma.

No. of Chrom.	54	49	45	44	43	41	40	39	38	37	36	35	34	33	Total
No. of cells obs.	1	1	1	1	2	3	8	7	1	2	1	2	1	1	32

3. Morphological analysis of chromosomes: It seems noteworty that, besides the cells with abnormal mitosis, cells showing regular mitosis were also observed. The latter are the tumor strain-cells. In the Takizawa quinone-carcinoma and the Ehrlich ascites carcinoma, the author has observed tumor strain-cells characterized by the presence of V-shaped chromosomes of medium size. In the tumor strain-cells of the MY-mouse carcinoma such V-shaped elements were never found. Small dot-like chromosomes, constricted chromosomes, and chromosomes with trabants were rather common. A fact worthy of notice is that the chromosomes with trabants are generally longer than those in the MY-mouse sarcoma cells described in the following report.

2. *Karyological Study on the MY-mouse Sarcoma*

(Report by Tosihide H. YOSIDA)

The MY-mouse sarcoma is a transplantable sarcoma established by the author (Yosida 1952). It is a kind of spindle-cell sarcoma originally developed in one mouse of the inbred So-strain, which was derived from the cross S-strain × mixed-strains. Observations on the chromosomes of this tumor have revealed the following facts:—

Various types of mitotic abnormalities, such as multinucleate and polyploid cells, multipolar mitosis, and atypical arrangement of the chromosomes, were observed. A noteworthy fact is that, besides showing abnormal mitosis, regular mitotic cells occur. These cells are undoubtedly the strain-cells of this tumor.

The number of chromosomes in these strain-cells may be counted in good metaphase figures. Cells having about 40 chromosomes are most common. This shows that the MY-mouse sarcoma, like the MY-mouse carcinoma, is characterized by having diploid strain-cells.

All the chromosomes were of the normal rod-type. No evidence has

been found for the presence of any V-shaped element such as observed in the Takizawa quinone-carcinoma and Ehrlich ascites carcinoma. Futhermore, constricted chromosomes, dot-like chromosomes and chromosomes with trabants were often observed, much as in the MY-mouse carcinoma described above. The differences may be found, however, that such abnormalities in chromosome configurations in this tumor occur with lower frequency than in the MY-mouse carcinoma, and that the chromosomes with trabants are much shorter than those in the latter. The cells of this tumor are characterized by possessing trabant-bearing chromosomes which are smaller than any of the other chromosomes. Such elements were never found in the MY-mouse carcinoma.

3. *Karyological Study of some Non-transplantable Tumors*

(Report by Tosihide H. YOSIDA)

We often encounter spontaneously developing or artificially induced tumors which do not show any transplantability to other hosts. It is well known that the transplantability is in many cases genetically controlled. It is highly probable, however, that besides such a genetical factor, some other factor affects the transplantability. This report deals with karyological observations of some non-transplantable tumors.

The materials used in the present study were No. 9 mammary carcinoma (originating in B-strain mouse), No. 10 mammary carcinoma (B-strain), No. 12 tumor (non-transplantable ascites tumor originating in B-strain), No. 20 mammary carcinoma (S-strain) and BE mammary carcinoma (E-strain). We have tried several times to transplant these tumors to animals of the same strain as that in which the tumer originated, but without any success.

Karyological observations of these tumors have revealed very few cells with regular mitosis. This observation suggests that the karyological state, is an important factor controlling transplantability of tumor cells.

4. *A Study on the Transplantability of the MY Mouse Sarcoma and Carcinoma*

(Report by Takaaki ISHIHARA and Tosihide H. YOSIDA)

The MY-mouse sarcoma is a spindle-cell sarcoma which developed in a So-strain mouse. This strain was originated from hybrids between S-strain and mixed strains, and has been inbred for many generations. The

transplantability of this tumor showed a considerable difference according to the strain of the host used. First, the transplantability was examined in four strains of mice and in a number of transplant generations (Table 1). As shown in the table, this tumor is difficult to transplant to the original So-strain mice. Its transplantability to the S- or D-strain increased with the transplant generations, while that to the B-strain decreased.

Next, experiments were undertaken to transplant the tumer to various other strains such as S^k, D, S, C3H, DBA/2, A, SWR and Swiss albino. The results obtained are shown in Table 2. The transplantability into C3H, DBA/2, A, SWR and Swiss albino was zero, whereas the highest transplantability (87.5%) was found for the S^k-strain mice. No strain having 100 per cent transplantability was found. The behaviour of the transplanted tumor was very characteristic to each strain. For instance, the tumor grafted into the S^k-strain mice showed an active growth, and the hosts usually died of the tumor 19–25 days after transplantation. The D-strain mice died of the tumor 24–84 days after inoculation. Although the transplantation to C3H, DBA/2, A, SWR and Swiss albino usually showed negative results, grafts inoculated into C3H and DBA/2 degenerated gradually, after showing some temporary developmental changes.

The MY-mouse carcinoma is a gland cell carcinoma which originally developed in a D-strain mouse. This tumor showed 100 per cent transplatability to the D-strain mice in the 1–7 transplant generations (Table 3), but decreased to 80.2 per cent in the later transplant generations. On the other hand, the growth rate of the graft in the S-strain mice was considerably lower in early transplant generations. From the above findings, it seems clear that this tumor undergoes changes in transplantability with the progress of the transplant generations, decreasing in the original strain and increasing in other strains.

Table 1. Transplantability of the MY-mouse sarcoma according to strains of mice and number of transplant generations.

Strain \ Transplant generation	1—8	13—24	32—38
	%	%	%
S	32.4 (71)	70.6 (17)	62.2 (57)
D	0 (1)	59.3 (32)	76.5 (34)
B	74.4 (47)	0 (13)	0 (12)
So	44.1 (34)	52.6 (36)	—

The figures in parentheses denote the number of mice used.

Table 2. Transplantability of the MY-mouse sarcoma according to various strains of mice.

Strain	No. of mice used	Results of transplantation		% of positive results
		Positive	Negative	
S^k	16	14	2	87.5
D	34	26	8	76.4
S	53	33	20	62.2
C3H	36	0	36	0
DBA/2	6	0	6	0
SWR	10	0	10	0
Swiss	8	0	8	0
A	10	0	10	0

Table 3. Transplantability of the MY-mouse carcinoma according to strains of mice and number of transplant generations.

Strain \ Transplant generation	1—7	13—24
	%	%
D	100 (12)	80.2(41)
S	60.8(46)	72.5(46)
B	77.7(18)	26.0(15)

The figures in parentheses denote the number of mice used.

5. *On a Non-transplantable Ascites Tumor in Inbred Mice*

(Report by Tosihide H. YOSIDA and Takaaki ISHIHARA)

An ascites tumor developed spontaneously in a B-strain mouse in the course of an inbreeding experiment in our laboratory. Transplantation experiments of this tumor to many mice of the original B-strain and also to animals of other strains were performed by using the usual transplantation technique for ascites tumors, but without success. The transplanted cells in all cases began to degenerate soon after transplantation. Various tumor tissues were also inserted under the epidermis of the host by the usual technique for solid tumor, but the transplantation was never

successful.

Autopsy revealed considerable hypertrophic change in the spleen, liver and lymphatic gland, but the kidneys had remained nearly intact. A tumor-like mass had developed in the dorsal part of the peritoneal cavity. The abdorminal cavity of the tumor-bearing animal was swollen by the accumulation of the hemorrhagic asctites.

Histological observations of various organs:

Liver: It was observed that lymphoid cells, lymphocytes and neutrophil leucocytes had infiltrated into Glisson's sheath, but none of them was found in the hepatic lobes.

Spleen: Remarkable infiltration of lymphoid cells was observed.

Kidney: In the Malpighian bodies many lymphoid cells were observed, especially in the Bowman's capsules or around these tissues.

Tumor-like mass in the dorsal region of the peritoneal cavity: The component cells looked very similar to lymphoid cells. There were many mitotic figures. Based on these observations, this tumor was assumed to represent a type of lymphatic leukemia, although any definitive conclusion is premature as yet.

Cytological observation: The ascites of the tumor-bearing animal was examined by using the acetic orcein smear technique. Many cells which had the character of lymphoid cells were found. The cytological features of these cells are somewhat similar to those of the ascites tumor in mice. Each cell contained one nucleus in many cases, more rarely two or three. Many mitotic figures were found, so that a chromosomal survey was feasible. It is noticeable that although agglutination, coagulation, displacement and stickiness of the chromosomes were often observed, no cell with a regular metaphase figure was found. From the above karyological observations it is concluded that the non-transplantability of this tumor is probably due to the lack of regular mitotic cells which should constitute the stem line of the tumor and play a decisive role in its growth.

D. SILKWORM GENETICS AND BIOCHEMISTRY

1. *Influence of the Incubation Temperature on the Development of Multilunar and Multistar Markings of the Silkworm*

(Report by Yoshimaro TANAKA)

The eggs of a given strain of silkworm were divided into two groups and incubated under different temperatures.

a) High temperature section: Eggs were incubated at 25°C for 11–14 days, from immediately after laying until they hatched.

b) Low temperature section: Eggs were kept at 15°C for 14 days, then removed to 25°C and kept for 4–5 days until the emergence of the caterpillars.

The larvae were reared at ordinary room temperature from July to August, and the markings in the adult stage were observed. The results are shown in Table 1.

Table 1. A List of Effects of Different Temperatures for Multilunar and Multistar Spots

Lot No.	High temperature				Low temperature		
	Standard type *		"Plus" *	"Minus" ψ	Standard *	"Plus" *	"Minus" ψ
		%	%	%	%	%	%
532 *l* 11	*L* 4—8	100.0	0	0	91.9	8.9	0
532 *l* 17	*L* 4—8	98.0	2.0	0	7.2	92.8	0
532 *l* 21b	*L* 4—10	100.0	0	0	100.0	0	0
532 *l* 41	*L* 4—9	22.4	0.8	76.8	75.1	24.9	0
532 *l* 62	*L* 5.8	94.1	5.9	0	49.2	50.8	0
533 *l* 11	*L* 4—8	82.1	0	17.9	98.0	2.0	0
533 *l* 17	*L* 4—8	95.3	4.7	0	35.4	64.6	0
533 *l* 62	*L* 5.8	97.1	2.9	0	4.7	95.3	0
532*ms*12	*ms* 6—10	73.7	0	26.3	91.2	8.8	0
532*ms*32	*ms* 8.9.10	88.2	0	11.8	53.3	19.6	27.1
532*ms*82	*ms* 8	100.0	0	0	98.8	1.2	0
532*ms*9	*ms* 0	100.0	0	0	65.1	34.9	0
533*ms*32	*ms* 8.9.10	81.2	18.8	0	55.1	44.9	0
533*ms*82	*ms* 8	100.0	0	0	100.0	0	0
533*ms*9	*ms* 0	100.0	0	0	99.6	0.4	0

* "Standard" of each strain.

* Larvae with more numerous spots than the standard type.

ψ Larvae with less numerous spots than the standard type.

It can be seen from the table that the high temperature reduces the number of spots, while the low temperature increases it, almost without exception. In lot No. 532 *l* 41, for example, the standard type and the "minus" type form 22.4% and 76.8% respectively of the whole high temperature group, while the "plus" type is only 0.8% in contrast to 75.1% standard type and 24.9% "plus" type but no "minus" type among the low temperature group. In No. 532 *l* 17, similarly, nearly all the larvae, 98%, reared at high temperature had the standard type markings, while in the low temperature section the great majority (93%) of larvae

were of "plus" type.

Exactly the same result has been obtained in the multistar lines. For example, in No. 532 *ms* 9 the 0 type occupied 100% of the high temperature lot, while it appeared in only 65% of the larvae reared at low temperature, the rest (35%) being provided with one or two star spots on the 8th segment.

2. *A Possible Sub-threshold Effect of Multistar Modifiers for the Development of Multilunar Spots*

(Report by Yoshimaro TANAKA)

As the multistar is a recessive gene (*ms*), and the multilunar strains usually have the diminant allele ($+^{ms}$) for multistar, it is natural that the F_1 between them develops no multistar spots. Nevertheless, I have found a case which suggests that the modifiers of the *ms* gene may induce the development of multilunar spots when *ms* is involved in the heterozygous state, though no *ms* marking itself appears.

The $L_{5\cdot6\cdot8}$ strain is characterized by brown spots appearing usually on the 5th, 6th and 8th segments. Rather exceptionally, the spots occur on either the 4th or 7th segment or on both, but never on the 9th and 10th segments.

In the F_1, the variability of the number of multilunar spots increased considerably, and the spots were often extended to the 9th segment. This was not the case with the F_1 between the $L_{5\cdot6\cdot8}$ strain and the normal ($+^{L}+^{ms}$).

3. *On the Intermediate Type Appearing in "Retarded" Strains*

(Report by Yoshimaro TANAKA)

In the dominant as well as the recessive "retarded" strains, and also in their hybrids with normals, there often appear some "intermediate" types. These larvae are somewhat smaller than the normal ones, but are larger than the retardeds, although the distinction is not always sharp. They are also intermediate between both types in growth rate.

The results of experiments with these individuals have disclosed that the intermediate animals can be either undergrown normals or well-developed retardeds. One cannot tell which until he has accomplished the progeny test of these animals. Another tool for making this distinction is the linkage relation between the retarded and the larval marking or blood-color genes in the second linkage group, because the retarded is

assumed to be due to a chromosome aberration in the second chromosome.

Thus it may be concluded that the cause of appearance of the intermediate type is mainly environmental. It is, however, premature to exclude a genic cause in this case, because there is a tendency for the frequency of the intermediate type among the offspring of intermediate parents to be higher than that among the offspring of other types. The existence of some modifiers influencing the growth of the silkworm seems to be plausible.

4. *Studies on the Action of the Gene Causing Malformation in the Silkworm*

(Report by Mitsuo TSUJITA and Bungo SAKAGUCHI)

The malformation in a mutant strain *om* appears on the dorsal and ventral sides of several segments ranging from the 5th segment anteriorly and posteriorly. Its characteristics were described in the Annual Report No. 1.

The previous report dealt with the effects upon the penetrance of this malformation gene of environmental conditions, especially temperature of 15°–25°C. and dilute hydrochloric acid in an early developmental stage. It has been shown that the rate of appearance of the cripped larvae can be changed within a wide range from 0 to 100%.

The experiments were performed as follows: Eggs were kept at 25°C. for 10–15 hours, incubated at 15°C for 7 days, and then kept in a cold room at 5°C for 60 days. After this refrigeration, they were subjected to treatment by dilute hydrochloric acid. The larvae hatched from these eggs were examined and the degree of penetrance of the gene was estimated. The results obtained are shown in the following table.

It is clear from the table that the most sensitive period of the malformation gene to environmental factors, especially temperature or stimulation by hydrochloric acid, is of very short duration, from 10 to 15 hours after oviposition. This sensitive period exactly corresponds to the early stages of embryo formation, i.e. the time during which cleavage nuclei migrate under the egg shell and form an epithelial layer, and some of them start to invaginate to prepare for blastoderm formation.

In order to make clear the mechanism of the appearance of the malformation in this period, we have undertaken biochemical studies of the early developmental stages of the mutant embryos of the period from 5 to 100 hours after oviposition. As the first step of the experiment the activity of cytochrome oxidase was examined.

Method: 10–15 hours after oviposition, each batch of the normal and

Table 1. The rate of appearance of crippled larvae after treatment of the eggs with low temperature (15°C)

T. O. / M. R. / E. L.	10—15 hours				15—20 hours				20—25 hours				25—30 hours			
	N	M	Total	MR	N	M	Total	MR	N	M	Total	MR	N	M	Total	MR
Lots treated with low temp.	350	0	350	0	456	25	481	5.2	256	198	454	43.6	284	278	562	49.5
Control	258	286	544	52.6	256	286	542	52.8	251	265	516	51.4	258	297	555	53.5

N: Numbers of normal larvae, m: Crippled larvae. MR: Ratio of crippled larvae.
T.O.: Time spent at 25°C after oviposition
E.L.: Experimental lots

mutant strains was halved, and one half was incubated at 25°C and the other at 15°C. The activity of cytochrome oxidase was measured after 5, 10, 15, 20, 40, 60, 80 and 100 hours. For this measurement 0.5 gr. of eggs was taken from each lot at the fixed time mentioned above, an isotonic solution of sucrose weighing three times as much as the eggs was added, and the whole was ground in a homogenizer. The homogenized solution was used as enzyme solution. Cytochrome C which was used as substrate was obtained from an extract of the heart muscle of cattle. The cytochrome oxidase was measured by a WARBURG manometer.

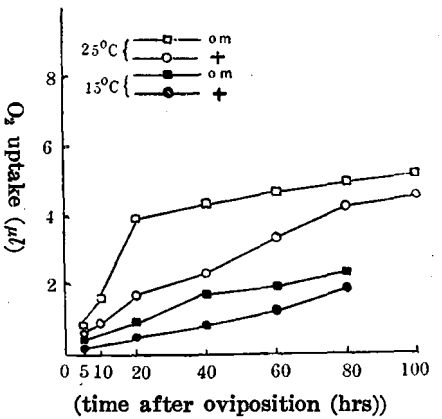


Fig. 1. Cytochrome oxidase activity in the eggs of normal and mutant strains.

Results: The experiment was repeated four times, and each time about the same result was obtained. In the lot of the mutant strain incubated

at 25°C, the activity of the enzyme increased rapidly for 10–20 hours after oviposition; later the rate decreased. In the normal strain, however, no rapid change in enzyme activity was found during the same period, and the increase was uniform and gradual (Fig. 1).

Both strains arrived at nearly the same rate of 0_2-uptake about 100 hours after oviposition.

In the lot incubated at 15°C, the enzyme activity was weaker than in the lot incubated at 25°C, in both the normal and mutant strains. As in Fig. 1, the activity in the normal strain showed a linearly rising curve, but in the mutant strain the increase was rather irregular during 30 to 40 hours after oviposition. No striking change as found in the high-temperature lot occurred, and the curve of increase resembled that of the normal strain.

It is clear from these findings, that the sensitive period of the malformation gene coincides with the time when the activity of the respiratory enzyme shows abrupt changes under high temperature. It is also shown that this change is less pronounced at the lower temperature. It is more than probable that these two phenomena, the sensitivity of the malformation gene and the abrupt change in the activity of the respiratory enzyme, are causally connected. Based on this finding we are now planning further studies along this line.

5. *Genetical and Biochemical studies on yellow lethal larvae (II)*

(Report by Bungo SAKAGUCHI and Mitsuo TSUJITA)

In a previous report (Annual Report No. 3, 1953), the maternal inheritance of a yellow lethal strain in the silkworm was described. Biochemical studies of the lethal yellow larvae with special regard to pterin pigment were continued. Our special interest was in the direct cause of the larval death which was apparently due to imperfect differentiation of the mandibular cuticle and incomplete hardening of the cuticle layer of the hypodermis.

Biochemical experiments with the normal and the mutant strains carried out in 1953 yielded the following results:—

Pterin: It was confirmed by Nawa et al. that the so-called leucoptrin-B was identical with isoxanthopterin. This pterin is contained in the epidermal tissue of normal silkworm larvae. The nature of the so-called xanthopterin-B remains obscure. We are now studying its chemical structure. The estimation of the amounts of xanthopterin-B in normal (+), lemon (*lem*), lethal yellow (lem^1), and white-egg lethal yellow (lem^1; w_1) was reported in the Annual Report No. 3.

Amino acids: In order to detect various kinds of bound and free amino acids, and to determine their relative amounts in normal and lethal yellow larvae immediately after the 1st moulting, extracts of the two amino acids obtained from the hypodermal tissue of the larvae were examined by paper chromatography following AWAPARA's procedure (1948). These amino acids were analyzed by means of two-dimentional paper-chromatography. The developments of extracts on the filter paper were saturated with solvents of phenol (0.1% NH_4OH, + 15%) butanol acetic acid (n-butanol 4: acetic acid 1: water 1), lutidine-collidine (lutidine 1: collidine 1) and pyridine. The positions of the amino acids developed were determined by spraying 2% ninhydrin.

Nearly twenty kinds of amino acids were detected in the epidermal tissue of both + and *lem*[1] strains. Remarkable differences were found for several amino acids shown in Table 1.

Table 1. Differences in amino acids extracted from epidermal tissue of normal and lethal yellow larvae.

Types of amino acids	Free amino acids		Bound amino acids	
Strain / Amino acids	+	*lem*[1]	+	*lem*[1]
Phenylalanine	++	±	±	++
Tyrosine	++	±	+	+
Serine	++	−	+	+
Cystine	+	−	+	+
Histidine	+	−	−	+

The table shows that the reaction of phenylalanine, tyrosine, serine, cystine and histidine is weaker in the lethal lemon larvae than in the normal larvae, and that the reaction of bound amino acid is weaker in the latter than in the former.

Phenoloxidase activity: By using a WARBURG manometer, the phenol-oxidase activity of the epidermal tissue in the larvae directly after the 1st moulting of the strains, +, *lem*, *lem*[1], and *lem*[1]; w_1, was measured. A homogenate of the tissue was prepared as the crude enzyme solution, and phenylalanine, tyrosine and dopa in 0.02 M. concentration were used as substrate. No activity of phenylalanine oxidase was recognized in the homogenates of any of the strains. No difference in the activity of tyrosinase could be found among the strains. The dopa oxidase activity in both the normal and the lemon larvae was a little stronger than in the lethal lemon or white egg-lethal lemon larvae.

Relation of pterin to phenolase activity: In order to ascertain the part taken by pterin in the phenolase activity, a homogenate prepared from larval epidermal tissue of the normal strain was used as a crude enzyme solution. The solution was poured into the small flasks of the manometer apparatus which contained in its side arms tyrosine or dopa and synthetic xanthopterin or isoxanthopterin solution. It was found from this in-vitro experiment that isoxanthopterin inhibited tyrosinase and dopa oxidase activity, and that xanthopterin promoted the activity of both enzymes.

Uric acid: The uric acid contained in the larvae immediately after the 1st moulting of the strains +, *lem*^{1}, *lem*^{1}; w_1 was measured by means of indirect FOLIN-WU and HARDEN methods.

The results of the experiment are presented in Table 2.

Table 2. Amounts of uric acid in +, *lem*^{1} and *lem*^{1}; w_1 strains.

Strains \ Methods	Indirect Folin-Wu mg/g dry matter	Harden mg/g dry matter
+	3.7	5.3
lem^1	2.2	3.0
lem^1; w_1	1.5	2.3

The table shows that with both methods the relative amounts of uric acid have been found to be +>*lem*^{1}>*lem*^{1}; w_1.

Cytochrome oxdase: The cytochrome oxidase activity during the 1st moulting in the + and *lem*^{1} strains was measured according to the procedure described by SCHNEIDER and POTTER (1943). The oxidase activity of the normal strain was significantly higher than that of the yellew lethal strain.

General consideration: It may be said from the experimental results reported above that the action of the gene *lem*^{1} is directly related to the excessive production of xanthopterin-B and that this abnormality has an effect upon other metabolic activities such as melanin-uric acid- and tryptophan-metabolism, and also upon the respiratory enzyme activity.

6. *On the Respiratory Enzymes and Several Other Enzymes Concerned with the T. C. A. Cycle in the Mid-gut Epithelium of the Silkworm (Bombyx mori L.)*

(Report by Bungo SAKAGUCHI and Mitsuo TSUJITA)

The complex of enzymes implemental in the reactions of the citric acid cycle is called cyclophorase. It has been established that the respiratory

enzyme activity and cyclophorase activity are closely related to mitochondria. Microscopic examination of cyclophorase has indicated that it is essentially a suspension of intact mitochondria, and the enzyme activity has an intimate relation to the structure of the mitochondria.

We have studied the relation of the respiratory enzymes to mitochondria, using *Paramecium caudatum* and the silkworm (Bombyx mori L.) as material. As far as we know, few studies on the cyclophorase system of these materials have been carried out.

This present report deals with the respiratory enzymes and several other enzymes belonging to the cyclophorase system found in the mid-gut epithelia of silkworms.

The mid-gut epithelia of silkworm larvae (3rd to 4th day after 5th instar) were ground in 0.25 M sucrose solution in a Waring Blendor, and this suspension was used as the enzyme solution. SCHNEIDER-ROTTER's method was applied for detection of cytochrome oxidase and succinic dehydrogenase, and GREEN's method was applied for the detection of enzymes belonging to the cyclophorase complex. Sodium succinate or cytochrome C was added to the enzyme solution as substrate, and the activities of cytochrome oxidase and succinic dehydrogenase were measured by the amounts of oxygen uptake (mm^3) for 1 ml of homogenate per hour. The results of the experiment are shown in Table 1.

Table 1.

Substrate	Concentration of substrate	Oxygen uptake mm^3 crude enzyme solution mm^3	Control mm^3
Sodium succinate	5/100 M	74.6	5.6
Cytochrome C	1/1000 M	120.9	8.8

In another experiment the reducing power of succinic dehydrogenase was measured in TUNBERG tubules by using methylenblue (M/15,000) as indicator. The presence of this enzyme was substantiated by the fact that the reducing power of the homogenate was stronger than that of the control, and its activity was suppressed by malic acid (M/30). The activity of cytochrome oxidase was suppressed by KCN (M/100).

The results of an experiment designed for detecting the enzymes concerned with the TCA cycle, and the effect of ATP upon them are shown in Table 2.

As shown in Table 2, five enzymes of the TCA cycle were detected in the homogenate, and the activity of these enzymes was strongly activated by ATP.

From the results of these experiments, the following conclusion may be

Table 2.

Substrate	Concentration (Mol)	Oxygen consumption (mm^3) (homogenate/ml/hour)
Pyruvate	2/100	7.6
+ ATP		44.0
Citrate	2/100	2.8
+ ATP		9.3
Oxaloacetate	2/100	39.5
+ ATP		89.0
Succinate	2/100	39.5
+ ATP		89.0
α-Ketoglutalate	2/100	27.8
+ ATP		49.0

deduced: The cellular respiration in the mid-gut epithelium of silkworm larvae is probably carried out according to WARBURG-KEILIN's system and KREBS' cycle. The fact that ATP, added to the homogenate of the mid-gut epithelium, increases the activities of several enzymes belonging to the TCA cycle supports this view. Furthermore, it seems that the complex of enzymes is closely related to mitochondria which are contained in abundance in the two kinds of cells, cylindrical and goblet, occurring in the mid-gut epithelium.

7. *Pterin Obtained from the Silkworm (Bombyx mori L.)*

(Report by Saburô NAWA)

We previously isolated a pteridine from eggs and larval epidermis of the normal strain of silkworm, and tentatively named it leucopterin-B. We have subsequently succeeded in obtaining this compound in a crystalline form. The following data provide evidence for the identity of leucopterin-B with isoxanthopterin (2-amino-4, 7-dihydroxypteridine).

Leucopterin-B has no characteristic melting point and decomposes at temperatures above 300°C. It is soluble in alkali, but almost insoluble in neutral or acidic media and common organic solvents. The presence of the pterin may be detected even in an extremely dilute aqueous solution on account of the strong fluorescence; an aqueous solution of leucopterin-B produces a strong purple fluorescence. The UV spectra of leucopterin-B and isoxanthopterin in 0.1 N NaOH are superimposable.

	max (mμ)	log ε	min (mμ)	log ε
Leucopterin-B:	255	(4.07)	290	(3.45)
	340	(4.16)		

Isoxanthopterin:	255	(4.09)		290	(3.48)	
	340	(4.19)				
Anal. Calcd. for	$C_6H_5O_2N_5$:	C, 40.22;	H, 2.80;	N, 39.11		
Found.		C, 40.77;	H, 2.96;	N, 39.24		

The various synthetic 7-hydroxypterins are closely related to one another in many respects (e.g., solubility, fluorescence, UV spectra), and paper chromathography provides the simplest means for their characterization. The compounds were submitted to several chemical reactions which attacked the side chain, only, and the Rf values of the respective compounds before and after the reactions were measured. Thermal decomposition, oxidation by alkaline MnO_2 or $KMnO_4$, reduction by aluminium amalgam and esterification were used for characterization. No inconsistency between leucopterin-B and isoxanthopterin could be observed with respect to the various reactions: the analytical data and UV spectra also agreed. The inertness toward all of the reactions suggested the absence of a functional group, and the lack of identity with compounds with simple alkyl side chains was apparent from paper chromathography. These findings show that leucopterin-B is identical with isoxanthopterin.

It seems that the yellow pigment contained in the larval epidermis of the mutant "*lem*", tentatively named xanthopterin-B, is a pteridine derivative, but its chemical nature is still obscure.

We are endeavoring to clarify the chemical nature and physiological properties of xanthopterin-B, and the relationship between the pterins and tryptophan metabolisms.

E. POPULATION GENETICS OF SOME INSECTS AND A LAND-SNAIL

(Report by Taku KOMAI)

1. The Lycaenid Butterfly Neozephyrus

As stated in the Annual Report No. 3, the females of *Neozephyrus taxila* have four color and marking types which are due to triple-allelic genes. This was shown by statistical analyses of thirteen samples from various localities in Japan (Amer. Nat. 87: 87–95, 1953). The fourteenth sample more recently obtained from Takatuki between Kyoto and Osaka also conforms to this interpretation, as indicated below:—

Type	O	A	B	AB	Total
Number	11	2	73	8	94
%	11.70	2.13	77.66	8.51	100

$p=0.055$ $q=0.628$ $r=0.340$

$p+q+r=1.023$ $2pq=0.0691$ $D=-0.023$ $\sigma D=0.023$

2. *The Land-snail Bradybaena*

The study of population genetics of this polymorphic land-snail was continued through the current year. The three genotypes AA (yellow banded), AB (brown banded) and BB (brown unbanded) were compared for growth rate and power of resistance to low temperature. A statistically significant difference has been found in the growth rate between the AB group and the AA or BB group, at least for the young stage reaching 4 mm in shell diameter. The double dominant AB group grow more rapidly than either single dominant homozygotic group (AA or BB). In the power of resistance to low temperature – 5.5°C, there is an apparent distinction between the AB and AA groups on the one hand and the BB group on the other.

The results of analyses of 97 samples from 81 localities conform well to the interpretation of polymorphism which assumes triple-allelic genes. On the basis of these findings, as well as of those by previous authors on other polymorphic animals the general mechanism of the formation and maintenance of polymorphism has been investigated.

3. *Drosophila rufa*

T. TAIRA is working on the dimorphism found in females of this species. This dimorphism may be recognized in the banding of abdominal segments. The "dark" type has banding similar to that in the male *melanogaster*, while the "light" type has banding like that in the female *melanogaster*; the dark marking is completely dominant over the light. Population-genetic studies are in progress on natural populations and laboratory populations. So far it has been found that the heterozygote has some selective advantage in competitive mating over either the homozygote.

F. GENETICS OF SOME CEREALS

1. *Tetrasomic Gigas-plants in the Offspring of Nullisomic Dwarfs and Analysis of their Additional Chromosome Pair*

(Report by Seiji MATSUMURA)

Gigas-plants with 42 chromosomes ($1_{IV}+19_{II}$) are called a~g-gigas, according to the orginal 7 different a~g-dwarfs having the sterile chromosome configuration 20_{II}. They could be called D-nulli- and AB-tetrasomics. These combinations are to some extent compensating, owing to the fact that the additional chromosome of a gigas-plant is in every case semi-

homologous to the lacking D-chromosome of the given dwarf line.

SEARS' nullisomics I~XIV, deficient in a chromosome pair of the A or the B-genome, have been used in crosses with my a~g-gigas, in an attempt to find out the chromosomes to which the additional chromsomes of the AB-tetrasomic gigas-plants correspond. From the results of crossing between Nulli-VII and a~g-gigas, it has been assumed that the additional chromosome in the a-gigas is the same as SEARS' VII, which belongs to the A-genome according to LARSON and hence can be called *a*A. According to SEARS' analysis, my *a*-dwarf corresponds to his Nulli-XXI, and the chromosome XXI is semi-homologous to VII. Thus, SEARS' finding that VII and XXI are semi-homologous has been confirmed by my results. As to the additional chromosomes in the *a*- and *f*-gigas, the disagreements between SEARS and myself await further investigation.

2. *Chromosome Aberrations in Einkorn Wheat Induced by X-rays*

(Report by Seiji MATSUMURA)

In order to study the relation between the frequency of chromosome aberrations and the quality of X-rays, dormant seeds of *Triticum monococcum* were exposed to X-rays of different wave lengths, at the same dose (8,100 *r*, 95 *r*/min.). For the sake of comparison, the thickness of the filter was adjusted in inverse proportion to the wave length; that is, at 80 KVP no filter was inserted into MATSUDA's Type KXC-17 apparatus (tube type STO-200-3), while at 130 KVP a filter of 0.3Cu+0.5Al, and at 180 KVP one of 0.8Cu+1.5Al was used. At 50 KVP, irradiation was applied by another apparatus, a Modified Type KR-75 (tube type XDW-10) with 0.5Al filter. The data are shown in Table 1. The results obtained

Table 1. Relation between wave length of X-rays and frequency of chromosome aberrations in *T. monococcum* (Dosage 8,100 r 95 r/min.) (1953)

Voltage (KVP)	Filter	No. of observ-ed ears	No. of ears with aberrations			No. of aberrations (%)
			$6_{II}+2_{I}$	④$+5_{II}$	⑥$+4_{II}$*	
Control	—	24	—	—	—	0 (0.00)
50	0.5Al	46	—	4	—	4 (8.70)
80	—	47	—	1	1	3 (6.38)
130	0.3Cu+0.5Al	61	1	2	—	3 (4.92)
180	0.8Cu+1.5Al	116	5	3	—	8 (6.89)

* Counted as two aberrations.

last year could not be confirmed. This unexpected outcome awaits further investigation.

3. *Measurement of X-ray Dosage for Inducing Mutations*

(Report by Hideo ETO, Seiji MATSUMURA and Taro FUJII)

The question to be answered was, whether irradiation was even in all points of the field of exposure in our MATSUDA's Type KXC-17 apparatus (200 KVP, 3 mA) with the tube Type STO-200-3. The dosages were measured at a distance of 15 cm at many points by MATSUDA's dosimeter or "*r*"-meter. The data are shown in Fig. 1, where the dosage in the center is arbitarily designated as 100 and is larger on the cathode side than on the anode side. Thus, it has been found advisable to place the exposed material on a rotating table, at a relatively short distances from the tube focus. Also, the data of measurements at different distances show that the dosage is exactly inversely proportional to the square of the distance.

The dosage was also measured of another apparatus for Grenz-rays with the tube Type TX-20 (20 KVP, 10 mA) by a Siemens' Universal Dosimeter. It was 140 *r*/min. at a distance of 23 cm. Therefore, the small Grenz-ray apparatus should be very useful.

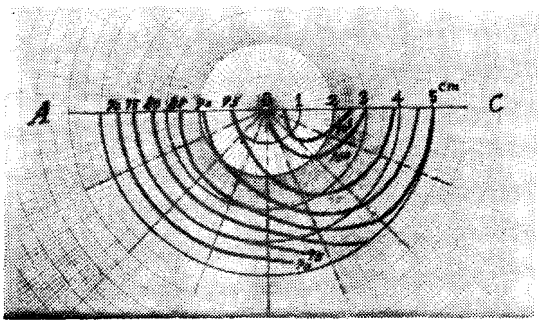


Fig. 1. Distribution of X-ray doses in tube STO-200-3. Distance 15 cm, A: anode, C: cathode.

4. *Inter-racial Differences Found in the Variety of Barley "Hosogara No. 2"*

(Report by Kanji GOTOH)

This experiment was designed to obtain answers to the following questions. First, to what extent has the differentiation between races of the same variety in crop plants, which have been grown in widely separated localities for a long time, progressed under the influence of the distinct environmental conditions? Second, how great is the difference between the original population of a variety and the geographical races derived from it? Third, to what extent has natural selection taken part in the racial differentiation?

A barley variety "Hosogara No. 2" was used for a preliminary series

of experiments. This variety was derived from the variety "Hosogara" more than 30 years ago, and it is known that it includes some distinct genotypes, such as, "Wechseltyp" and the "pure winter habit type". Seven population samples were obtained from various localities in Japan, ranging from Kitami, Hokkaido (44°N) to Kurashiki, Chugoku district (34°N).

Progeny of these local samples grown in Mishima under the same conditions were compared with one another. The progeny of local samples from the southern localities have more tillers than those from the northern localities. The average ear length of the progeny of southern samples was greater and the density of spike was lower than those of northern samples, and the differences were statistically significant. The difference in yield among these local samples was also significant, and the southern samples showed the general tendency of having smaller but more numerous grains per plant than the northern samples. Of the plants developed from seeds sown in our experimental field on February 28 and on March 30, all of the three southern races produced ears, while very few or none of the northern races did. These data show that there is an inter-racial difference in the photoperiodic response. Futhermore, it was found that this variety had higher competitive ability than the three tester varieties, but the inter-racial difference in this character was not significant, so far as shown by this experiment. Finally, it has been found that the *Hs* gene, which controls the development of hair on leaf sheath, is distributed in the northern races, and the *s* gene, which controls the appearance of short basal bristles, is distributed mainly in the northern races though in low frequency. Further work is in progress to elucidate the mechanism of differentiation of these races, as well as of some other varieties.

G. CYTOLOGY AND GENETICS OF *Nicotiana*

1. Cytogenetical Problems in Nicotiana

(Report by F. A. LILIENFELD)

a. A new amphidiploid.

F_1 hybrid between N. paniculata and N. plumbaginifolia exhibited at MI mostly $0_{II}+22_I$ or $(1\text{–}2)_{II}+(20\text{–}18)_I$. Sometimes single trivalents occurred. In early MI the univalents were concentrated at the poles. Spindle often curved or tripolar. All pollen empty, unreduced giant grains very rare. The amphidiploid—two plants—in general habitus very similar to F_1 hybrid, scarcely more vigorous. Flowers larger, also stomata, not much but significantly, larger. Meiosis was examined in one of the two plants. MI showed regular pairing, except for occasional 1–2 univalents. One

large bivalent was usually found off the equatorial plate. Its division was delayed. Furthermore, a small fragment could be detected in almost all PMC's.

The amphidiploid was on both sides fertile. Pollen fertility, evidently highly dependent upon the supply of nourishment, extremely variable, from 0 to 36% (of healthy looking pollen grains). Seed setting likewise variable, best results obtained with the plumbaginifolia parent as pollinator.

b. Continued investigation of synthesized N. tabacum.

The amphidiploid of the hybrid N. sylvestris × tomentosiformis was crossed with Dixie Bright (as mother), a commercial variety. As previously reported, F_1 showed pronounced hybrid vigor and was very healthy throughout the whole vegetation period. Pollen fertility on the average reaching up to that of Dixie Bright, but female fertility much lower. Most of the 29 plants taken at random from a larger F_1 had 15–19% of the seed fertility of Dixie Bright (grown side by side), the whole range extending from 7 to 29%.

c. Octoploids of synthesized N. tabacum.

In contrast to the normal appearance of octoploids of normal N. tabacum, this octoploid made a highly abnormal impression with its thick, deformed leaves. From 25 plans exhibiting such a habitus, 12 flowered very late in autumn. Five were more closely examined. Three of them were true octoploids, one had an octoploid subepidermal layer under a tetraploid epidermis, and in the remaining one the situation was just the other way, i.e. a tetraploid subepidermal layer and an octoploid epidermis. No seeds were obtained, probably as a result of unfavorable conditions (low temperatures) at the time of seed setting.

2. Cytogenetic Studies on the Genus Nicotiana. V.

(Report by Yô TAKENAKA)

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

The three species, *N. trigonophylla*, *N. undulata* and *N. rustica* were crossed reciprocally with *N. tabacum*. The crosses *N. tabacum* × *N. trigonophylla*, *N. undulata* × *N. tabacum* and *N. tabacum* (Odaruma) × *N. rustica* (Afghanistan) gave some seeds, while no seeds were obtained from the three remaining crosses. No report on the hybrids *N. tabacum* × *N. trigonophylla* and *N. undulata* × *N. tabacum* or their reciprocal crosses has been published, so far as I know.

In external characters the F_1 *tabacum-trigonophylla* is somewhat smaller than *N. tabacum*, the leaf form is intermediate between those in the

parents, and the flower colour is a pale red. F_1 *undulata-tabacum* is more similar to *N. tabacum* than to *N. undulata*, but its flowers are pale yellowish red, showing an intermediate color between the two parents. The morphology of F_1 *tabacum-rustica* agrees with that of KOSTOFF's description of the reciprocal hybrid (*N. rustica*×*N. tabacum*). These three hybrids were all vigorous.

All the hybrids mentioned above showed considerable irregularities in the meiotic behaviour of the PMC's. Polysporous PMC's were often observed, and the hybrids were completely sterile.

At first metaphase in F_1 *tabacum-trigonophylla*, 0–11 bivalents, mostly 5–6, were counted. In F_1 *trigonophylla-tomentosa* and F_1 *trigonophylla-tomentosiformis*, KOSTOFF (1941–43) observed 2–10 and 0–8 bivalents, respectively. Accordingly, the chromosome conjugation in F_1 *tabacum-trigonophylla* is assumed to be caused mostly by semihomologous chromosomes between the *trigonophylla* genome and the *tomentosa* subgenome, not the *sylvestris* subgenome, of *N. tabacum*. In F_1 *undulata-tabacum*, 0–8 bivalents, mostly 3–5, were observed. In F_1 *tabacum-rustica*, 1–10 bivalents (also multivalents) were formed at the first metaphase and many secondary associations were observed at first and second metaphases. CHRISTOFF (1928) and TRENOVSKY (1935) also observed a small number of bivalents in F_1 *rustica-tabacum*, while KOSTOFF (1941–43) found many bivalents, as many as 5–24, in the same hybrid. The cause of these different results was not determined.

b. Reduction divisions in the hybrids between the *N. tomentosa* group and three other species.

The three species, *N. glauca*, *N. sylvestris* and *N. paniculata*, were crossed each with *N. tomentosiformis* or *N. otophora* as follows; *N. glauca* × *N. otophora*, *N. sylvestris* × *N. otophora*, *N. sylvestris* × *N. tomentosiformis*, *N. paniculata*×*N. otophora* and *N. tomentosiformis*×*N. paniculata*. Among the above five crosses, *N. glauca*×*N. otophora*, *N. sylvestris*×*N. tomentosiformis* and *N. paniculata*×*N. otophora* produced some germinating seeds.

The hybrids *N. glauca*×*N. otophora* and *N. paniculata*×*N. otophora*, to my knowledge, have never been reported. The hybrid *N. sylvestris*× *N. tomentosiformis* was studied by KOSTOFF (1938), GREENLEAF (1938) and CLAUSEN (1941). Among the hybrids closely related to it, *N. sylvestris* ×*N. tomentosa* was studied by GOODSPEED and CLAUSEN (1928) and KOSTOFF (1930), and *N. sylvestris*×*N. Setchellii* by CLAUSEN (1941).

The leaf form of F_1 *glauca-otophora* resembles that of *N. glauca* but is not as shiny as that of *N. glauca*. The flower form is somewhat campanulate zygomorphic, showing a resemblance to *N. otophora*. The flower colour is yellowish green.

The external characters of F_1 *sylvestris-tomentosiformis* agree with the description of many previous investigators. The F_1 *paniculata-otophora* is intermediate between the two parents regarding size and shape. The flower shape is somewhat campanulate zygomorphic, resembling *N. otophora*, but the flower colour is of a greenish yellow similar to *N. paniculata*.

The reduction divisions in the PMC's of the above three hybrids are very irregular, and the PMC's are frequently polysporous. Accordingly, no seed was yielded.

At the first metaphase of F_1 *glauca-otophora*, 1–9 bivalents, mostly 4–5, were observed. Trivalents were rarely observed; still more rarely multivalents. At prophase, until diakinesis, remarkable heteropycnotic bodies were seen in the nucleus, usually one, and rarely two.

At meiosis of F_1 *sylvestris-tomentosiformis*, 0–7 bivalents occurred with the mode at 4. These chromosome configurations generally agree with the findings of KOSTOFF (1941–43) and GOODSPEED (1934).

At the first metaphase of F_1 *paniculata-otophora*, 3–10 bivalents were observed. GOODSPEED (1934) observed 0–4 bivalents in F_1 *paniculata-tabacum*, while KOSTOFF found 2–12 bivalents in the same hybrid. From the author's investigations of F_1 *paniculata-otophora* and KOSTOFF's studies on F_1 *paniculata-tabacum*, the *paniculala* genome is believed to conjugate mostly with the *tomentosa* genome rather than with the *sylvestris* genome.

c. Reduction divisions in hybrids between *N. Sanderae* and three other species.

N. longiflora ($n=10$), *N. plumbaginifolia* ($n=10$), *N. repanda* ($n=24$) and *N. suaveolens* ($n=16$) were crossed with *N. Sanderae* as the pollinator. The crosses *N. longiflora*×*N. Sanderae*, *N. plumbaginifolia*×*N. Sanderae* and *N. suaveolens*×*N. Sanderae* gave some seeds.

In external characters, the F_1 *longiflora-Sanderae* and F_1 *plumbaginifolia-Sanderae* are intermediate between the respective parents but the flower colors are red, resembling the fathers. F_1 *longiflora-Sanderae* was examined by CHRISTOFF (1928) and ISAKOVICH (unpublished, after KOSTOFF 1941–43), but F_1 *plumbaginifolia-Sanderae* has never been reported, so far as I know. The morphology of F_1 *suaveolens-Sanderae* agrees with that of KOSTOFF's description of the same hybrid.

At diakinesis in the PMC's of F_1 *longiflora-Sanderae*, the chromosome conjugations $9_{II}+1_I$, $1_{III}+8_{II}$ and $1_{III}+7_{II}+2_I$ were most frequent. At the first metaphase the chromosome conjugation $9_{II}+1_I$ was mostly observed, followed by the conjugations $1_{III}+7_{II}+1_I$, $1_{III}+8_{II}$ and $8_{II}+3_I$, in the cited order. The chromosome conjugations agree with ISAKOVICH's findings in the same hybrid (after KOSTOFF 1941–43). The meiotic chromosome behaviour of F_1 *plumbaginifolia-Sanderae* is the same as in F_1 *longiflora-Sanderae*.

According to the observations on the meiosis in the F_1 hybrids *longiflora-Sanderae* and *plumbaginifolia-Sanderae*, it is assumed that there are some homologous or semihomologous chromosomes between the *sanderae* genome on one hand and the *longiflora* or *plumbaginifolia* genome on the other.

At the first metaphase of F_1 *suaveolens-Sanderae*, 1–7 bivalents, mostly 3–4, were found. KOSTOFF (1941–43) observed 0–4 bivalents in the same hybrid. The cause of the difference between the author's and KOSTOFF's observations has not been studied.

3. *Genetical Studies on the Mid-rib Proportion and the Leaf-shape in Tobacco Plants.*

(Report by Kan-Ichi SAKAI and Shin-ya IYAMA)

In the Annual Report No. 3 for 1952, we have reported the result of our experiment dealing with the genetic analysis of the mid-rib proportion and the leaf-shape in tabacco hybrids in the F_2 and two backcross populations.

In order to pursue further studies on the same problem, 47 kinds of F_3 progeny together with their parental varieties were examined for these characters in a randomized block experiment with four replications.

One of the two parental varieties, White Stem Orinoco, is very high in the mid-rib proportion and has slender leaves, while the other, the Holmes variety, is the lowest in this proportion and has rather round leaves.

The mean values as well as the second degree statistics were computed in the plant populations for these two characters; the results obtained are presented in Table 1.

Table 1. Mean values and estimated variances of mid-rib proportion and leaf-shape in P_1 (White Stem Orinoco) and P_2 (Holmes), estimated variances of F_3 progeny means ($V_{\bar{F}_3}$), estimated covariances between F_2 individuals and their F_3 progeny means (W_{F_2/F_3}) and means of estimated variances within F_3 progenies ($\bar{V}_{F_3}$).

	Mid-rib proportion		Leaf-shape index	
	Mean (%)	Second degree statistics	Mean	Second degree statistics
White Stem Orinoco	35.46	4.1701	35.97	5.9423
Holmes	23.85	3.0607	57.61	12.7269
$V_{\bar{F}_3}$	—	6.8134	—	50.9189
W_{F_2/F_3}	—	5.9006	—	51.9089
$\bar{V}_{F_3}$	—	8.5042	—	38.5153

According to Mather's principles, the variance component due to additive effects of genes (D), that due to their non-additive effects (H), and that due to the non-genetic effects of the environment for individual plants (E_1) and for progeny means (E_2) were partitioned. The estimated values of such components of variance are presented in Table 2.

Table 2. Estimated values of D, H, E_1 and E_2 for the mid-rib proportion and leaf-shape in tobacco plants in the 1953 experiment.

	D	H	E_1	E_2
Mid-rib proportion	8.0355	18.4813	3.9003	1.3557
Leaf-shape	90.9457	51.4884	9.3428	2.2280

From these estimated values, the heritability of the character in the F_2 and F_3 populations as well as that of the progeny means in the F_3 generation and the number of effective factors for the two characters were computed (Table 3). The three heritability values were obtained from $\frac{\frac{1}{2}D}{V_{F_2}}$, $\frac{\frac{3}{4}D}{V_{F_3\text{ bulk}}}$ and $\frac{\frac{1}{2}D}{V_{\bar{F}_3}}$, respectively. The number of effective factors was computed as $K_1=\frac{(\bar{P}_1-\bar{P}_2)^2}{4D}$ and $K_2=\frac{(\bar{V}_{F_3})^2}{V_{V_{F_3}}}$.

Table 3. Heritability values and number of effective factors for the mid-rib proportion and the leaf-shape in tobacco plants.

	Heritability in			Number of effective factors	
	F_2 bulk	F_3 bulk	F_3 progeny	K_1	K_2
Mid-rib proportion	0.320	0.407	0.590	4.19	4.77
Leaf-shape	0.672	0.784	0.893	1.29	1.85

Correlation between mid-rib proportion and leaf-shape in the F_3 progenies has been found to be

$$r=-0.724$$

which is highly significant at the 1 per cent. level.

It is concluded from this experiment that the number of effective factors for the mid-rib proportion is four or five, and that for the leaf-shape is one or two. These results are in good agreement with those obtained in

1953.

For the purpose of breeding tobacco strains with low mid-rib proportion, direct selection of individuals among F_2 populations with respect to that character seems to bring about little success on account of the low heritability found. It is suggested that the breeder should make line selection among the F_3 progenies or plant selection within the F_2 or F_3 bulk, by taking leaf-shape into consideration with the aid of an appropriate selection index, because the leaf-shape shows a rather high negative correlation with the mid-rib proportion and has a high heritability.

The appropriate selection index, I, constructed from the present data is shown below:—

$$I = X_1 - 0.352X_2 ,$$

where X_1 and X_2 stand for the mid-rib proportion and the leaf-shape index, (width of the leaf/length of the mid-rib)×100, respectively.

4. *Mutations in Tobacco Induced by X-rays*

(Report by Seiji MATSUMURA and Taro FUJII)

Dormant seeds of *Nicotiana tabacum* (Dixie Bright 101) were exposed to hard X-rays at 180 KVP, 3 mA, without filter. The dosage was 15,000, 30,000 and 50,000 *r*. At the highest dosage, the germination rate of the seeds was reduced. Table 1 shows the relation between the frequency of chromosome aberrations in the PMC's and the X-ray dosage.

Table 1. Relation between X-ray dosage and frequency of chromosome aberrations in Dixie Bright 101

Dosage	No. of observed plants	Normal 24_{II}	No. of plants with aberrations					Frequency of translocation per PMC
			$1_{IV}+22_{II}$	$2_{IV}+20_{II}$	$3_{IV}+18_{II}$	$23_{II}+2I$	$24_{II}+fr.$	
—	11	11	—	—	—	—	—	0.00
15,000	66	52	12	1	—	1	—	0.21
30,000	52	38	18	5	—	1	—	0.54
50,000	25	10	8	2	2	1	2	0.72

The frequency of chromosome aberrations especially translocations per PMC increased in proportion to the dosage. Identical chromosome aberrations were observed in two or three inflorescences of the same plant.

Many X_2-plants of both varieties, Bright Yellow and Dixie Bright 101

were examined. Various kinds of morphological abnormalities were detected among them. Several X_3- and X_4-pedigrees were bred, and several kinds of mutants, such as early, small round, narrow, dwarf, shrivelled, variegated, mottled, yellowish green, yellow petiole, etc. appeared by segregation.

The "early" mutant flowered about 2 weeks earlier and was a little smaller than the normal plants. But its leaves seemed to be of good quality and its early flowering may be of great advantage in tobacco breeding.

H. GENETICS, CYTOLOGY AND BIOCHEMISTRY OF SOME PHANEROGAMS

1. *Sex Relations in Artificially Produced Tetraploids of Melandrium album*

(Report by Yô TAKENAKA)

Seeds and young plants of *Melandrium album* were treated with colchicine in the spring of 1950. The most favorable concentration and duration of treatment for inducing polyploidy were found to be 0.02% and 48 hours, respectively. Of the 56 treated plants which attained maturity, 27 were tetraploid, including 12 females and 15 males. These polyploids were used in crossing experiments carried out in 1951–1953. The results obtained are as follows:

Crossing experiment I.

Parents	Offspring
4x♀ × 4x♂	11♀ and 76♂ plants

Crossing experiment II.

Parents	Offspring
4x♀ × 2x♂	12♀ and 10♂ plants

The chromosome complement of *M. album* is 22a+2X in the female and 22a+X+Y in the male. Accordingly, the tetraploid males and females mentioned above must have 44a+2X+2Y and 44a+4X constitutions respectively. This chromosome constitution in the male was confirmed cytologically.

Under neither the wild nor the cultured condition has any noticeable numerical difference between male and female plants been observed. The author has found 56 females and 54 males among the 110 plants cultivated in 1951, and 41 females and 46 males among the 87 plants which matured in 1952.

These ratios are approximately to 1:1. However, Crossing Experiment

I yielded 11 females and 79 males among 90 plants, while Crossing Experiment II resulted in 12 females and 10 males, approximately a 1:1 ratio, among the 22 plants. There must be some special cause for the considerable discrepancy from the 1:1 ratio found in Experiment I.

WARMKE and BLAKESLEE (1939) suggested from the results of their observations in *M. dioicum* that XY pollen grains represent 90% or even more of all the pollen grains produced by the male tetraploid plants of the 44a+2X+2Y constitution. T. ONO (1939) reported that the proportion of XY pollen grains could reach as high as 91% in the colchicine-induced tetraploid males of *M. album*.

Of the 101 PMC's observed by the author, 86 had the chromosome conjugation type producing XY at both poles; 9 were of the XX- (or YY-) type, and 6 belonged to various other modification types. These figures are in very good agreement with those found by WARMKE and BLAKESLEE, and ONO.

According to these observations, the above Crossing Experiment I should give about 90% plants of 44a+3X+Y type, 5% of 44a+4X type and 5% of 44a+2X+2Y type, while Crossing Experiment II should produce females of 33a+3X type and males of 33a+2X+Y type in equal numbers.

The results of chromosome counts on the root tips of 32 male plants (including two male intersexes) among the 79 offspring of Crossing Experiment I were as follows:

Chromosome number	Chromosome complement	Number of plants
4x+1	45a+3X+Y	3
	45a+2X+2Y	1
4x	44a+3X+Y	21
	45a+2X+Y	1
	44a+2X+2Y	1
4x−1	43a+3X+Y	2
	44a+2X+Y	1
4x−2	42a+3X+Y	1
3x+4	35a+3X+2Y	1

The above chromosome survey reveals that 27 males arose from fertilization by XY pollen and 3 by YY pollen; and that in the remaining two males, pollen grains of other chromosome constitutions participated in fertilization. These results are in good agreement with the expectation, and sustain the findings by Westergaard in *M. album* and by WARMKE and BLAKESLEE in *M. dioicum*.

The 11 females obtained in this experiment had the chromosome complement 44a+4X. The number of female plants surpasses the expected figure, and the XX pollen may be assumed to have an advantage over the XY- and YY-pollen at fertilization.

In the third experiment, a female tetraploid with 44a+4X was crossed with a tetraploid male with 44a+3X+Y, and the following result was obtained:—

Crossing Experiment III.

Parents	Offspring
4x♀ × 4x♂	26♀ and 21♂ plants

The sex ratio in the offspring of this cross is nearly 1:1. The tetraploid male, 44a+3X+Y, should produce XX and XY pollen in equal numbers and a sexual of 1:1 ratio should be expected in the absence of certation.

The results obtained by these experiments may be summarized as follows:—

1) The Y chromosome has a strong male-determining potency and 2) stable and normal tetraploid dioecious plants can be produced.

2. *Ring Formation in the Meiosis of Allium Scorodoprasum var. viviparum*

(Report by Yô TAKENAKA)

The somatic chromosome number has been determined as sixteen by many investigators, e.g. KATAYAMA (1928), MORINAGA and FUKUSHIMA (1931), TAKENAKA (1931), Y. ONO (1935) and KURITA (1951). In a previous paper the present author hinted at the possible hybrid origin of this species from his observations of rings at meiosis and two chromosomes of strikingly different shape and size represented singly in the somatic chromosome complement. According to KURITA, the two somatic chromosome sets should be identical in both size and shape. The author tried to elucidate this discrepancy by a study of meiosis in this plant.

The 16 chromosomes may be classified into 7 types designated by the first 7 letters of alphabet. The somatic chromosome complement has been found to consist of 4a and 4b, 2c, 2d and 2e, plus one f and one g. The g-chromosome has a strikingly large satellite on its short arm.

In diakinesis of the PMC's, a large chromosome ring consisting of 6 chromosomes usually appears, besides 5 bivalents of which two frequently show a secondary association or formation of a quadrivalent. At MI, one large chromosome ring and 5 bivalents are usually found. The ring consists of 6 chromosomes, which do not show a zig-zag arrangement of the *Oenothera*-type. Therefore, disjunction at AI does not separate the participating members of the two sets as in *Oenothera*. All the division figures following AI show a somewhat irregular behaviour, but the young pollen grains are not abortive. Nevertheless, no seeds are obtained, since the plant is predominantly viviparous. Small bulbils are formed from

most of the young flower buds, and only exceptionally a few open flowers are to be seen.

From the above results the author assumes that the plant is a hybrid originated from a natural or artificial cross between two related species whose chromosome complements have become differentiated by translocations, inversions or deficiencies in the course of evolution.

3. *Inheritance of Flower Color of F_1 Hybrids in the Swiss Giant Pansy*

(Report by Tôru ENDO)

In a previous report, paper-chromatographic analyses of the pigment constituents in the petals of ten varieties of pansy, and the inter-relationships between them were briefly described. The present report deals with the analysis of the genetical behaviours of the pigment components found in nine F_1 hybrids, which were produced by the crossing of Pure White (as the pollen parent) with the remaining nine varieties.

The results show that the flower color of F_1 hybrids of the two maternal varieties, Coronation Gold and Giant Orange, was pale yellow, and this coloration was brought about by a smaller quantity of the same three kinds of xanthophylls as those occurring in the maternal plant, Coronation Gold. However, the two carotenes detectable in the maternal Giant Orange have vanished completely in the F_1 hybrids.

In the F_1 hybrids from the red-flowering pansies, Raspberry Rose, Fire Beacon and Alpenglow, segregation has been observed to a certain extent, probably owing to insufficient fixation of the parent strains. The flower colors of the hybrids were generally pale reddish-purple or purplish, and the pigments were localized in the posterior petals or in the marginal portion of all petals. The F_1 hybrids from Lake of Thun also underwent slight segregation, and the pigments were completely or incompletely eliminated from the area outside of the smaller blotches. The F_1 hybrids of Berna segregated into three types: Berna, Lake of Thun, and rarely the Mont Blanc type. The hybrid of the Berna type is different in pattern from the parent Berna in that the blotched area is smaller and the adjacent portion is purplish white.

The F_1 hybrids derived from Mont Blanc, which is orginally of the blotched type, have developed the so-called felix or cat's whisker, composed of many stripes in the blotched parts. In F_1 hybrids of Coronation Gold and Lake of Thun, all petals were pale yellow and the blotched parts were small. However, in some of them a bluish pigmentation appeared in the marginal portion of the petals.

4. *Analysis of Coronal Anthocyanins in Various Strains of the Japanese Morning Glory (Pharbitis Nil).*

(Report by Yukihide ABE)

Aiming at the elucidation of the genetical behavior of anthocyanins in the Japanese morning glory, in which the genes controlling the flower color variation have been analysed in detail by T. HAGIWARA and others, I have recently carried out some basic experiments concerning the analysis of anthocyanin-constituents in flower petals of thirty strains and of their F_1 hybrids, by the paper-chromatographic method. In these studies the original spots were marked on chromatographic paper with 1% methanolic hydrochloric acid extracts of fresh petals, and various solvent-mixtures were used for irrigation. The results obtained are briefly summarized in the following lines.

1) Throughout all the plant materials employed, some 16 different kinds of anthocyanin-spots were detected, which could be classified into three main groups with regard to the glycoside types:

(A) Free (or acylated) glycoside group, in which are included:

Pelargonidin-	glycoside (two kinds)	Pelargonin
Peonidin-		Peonin
		Cyanin

(B) Complex glycoside group, rather intimately combined with some unknown substances (X, X′, or X″):

Pelargonin	+X
Peonin	+X+X′
	+X″

(C) Co-pigmented glycoside group (co-pigments more or less loosely combined with the substances):

Pelargonin	
Peonin	+co-pigment
Cyanin	

2) The anthocyanins appearing in the corollas of different flowers, which were used for analysis at their full blooming stages, are shown in the following table.

In all the strains belonging to the broken-colored group, the total anthocyanin content was usually smaller than in the pure-colored group. The anthocyanins occurring in the parts of the petal that have undergone a striking change from purple to red in an otherwise purple corolla, and from blue to purple in a blue corolla, and also from red to purple in a red corolla, have been found to belong to the same pigment system as that found in the basal part of the ground color.

3) Analysis of anthocyanins occurring in F_1 hybrids have given the

Flower Colors	Anthocyanin-system			
	Type of Glycoside	Aglycones		
		Pelargonidin	Peonidin	Cyanidin
(a) Broken blue ($+^{mg}+^{pr}$)—broken purple ($+^{mg}$ *pr*) group	A,AB,BC,C	0 ~ 0	10 ~ 8	0 ~ 2
(b) Pure blue ($+^{mg}+^{pr}$)—pure purple ($+^{mg}pr$) group	AB,ABC,AC,BC	0 ~ 0	10 ~ 6	± ~ 4
(c) Broken magenta (*mg*$+^{pr}$)—broken red (*mgpr*) group	A,AB,B,BC	9 ~ 7	1 ~ 3	0 ~ 0
(d) Pure magenta (*mg* $+^{pr}$)—pure red (*mg pr*) group	AB,ABC,BC	10 ~ 7	± ~ 3	0 ~ ±

1) Genotype proposed by T. HAGIWARA; 2) AB represents the anthocyanin of both (A)-type and (B)-type as major pigment constituents, etc. 3) Relative amount (in 10 parts) of anthocyanins in the petals; 4) Wild type is pure blue (P8Cy2BC).

following results:

Blue ($+^{mg}+^{pr}$, P[2])8Cy2 BC[1]) × Purple ($+^{mg}pr$, P5Cy5 ABC) F_1 Blue (P9Cy1 BC)
Blue ($+^{mg}+^{pr}$, P8Cy2 BC) × Red (*mgpr*, P18p 2 AB) Blue (P8Cy2 BC)
Red (*mgpr*, P18P 2 BC) × Purple ($+^{mg}$, P5Cy5 ABC) Purple (P6Cy4 ABC)
Blue ($+^{mg}+^{pr}$, P8Cy2 BC) × White ($+^{mg}pr+^{ca}c+^{r}$) Blue (P7Cy3 BC)
Red (*mgpr*, P18P 2 AB) × White ($mgpr+^{ca}+^{c}r$) Red (P19P1 BC)
White ($+^{mg}m+^{ca}c+^{r}$) × Red (*mgpr*, P18P 2 AB) Purple (P7Cy3 ABC)
White ($mgpr+^{ca}+^{c}r$) × White ($mgpr+^{aa}c+^{r}$) Light red (P19P1 AB)

1) Glycoside type. 2) Pl-pelargonidin, P-peonidin, Cy-cyanidin.

4) From these paper-chromatographic analysis, it may be surmised that throughout the whole pigmentation stage of petals, the relative amount of peonidin to pelargonidin remains unchanged in every strain of the broken and pure magenta-red group, but that the proportion between the cyanidin- and peonidin-content varies to a remarkable extent, as has been observed in some strains of the broken and pure blue-purple group, where pelargonidin was never found. With regard to the glycoside type, the AB-type appeared in place of the original A-type, and the total anthocyanin content (about 2 times in maximal amount) showed a rapid increase during the 24 hrs. up to full blooming, accompanied also by the production of (C)-type anthocyanins. A decrement of the (A)-type anthocyanins takes place during the same period. In the strains in which such pigment increment could not be observed, the total anthocyanin content was usually small,

and the glycoside type in the corolla was either A or AB.

Moreover, it may be pointed out that a bluish tinge caused by $+^{pr}$ is by no means due to the combination of different anthocyanins, and that $+^{mg}$ or *mg* controls the number of OH-groups in the side benzene nucleus. Methylation or demethylation seems to be effected by other factors.

5. *Inheritance of Doubleness in Zinnia elegans L.*

(Roport by Kanji GOTOH)

It is well known that in *Zinnia elegans* the frequency of flower heads exhibiting doubleness in a population rapidly decreases in a few years, when seeds are taken continuously under an open-pollinated condition. The object of the present study was to investigate this degenerating process from the genetic standpoint. In 1950, a reciprocal cross was carried out in the variety Pumila scarlet between the completely double, No. 4–5, and the completely single, No. 4–1. The F_1 hybrids between the completely double (B type) and the completely single (A type) showed the so-called E type described previously (Annual Report of this series, No. 2). No difference was observed between the reciprocal F_1 hybrids. The 208 F_2 plants were grown in 1951 and were classified according to their flower types into ten classes. It has been confirmed by observation of these plants that the difference between the completely single (A) and completely double (B) types was governed by three Mendelian genes. Thus, the B type might be recovered in the F_2 population in the ratio, 1 : 64. This type yields only a few seeds. The A type, as well as the subtypes similar to it, shows a much higher fecundity than the B type or its subtypes, because they have many tubular florets. Thus, if the pollination by insects is carried out at random, a general degeneration of an open-pollinated *Zinnia* population will inevitably ensue.

6. *Karyotaxonomic Studies in Poaceae I.*

(Report by Tuguo TATEOKA)

Various investigators have made cytological studies concerning both the morphology and behavior of the chromosomes in various grass species in Europe and the United States, while only a few works along such lines have been done on Japanese materials. The prsent author is studying the somatic chromosomes of Japanese grasses and endeavoring to elucidate their taxonomic relationships from the karyological standpoint. In 1953,

the somatic chromosomes of the following species of Poaceae were observed:

Species	Somatic chromosome number
Pleioblastus chino	48
Sasa purpurascens	48
Sasa sp.	48
Hordeum murinum	28
Brachypodium sylvaticum var. *luzoniense*	18
Elymus sibiricus	28
Bromus japonicus	14
Calamagrostis Langsdorffii	28
C. hakonensis	28,56
C. arundinacea var. *brachytricha*	42,56
Agrostis flaccida	56
Phleum pratense	42
Alopecurus japonicus	28
Beckmannia syzigachne	14
Helictotrichon Hideoi	14
Trisetum spicatum	28
Koeleria cristata	14+1 supernumerary chromosome
	14+2 supernumerary chromosomes
Deschampsia flexuosa	28
Milium effusum	28
Anthoxanthum odoratum	20
A. japonicum	70
Hierochloe alpina	56
H. odorata	42
Phalaris arundinacea	28
Leersia oryzoides	48
Dactylis glomerata	28
Festuca rubra var. *pacifica*	42
F. parvigluma	28
F. japonica	28
Poa Komarovii var. *shinanoana*	ca. 77
P. annua	28
P. acroleuca	28
P. nipponica	28
P. Matsumurae	70
P. pratensis	56
Briza minor	10

B. maxima	14
Melica nutans	18
M. Onoei	18
Glyceria acutiflora	20
G. ischyroneura	40
G. lithuanica	20
G. alnasteretum	20
Torreyochloa viridis	21
Moliniopsis japonica	50
Hakonechloa macra	50
Phragmites japonica	48
Eragrostis ferruginea	80
Cynodon Dactylon	40
Arundinella hirta	56
Echinochloa Crus-galli	54
Panicum bisulcatum	54
Setaria viridis	18
S. autumnalis	36
Isachne globosa	60
Imperata cylindrica var. *Koenigii*	20
Miscanthus sinensis	40
Microstegium japonicum	20
Arthraxon hispidus	36
Bothriochloa parviflora	40
Ischaemum anthephoroides	72
Coix Lacryma-Jobi	20

From observations of the somatic chromosomes of the species listed above, some taxonomic information was obtained for the following groups: the tribe *Chlorideae*; the genera *Torreyochloa, Poa, Milium, Calamagrostis, Beckmannia. Bromus* and *Briza.*

I. GENETICS AND CYTOLOGY OF SOME LOWER ORGANISMS

1. Studies on the Methionine-requiring Strain of Ustilago maydis

(Report by Tetsuo IINO)

a. Reversion of methionine-requirement by two-step mutations.

The inferior reversion type (IR), originating from the methionine-requiring strain (me_1) 4-24 of *U. maydis*, is characterized by its restricted growth in media lacking in methionine, but it sometimes happens that

successive subcultures recover a normal growth rate and change into the stable prototrophic type (IR′) (Annual Report No. 3, 1952). In order to throw some light upon the genetic background of this phenomenon, the change of the growth type was thoroughly investigated, and the genetic segregation carefully analyzed.

Each stock of fourteen IR-clones, which had been screened out from a minimal plate culture of strain 4–24, was subcultured (at 30°C) in several series of test tubes containing 1 ml of liquid media which was renewed every 72 hours. For all the series and generations, a part of each subculture was sampled and the change in growth type of the cells was observed on plate cultures.

In 93% of the series examined, IR′-cells appeared in one to six subcultures, and being at a selective advantage, they increased rapidly in proportion to the IR-cells, and eventually the whole culture changed into the IR′-type. During this process, the mixed culture of IR- and IR′-cells did not contain any cells of the intermediate type between IR and IR′, and the single-cell cultures isolated from the mixed culture produced only pure IR- or IR′-clones.

The analysis of genetic segregation was carried out with chlamydospores which had been produced by the conjugation of IR or IR′ with the wild strain (+)5 or isoleucine-requiring strain (il_1)5–91. The chlamydospores were obtained by injecting the desired combination of cultures into a young pop-corn plant in a greenhouse. They were sown on complete agar media and each segregant was isolated by micromanipulation. The nutritional requirement was examined by means of auxanography.

Eleven out of the fifteen chlamydospores derived from (IR × +) segregated IR and + in a 2:2 ratio, and the remaining four produced + alone. All fourteen chlamydospores derived from (IR′ × +) produced + alone, since IR′ is phenotypically indistinguishable from +. The chlamydospores derived from (IR × il_1) segregated IR and il_1 in one case, and IR·il_1 and + in six cases; in every case two IR or il_1 segregants were found among the four. Finally, four out of the five spores derived from (IR′ × il_1) segregated + and il_1 in a 2:2 ratio, while one spore produced IR·il_1 besides + and il_1.

These data of the segregations lead us to assume that me_1 and IR, as well as IR and IR′, differ from each other in one gene, and that the genes controlling the me_1 and IR characters are closely linked, or else are allelic together. The production of + alone from all the four chlamydospores derived from (IR × +) may be explained as due to mutation of IR to IR′ during the course of the crossing experiment. Further investigation is needed to decide whether the segregation of IR·il_1 from IR′ × il_1 is due to a suppressor mutation of IR to IR′ or it is a back mutation of IR′ to IR.

From the observations of the successive clone cultures and from the

segregation analysis, it is assumed that the methionine-requiring strain 4-24 changes to the IR-type by a mutation of the me_1—locus or of another locus closely linked to it, with the IR-type mutates in turn to the IR′-type: thus the dispensability for supplemented methionine has been recovered by a two-step mutation.

b. Methionine-requiring and sulfonamide-resistant characters.

The concurrence of sulfonamide-resistance with methionine-requirement has been observed on the m-1 step mutants in some bacteria such as *Escherichia coli* (KOHN and HARRIS, 1942) and *Salmonella enteritides* (FUJINO and others, 1950). This phenomenon has been regarded as an evidence of the fact that para-aminobenzoic acid (PABA), an antagonist of sulfonamide, takes part in the m-1 reaction.

From the data examining the response to nutrients of the methionine-requiring strain 4-24 of *U. maydis*, it is assumed that the m-1 step is blocked. This strain shows a distinct response to methionine, but responds neither to cysteine, homocysteine, choline, PABA nor to any combination of these (IINO, 1952). This strain and the wild strain 5 were cultured in several series of synthetic methionine-glucose media (MG-media) and minimal media, both containing 0 to 2×10^{-2} mol of sulfanilamide (SA). The growth rates of these strains were compared by counting the number of viable sporidia in the cultures. No significant difference such as that found in bacteria was observed between these strains in either set of cultures. Inhibition of the growth appeared at the conc. of 10^{-4} mol, and came to the maximum at 2×10^{-3} mol. The inhibition was not of a fungicidal but of a fungistatic nature in the range under 2×10^{-2} mol and 120 hours.

A SA-resistant mutant M5008 has been screened out from the wild strain 5 which had been grown on MG-media containing 2×10^{-3} mol of SA. The strain M5008 showed resistance against SA which was twenty times greater than that of the original strain. Although the requirement of this strain for methionine was tested, no experiment gave a positive result, showing that this strain has no methionine-requirement.

The most effective antagonist against SA-inhibition is PABA in all the strains mentioned above. Adenine and hypoxanthine show less effective antagonisms against SA. Methionine has, however, no antagonistic effect when supplemented alone.

2. *Electron-microscopical Studies on the Structure of Paramecium caudatum by Means of Ultra-thin Sections*

(Report by Mitsuo TSUJITA, Kyozo WATANABE and Seizo TSUDA)

Cytological and genetical studies of *Paramecium aurelia* have been carried out by many authors, notably by CHEN ('40), PREER ('50) and

SONNEBORN ('49–'50). Few studies, however, have been made on *Paramecium caudatum.*

The present report deals with the results of an electron-microscopical study of the fine inner structures of *Paramecium caudatum* carried out by us by means of ultra-thin sections.

A strain of *Paramecium caudatum* was used as material. The protozoa were kept in the nutritional liquid in test tubes in a constant-temperature-room regulated at 25°C.

The animals to be fixed were gathered by utilizing their galvanotaxis. Several fixatives were used: neutral osmic acid, Champy's fluid, Flemming's fluid without acetic acid, Carnoy's solution and 95% alcohol. Of these neutral osmic acid gave the most favorable results for ultra-thin sectioning. The fixed materials were dehydrated by passing them through the alcohol series (50%, 70%, 80%, 90% and absolute), and then imbedded in n-buthyl methacrylate catalyzed by 2% benzoyl peroxide. The materials in this plastic substance were cut into thin sections of about 0.1 μ thickness with a Spencer's ultra-microtome. The sections were examined by means of a J. E. M. III type electron microscope at 50 K.V.

The results of observations of these ultra-thin sections may be summarized as follows:—

The cilia cover all the pellicle as well as a part of the inner surface of the gullet. A kinetosome is present at the base of each cilium. Some of the kinetosomes of the pellicle are attached to two cilia (fig. 1). Each cilium shows a fibrillar structure which is apparently similar to that of a myofibril.

The macronucleus (vegetative nucleus) is filled with granular or filamentous bodies, mixed with granules of smaller sizes. These granules are often found in abundance in the peripheral region of the nucleus, and they seem to migrate into the cytoplasm through the nuclear membrane.

The micronucleus (sexual nucleus) is filled with small granular or filamentous bodies, but large granules like those observed in the macronucleus are not found.

A great number of mitochondria-like granules, spherical, ellipsoidal or filamentous in shape, are scattered in the cytoplasm. The ectoplasm immediately beneath the pellicle has a fibrous structure, in which filamentous granules run almost parallel.

The examinations of the longitudinal and horizontal sections of the granules, reveal that the periphery of the granule appears to be of dense structure, into which the electron rays do not penetrate. The inner structure of the granule seems to consist of fine particles. But this inner structure of the granule requires further study.

In the living material, the endoplasm granules stain with Janus green B, but not with neutral red. The majority of them show a positive NADI's

reaction, turning bluish, which indicates that they belong to the cytochrome oxidase and cytochrome complex. Furthermore, the granules isolated by the ultracentrifuge show their similarity to the respiratory enzymes.

In the sections of the material fixed with Champy's fluid or Flemming's fluid without acetic acid, the granules turn reddish purple or reddish by Altman's or Altman-Kull's test, and they stain bluish black or black with iron haematoxylin. The granules are dissolved in Carnoy's solution or 95% alcohol.

From the observations described above, it may be safely said that these granules represent a kind of cell organ which is comparable to the mitochondria in the cells of higher organisms.

Minute particles scattered in the cytoplasm between the mitochondrial granules seem to represent the so-called microsomes.

Two types of food vacuoles can be discriminated under the electron microscope. One has vesicles with food substance surrounded by a protoplasmic layer which contains short thread-like bodies. These seem to be disintegrated mitochondria which participate in the secretion of digetive enzymes. The other type of food vacuole contains many granules, spherical or ellipsoidal. These granules do not dissolve in Carnoy's solution, and they can easily be distinguished from the mitochondrial granules in the cytoplasm.

3. *Formation of Tobacco Mosaic Virus in Plastids*

(Report by Mitsuo TSUJITA and Seizo TSUDA)

This work was carried out in collaboration with J. HIDAKA and H. MURANO in Hatano Tobacco Experiment Station, Japan Monopoly Corporation.

Since KAUSCHE and RUSKA (1940) assumed the formation of tobacco mosaic virus in green plastids, and published an electron micrograph showing filamentous particles protruding from a chloroplast fragment, the idea that chloroplasts are the site of virus formation has been supported by some investigators. Recently LEYON (1953) examined leaves infected with beet yellows virus with an electron microscope, and he observed the characteristic filamentous particles. As these filamentous particles were found in association with chloroplasts, LEYON thought that the former had been extruded from the latter. He also carried out investigations of the leaves infected with the virus and came to the view that at least some viruses are formed within the chloroplasts.

We have examined the relation between the virus development and chloroplasts with the help of ultra-thin sections, and the following results were obtained.

Material and method: Normal young healthy tobacco leaves, and leaves

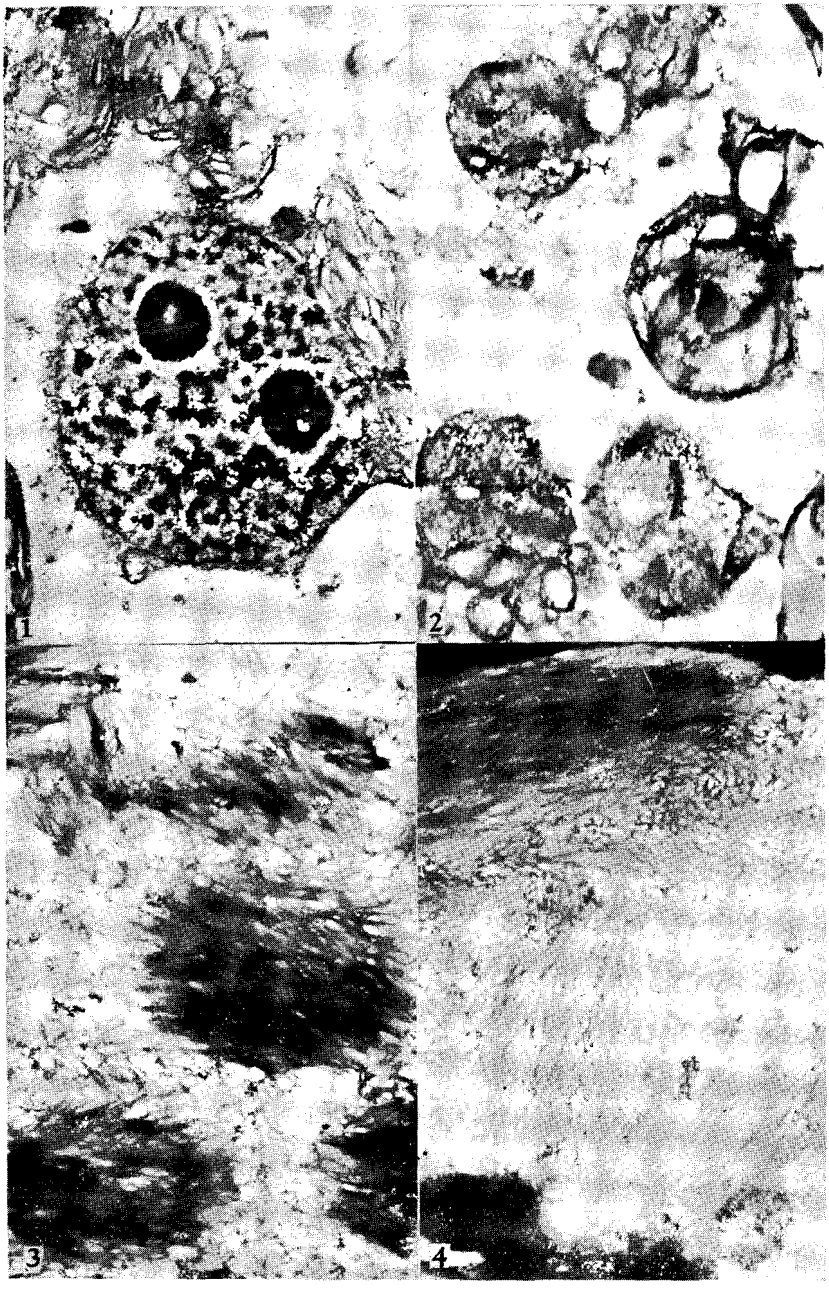


Fig. 1. Chloroplasts in normal condition.
Fig. 2. Chloroplasts in leaves 5 days after infection. Leaves 10 days after infection.
Fig. 3. Masses of filamentous bodies can be seen here and there in the cytoplasm. The masses containing filamentous substances show hexagonal shape.
Fig. 4. Leaves 10 days after infection. A great amount of filamentous substances can be seen in the cytoplasm.

5 and 10 days after infection were used as materials. As fixing fluid, neutral osmic acid of various concentrations was tried. This fixative, however, preserved the cells very poorly, especially the cytoplasm. Next LEYON's fixation method was followed:—The material was fixed in 0.5% osmium tetraoxide at pH 7.0 for 1 hr., washed in running water for 2 hrs, treated with 0.5% formaldehyde for 1/2 hr, washed in running water for 1 hr, then treated with 0.3% chromic acid for 1 hr and washed.

This fixation procedure gave fairly good results. Therefore, healthy and diseased leaves were fixed according to this procedure, and the changes in the chloroplasts were examined.

The fixation procedure in the healthy and diseased leaves was followed by dehydrating and embedding. The specimens were embedded in n-butyl methacrylate. The material in methacrylic resin was cut with a knife made by the Japan Microtome Institute. The sections were treated with amyl acetate to dissolve the methacrylate.

Results: Chloroplasts in normal conditions are shown in Fig. 1. They have no membrane, and contain grana and stroma. Chloroplasts in leaves 5 days after infection are shown in Fig. 2. The chloroplasts have undergone some changes, with a small quantity of filamentous material present within them. However, we have not ascertained the relation which these filamentous bodies might have similar bodies, shown in Fig. 3.

In leaves 10 days after infection a great amount of the filamentous material is found. Some of these bodies are scattered in the cytoplasm, and they look as if they were protruding from the chloroplasts. Masses of filamentous bodies are also seen here and there (Fig. 3). The masses, which are bundles of filamentous bodies, show a hexagonal shape in cross section. These masses seem to be derived from the infected chloroplasts.

In another section a great number of filamentous bodies were present in the cytoplasm (Fig. 4). Whether these represented artificially disentangled bundles of filamentous bodies or natural disintegration remains obscure.

As already reported by several authors, the production of filamentous substance in infected cells was recognized also in our preparations. However, it was rather interesting that conspicuous masses of filamentous substance were found in the infected cells.

Since IWANOWSKI (1903) first described intracellular amorphous and crystalline inclusions particularly in the epidermal cells of tobacco plants infected with mosaic virus, they have been made the object of investigations by many authors. Recently, STEERE and WILLIAMS (1953) studied the crystalline inclusions occurring in hair cells of Turkish tobacco and found these crystals to consist apparently of nothing else than particles of tobacco mosaic virus and volatile solvent. It is interesting that the shape of the dissected masses of filamentous substance is almost hexagonal, suggesting

that they are crystalline bodies.

4. *Electron-microscopic studies of a bacterial virus attacking Pseudomonas solanacearum E. F. Smith*

(Report by Mitsuo TSUJITA and Chiaki MATSUI)

A strain of bacterial virus infecting *Pseudomonas solanacearum* E. F. SMITH B19 was purified by differential centrifugation at 26,000 g for 60 min, 59,000 g for 60 min. and 20,000 g for 120 min. The electron-microscope observation of this virus showed that it is a spherical particle approximately 70~80 μm in diameter. The virus particle shows a rather rough surface, and apparently has some internal structure.

The host bacterial cell infected with the virus is filled with very small protoplasmic particles, as well as some new virus particles. It was further observed that the membrane of the infected bacterial cells had ruptured after a latent period, and liberated the proliferating new virus particles.

J. IMPROVEMENT OF SOME USEFUL PLANTS

1. *Improvement of Sugar Beets by means of Induced Triploidy*

(Report by Seiji MATSUMURA)

The results of the experimental work carried out during 1940–'52 by several collaborators were published in a book, written in Japanese with an English summary. The contents are as follows:

I. History of our studies on triploid sugar beets (1940–'52); by S. MATSUMURA.
II. Induction of triploid sugar beets and their characteristics; by S. MATSUMURA and A. MOCHIZUKI.
III. Genetic and cytological studies on the genus *Beta*. V. Production of triploid seeds; by A. MOCHIZUKI.
IV. Studies in polyploid varieties of sugar beets; by S. NAGAO and M. TAKAHASHI.
V. Physiological characteristics of early developmental stages of diploand tetraploid sugar beets in water culture; by S. HOSOKAWA, I. SAWAI and M. SHICHIJI.
VI. Results of experiments with polyploid sugar beets; by S. HOSOKAWA, J. NAKAJIMA, K. KATO and T. TAKEDA.
VII. Results of various experiments with triploid sugar beets; by Agricultural Department, Nippon Beet-Sugar Manufacturing Company.

VIII. Improvement work with sugar beets by means of triploidy. A concluding review; by S. MATSUMURA.

Since the weather was unusually unfavorable, especially in the summer of this year, the results of experiments performed were somewhat divergent. Some of the American resistant varieties showed the best results and next to them our triploid variety, 3n-1, a combination of No. 4398 (398-4x)×162 (2x). This combination was compared with the original diploid hybrid No. 398×162 in only one experimental field. There was no significant difference between the two. Further investigation must be awaited. By comparison of several other triploid varieties, 2 new triploid combinations seem promising.

2. *Further Investigations Regarding the Degree of Heritability and the Number of Effective Factors in the Eggplant*

(Report by Kanji GOTOH)

Adequate estimation of heritability of quantitative and aggregate characters is important for effective selection. In the present study the heritability of three characters in the eggplant was estimated by means of the parent-offspring regressions in the F_2 and F_3 generations. It was also attempted under the same design to resolve the variation into its components, and to estimate the number of effective factors governing the given traits by the procedure proposed by MATHER (1949).

Two crosses out of five previously examined, namely, Florida high bush × Sendai-naga No. 1 and Turuboso-sen-nari × Taiwan-naga were chosen as materials. The degrees of heritability for the period from seeding to flowering, fruit shape index, and fruit weight were 65~78, 60~75, and 40~60% respectively. The heritability for the fruit shape index and fruit weight estimated by parent-offspring regression was relatively low, as compared with the heritability in the broad sense, where the variances of F_1 were used for the estimation of the environmental variances. From this finding it has been concluded that the F_1 variances used as environmental variances of the segregating generations give too large or too small values, according to the traits.

The marked differences between the F_2 variances of both crosses may be interpreted as due to the effects of individual genes governing such traits. This assumption has been verified by the comparison of frequency distribution in the F_2, and its appropriateness has been shown by examination of the relationship between the effects of genes and the degree of heritability.

The estimated number of effective factors is generally low, but it has been found that the factors having plus or minus effects are probably

concentrated in one of the parent varieties.

Various segregants or recombinants were observed in the F_3 of the cross, Florida high bush × Sendai-naga No. 1, and the range of the segregation reached almost the whole range of variation occurring in the cultivated varieties of this plant found in Japan. It may be expected in practical breeding to obtain many new variants from such cross combinations.

3. *Studies on polyembryony in Citrus*

(Report by Kazuo FURUSATO)

Observations on the number of nucellar embryos per seed were carried out. After fertilization, formation of embryos from the nucellar cells begins in the neighborhood of the embryosac. It seems that fertilization is necessary for nucellar embryogenesis, since in fruits developed by hormone treatment no seeds are formed. Fertilization in *Citrus* requires a longer time than in many other phanerogams, taking 2–8 days to accomplish. The zygote remains dormant for about one month, and then begins to develop. Nucellar embryogenesis starts either shortly before or after the beginning of development of the fertilized egg. Gradually more and more nucellar embryos are formed. They do not develop simultaneously, and embryos in various growth stages may be found.

The number of embryos per seed varies according to species. In *C. Unshu* × *C. Natsudaidai* the average number of embryos was found to be 12.3, with a wide variation ranging from 1 to 29. In *C. Unshu* × *C. leiocarpa* var. *tumida* the number averaged 14.3, and in *C. Unshu* × *Poncirus trifoliata* 16.8. It remains to be investigated whether different pollinators are responsible for these differences.

Sometimes monoembryonic seeds are found in usually polyembryonic seed species. It is difficult to decide whether these embryos are derived from fertilized eggs, or are of nucellar origin. For this one must observe the morphology of the seedlings.

The possibility of artificial control of the number of embryos in polyembryonic species is being examined. It has been found that water and growth hormones have a reducing effect on the number of embryos. In the fruits of *C. Unshu* and *C. Natsudaidai* injected with water or MH–30 at an early stage, seeds containing decreased numbers of embryos, even to a single embryo, were found. The cause of this change is unknown. It remains to be investigated whether the reduction in number of embryos goes hand in hand with that of the nucellar embryos when the fruits are treated with water or hormones.

K. STUDIES ON COMPETITION

1. *Competitive Ability of F_1 Hybrids in Barley*

(Report by Kan-Ichi SAKAI and Kanji GOTOH)

Five inbred barley varieties and ten F_1 hybrids were tested for their competitive ability by using two tester varieties. The competitive ability was measured by the increments or decrements in dry plant weight, number of culms and weight of heads in mixed plantings in relation to the two testers, and by comparing them statistically with the results obtained in pure stands concerning the same characters. Planting was made individually in rows 50 cm apart with 12 cm inter-hill spacing. Experiments were conducted by the split-plot design with four replications.

F_1 plants in pure-stand plots generally showed a marked heterosis for various characters in comparison with the parental varieties in pure-stand plots. Their heading was generally one to six days earlier than the corresponding average of their parents. The competitive ability of the F_1 hybrids, however, was on the average inferior to that of their parents, and only in relatively few cases did the F_1 hybrids surpass the parental varieties in that ability. It was found also in this experiment that in general the most vigorous hybrids had the lowest competitive ability, and those with rather moderate heterotic vigor were best in competition.

It is therefore concluded from this experiment that the competitive ability of plants of this species should be considered as a character quite independent from vigor.

2. *Further Studies on Competition between Diploid and Autotetraploid Plants of Barley*

(Report by Kan-Ichi SAKAI and Yasuo SUZUKI)

In 1953, diploid and autotetraploid races of seven varieties of barley were examined for their competitive ability. The seeds of the races to be examined and of the plants with which they had to compete were seeded in alternate hills in rows. The inter-hill spacing was 12 cm and the distance between adjacent rows was 40 cm. The experimental plots were arranged according to the split-plot design with three replications. The experiment involved, besides single and mixed plantings of diploid and autotetraploid races of each variety, mixed plantings of both races with the diploid race of a standard tester, for the sake of comparison of the competitive ability between fourteen chromosome races. Data on an individual plant basis were taken for dry plant weight, number of culms, and number and weight of heads.

Analysis of variance of the data suggested that the effect of competition, as well as the interaction between competition and chromosome race, was highly significant for all the four characters. It was found throughout the experiment that the autotetraploid races were almost always inferior in their competitive ability to the diploid prototypes, and that the autotetraploids derived from diploid races of high competitive ability had higher competitive ability, and those derived from diploid races of low competitive ability had lower competitive ability.

It is interesting to find whether doubling of the chromosome number in homozygous races of plants tends in general to decrease the competitive ability of the plants, since this ability would surely play a certain role in the evolution of plant species.

3. *Competition Studies on Diploid and Autotetraploid Plants of Rice*

(Report by Kan-Ichi SAKAI and Yasuo SUZUKI)

The diploid and autotetraploid races of four varieties of rice plant, *Oryza sativa L.*, were examined for their competitive ability. Seedlings of the races to be examined and of the races with which they had to compete were planted in alternate hills in the row. The inter-hill spacing was 12 cm and the distance between adjacent rows was 30 cm. The experimental plots were arranged according to the split-plot design with four replications. Data on an individual plant basis were taken for dry plant weight, number of culms, weight and number of panicles, and weight of grains.

Analysis of variance of the data showed that the effect of competition was highly significant for all the five characters. It was found that the autotetraploid races in this species tended to be more or less inferior in their competitive ability to the diploid prototypes.

It is found that the conclusion drawn from another experiment that the autotetraploid barley races were bad competitors against the diploid prototypes also holds true for the present species.

4. *Competitive Ability of Thirty Varieties of Upland Rice Against the So-called "Red Rice"*

(Report by Kan-Ichi SAKAI, Shinya IYAMA and Takeo MEGURO*)

As was reported in the Annual Report No. 3 for 1952, the upland-rice

* Head agronomist of the Laboratory of Upland Rice Breeding of the Ibaraki Prefectural Agricultural Experiment Station.

cultivation in Japan is often contaminated by the so-called "red rice". This variety belongs to the subspecies *Indica* and is inferior to the ordinary rice both in quality of grain and in yield. It often gets into the field and grows as a weed among the upland rice. It is likely that some upland rice varieties would suffer relatively less from this contamination than others.

In 1953, we undertook an experiment to examine intervarietal differences in competitive ability of varieties of upland-rice at the Experimental Farm of the Agricultural Experiment Station in Isioka, Ibaraki Prefecture. This report deals with the results of the experiment.

Seedlings of thirty varieties of upland rice were transplanted to the experimental farm plots both in pure-stand and in mixture with the seedlings of the "red rice". Plants were grown in a split-plot experiment with three replications. Data were taken on plant height, plant weight, number and weight of panicles as well as weight and number of grains, each on an individual plant basis.

Analysis of variance of the data obtained showed, without exception, that the effect of competition was highly significant. It was found that most of the uplant rice varieties were bad competitors against the red rice, while a few, not more than five, were found to have higher than, or at least equal competitive ability to, the latter.

5. *Competition and Spacing in One Dimension in Plants*

(Report by Kan-Ichi SAKAI and Yasuo SUZUKI)

Competition between two varieties of barley differing in competitive ability was examined under various interplant spacings. Seedlings of the stronger variety (*SZ*) were individually space-planted in rows, either alone in pure stand plots or alternating with plants of the weaker variety (*SS*) in mixed plots. The distance between the adjacent rows was fixed at 70 cm in order to avoid any influence from this factor. Spacing between the adjacent plants in a row was made at 2, 4, 8, 16, 32 or 64 cm, according to the experimental plan. Plants were grown in a split-plot experiment with three replications. Measurements were made on dry plant weight, number of culms, and number and weight of heads on an individual plant basis.

Data obtained from this experiment were analysed statistically. It has been found that the effect of distance between plants within rows upon the competition pressure operating between them is very marked: the increments due to competition in plant weight, number of culms, and in number and weight of heads have been shown to be adversely proportionate to the logarithm of the logarithm of the distance between plants.

The relation has been found to fit well to the equations of a straight line as follows:

$Y_1=113.4007-136.0274\ X_1$ for number of culms,
$Y_2=109.3955-130.3571\ X_2$ for number of heads,
$Y_3=171.6113-215.2887\ X_3$ for weight of heads, and
$Y_4=248.2951-189.0116\ X_4$ for plant weight.

The X_i in the above formulas stands for $\log_{10}(\log_2 x_i)$, where x_i represents the distance between adjacent plants in the row.

It is conceivable from the result of the present experiment that as long as individuals of a wild species are few in number and scattered in a spacious area, they will not suffer from any intraspecific competition. However, when the population becomes fairly dense, the individuals will inevitably have to compete with one another. Thus it is likely that when a plant species continues to propagate within a small range of habitats, and increase in density within a limited area, natural selection due to intraspecific competition will become quite effective.

L. THEORETICAL STUDIES AND TECHNICAL NOTE

1. Theoretical Studies in the Breeding Technique of Autogamous Crop Plants

a. Change in the Value of Heritability of Quantitative Characters in Hybrid Bulks and Plant Progenies in Autogamous Plants

(Report by Kan-Ichi SAKAI)

In the breeding practice of agricultural crop plants, how to make successful selection with regard to any economic character is often one of the most important problems. This is because most economic characters show extremely low heritability. This report deals with theoretical investigations on the possible change in the value of the heritability of a given character in hybrid bulks and in plant progenies of autogamous plants.

(A) Heritability in hybrid bulks.

If the phenotypes corresponding to the three genotypes, *aa*, *Aa* and *AA* from an *Aa*-hybrid are related as $-d_a$, h_a and d_a, respectively, the mean phenotypic value of a given F_n bulk will be $\frac{1}{2^{n-1}}h_a$, since the three genotypes in that population appear in a frequency of $\frac{1}{2}\left(1-\frac{1}{2^{n-1}}\right)$:

$\frac{1}{2^{n-1}}$: $\frac{1}{2}\left(1-\frac{1}{2^{n-1}}\right)$. The contribution of gene $A-a$ to the variance of F_n bulk must then be

$$\left(\frac{2^{n-1}-1}{2^{n-1}}\right)d_a{}^2+\left(\frac{2^{n-1}-1}{4^{n-1}}\right)h_a{}^2+E_1\,,$$

where E_1 stands for the non-heritable component of variation of single individuals.

Provided that a number of polygenes besides $A-a$ affect the same character and the total heritable variation within the bulk is the simple sum of the contributions made by the individual genes, the total variance becomes

$$V_{F_n\,(\mathrm{Bulk})}=\left(\frac{2^{n-1}-1}{2^{n-1}}\right)D+\left(\frac{2^{n-1}-1}{4^{n-1}}\right)H+E_1\,,$$

in which $D\equiv\sum_i d_i{}^2$ and $H\equiv\sum_i h_i{}^2$.

The heritability of the character must then be,

$$h^2{}_{F_n\,(\mathrm{Bulk})}=\frac{\left(1-\frac{1}{2^{n-1}}\right)D}{D\left(1-\frac{1}{2^{n-1}}\right)\left(1+\frac{K}{2^{n-1}}\right)+E_1}\,,$$

where K stands for H/D. For a given value of heritability, h^2, for the F_2 generation and a value of K, relative values of D and H are readily computed from the following relation, taking the value of E_1 as unity,

$$D=\frac{4}{\frac{2}{h^2}-2-K}\,,$$

which is derivable from the relation,

$$h^2{}_{F_2}=\frac{\frac{1}{2}D}{\frac{1}{2}D+\frac{1}{4}H+E_1}\,.$$

(B) Heritability of means of F_n plant-lines derived from selected plants in F_{n-1} bulk population.

F_n lines derived from F_{n-1} plants consist of either one of the following three genotypes with respective frequencies and with respective mean values as follows:

Lines:	from AA-individuals	from Aa-individuals	from aa-individuals
Frequencies:	$\frac{1}{2}\left(1-\frac{1}{2^{n-2}}\right)$	$\frac{1}{2^{n-2}}$	$\frac{1}{2}\left(1-\frac{1}{2^{n-2}}\right)$
Mean value of lines:	d_a	h_a	$-d_a$

The whole mean of mean values of plant-lines is $\frac{1}{2^{n-1}}h_a$. Variance of means of F_n plant-lines is then

$$\left(1-\frac{1}{2^{n-2}}\right)d_a^2+\left(\frac{2^{n-2}-1}{4^{n-1}}\right)h_a^2+E_2,$$

where E_2 stands for the non-heritable components of variance of the means of lines. The formula is again rewritten, as in the previous case, as:

$$V_{F_n\,(\text{Line})}=\left(\frac{2^{n-2}-1}{2^{n-2}}\right)D+\left(\frac{2^{n-2}-1}{4^{n-1}}\right)H+E_2.$$

The heritability of the mean values of a given character observed in the F_n lines will be

$$h^2_{F_n\,(\text{Line})}=\frac{\left(1-\frac{1}{2^{n-2}}\right)D}{D\left(1-\frac{1}{2^{n-2}}\right)\left(1+\frac{K}{2^n}\right)+E_2}.$$

Table 1 shows an example of results of some computations in several successive generations. The computation is made, as is seen in the table, for $h^2_{F_2}=0.1$, the K values being taken as 0, 1, and 10. Each plant line is supposed to include twenty plants for measurement.

Table 1.

	Bulk	Line	Bulk	Line	Bulk	Line	Bulk	Line
K	0		1		5		10	
H	0		0.235		1.55		5	
D	0.22		0.235		0.31		0.5	
E	0.1	0.05	1	0.05	1	0.05	1	0.05
F_2	0.1		0.1		0.1		0.1	
F_3	0.14	0.69	0.15	0.64	0.15	0.51	0.16	0.41
F_4	0.16	0.77	0.17	0.74	0.19	0.65	0.22	0.57
F_5	0.17	0.80	0.18	0.78	0.21	0.75	0.27	0.70
F_6	0.18	0.81	0.19	0.80	0.22	0.80	0.30	0.79
F_7	0.18	0.81	0.19	0.81	0.23	0.83	0.31	0.84
F_8	0.18	0.81	0.19	0.82	0.23	0.85	0.32	0.88
F_9	0.18	0.82	0.19	0.82	0.23	0.85	0.33	0.89
F_{10}	0.18	0.82	0.19	0.82	0.24	0.86	0.33	0.90
F_{11}	0.18	0.82	0.19	0.82	0.24	0.86	0.33	0.90
F_{12}		0.82		0.82		0.86		0.91

It is suggested from these computations that the most efficient method for the successful breeding of superior genotypes with respect to any character with heritability as low as 0.1 should make use of the bulk-method for several generations followed by selection for that character among the greatest possible number of plant-lines.

b. Heritability and the Number of Individuals to be Selected

(Report by Kan-Ichi SAKAI)

The number of individuals or lines to be selected among a given population of individuals or lines of a segregating generation is a function of the value of heritability of the character.

Let the phenotypic value of each individual or line in the F_n generation be Y, which would distribute normally with variance V. If we would select any individuals or lines having Y greater than a fixed value Y', they will be those falling in the shaded area q in Fig. 1A, having a mean value of $\overline{Y}_s$. If Y be transformed to a variate, u, with unit variance and mean at zero, that is $u=(Y-\overline{Y})/\sqrt{V}$, then the value of $u'=(Y'-\overline{Y})/\sqrt{V}$ corresponding to any given value of q may be ascertained from a table of the normal probability integral.

The selection differential expressed in the standard deviation, i, then becomes

$$i=\frac{\overline{Y}_s-\overline{Y}}{\sqrt{V}}=u_s=\frac{z}{q},$$

where z is the ordinate of the unit normal curve at the deviate u'. The expected genetic gain, ΔG, in the next F_{n+1} generation will then be

$$\Delta G=h^2 i\sqrt{V}=h^2\sqrt{V}\frac{z}{q},$$

in which h^2 is the corresponding heritability of that character in the F_n population. Assuming that the values of the character of each F_{n+1} line, P, are normally distributed with a mean value $\overline{P}$ and variance V', the proportion of progenies having P greater than Y', that is the shaded area Q in Fig. 1B, will be calculated as

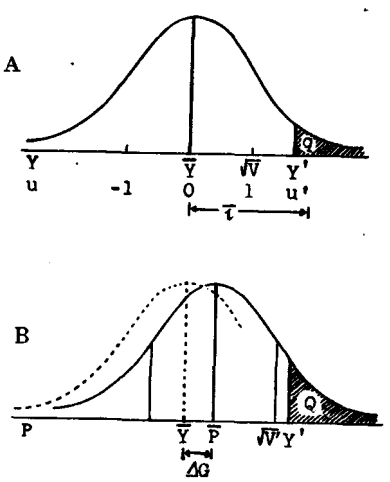


Fig. 1

$$Q=\int_{P=Y'}^{\infty}\frac{1}{\sqrt{2\pi V'}}e^{-\frac{1}{2}\left(\frac{P-\bar{P}}{\sqrt{V'}}\right)^2}dp=\int_{t=t'}^{\infty}\frac{1}{\sqrt{2\pi}}e^{-\frac{1}{2}t^2}dt\,,$$

in which $t=\frac{P-\bar{P}}{\sqrt{V'}}$ and $t'=\frac{Y'-\bar{P}}{\sqrt{V'}}=\frac{Y'-\bar{Y}-h^2\sqrt{V}\frac{z}{q}}{\sqrt{V'}}$. If we assume that $V'=V$ in case of any character with low heritability, then $t'=u'-h^2\frac{z}{q}$.

The value of Q becomes

$$Q=\int_{t=u'-h^2\frac{z}{q}}^{\infty}\frac{1}{\sqrt{2\pi}}e^{-\frac{1}{2}t^2}dt\,,$$

which may also be ascertained from a table of the normal probability integral.

Now any one line among the progeny of the selected individuals or lines has a chance of Q of having a greater value than Y'. The minimum number (N) of individuals or lines which should be selected in the F_n generation in order that the F_{n+1} progeny would contain at least one superior line of that level in 99 cases out of 100 is given by the solution of the equation

$$N\geqq\frac{\log 0.01}{\log(1-Q)}\,.$$

The following table (Table 2) presents results of some calculations con-

Table 2. The minimum number of individuals or lines to be selected (a) and of those to be grown for the selection (b) in relation to various values of heritability (h^2) and intensities of selection (q)

h^2	q					
	0.1		0.05		0.01	
	a	b	a	b	a	b
0.01	43	420	88	1741	428	42728
0.05	37	370	74	1475	329	32900
0.10	32	318	61	1206	238	23717
0.20	24	238	41	810	188	18778
0.30	18	179	29	571	73	7222
0.40	14	140	21	406	43	4279
0.50	11	108	16	301	28	2711

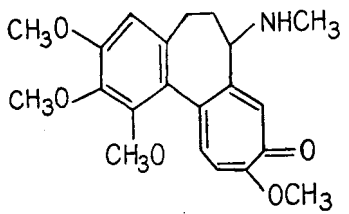
cerning the minimum number of individuals or lines to be selected in the bulks or groups of plant lines of some successive hybrid generations.

2. *A new Chemical "Substance F" for Inducing Doubling of Chromosomes*

(Report by Yô TAKENAKA)

Colchicine, as a chemical agent causing doubling of chromosomes, was first discovered by BLAKESLEE (1937) and is still much in use. Also acenaphtene introduced by KOSTOFF (1938) serves the same purpose and is being used extensively. WITKUS and BERBER (1944) have reported veratrine and LEVAN (1947) recommends iso-colchicine also as polyploidy inducing agents. According to PARTHASARATHY (1941), fresh extract of *Gloriosa superba* caused doubling of chromosomes in root tips, and KUMAR (1953) reported that gloriosine extracted from its tubers gave similar results.

The "Substance F" was first extracted from tubers of *Colchicum* and named by ŠANTÝ and REICHSTEIN (1950), and its chemical structure was determined by UENO (1953) as follows:—



The author examined the effect of this alkaloid upon plant tissues by the following methods:

(1) Tumor tests on growing root-tips soaked in water solutions of this substance in different concentrations.

(2) Dwarf-Tumor tests in seedlings germinated from seeds soaked in the same solutions.

(3) C-mitosis tests in growing root-tips dipped in the same solutions.

The tumor tests on growing root-tips were repeated six times with materials of *Allium Cepa*, *Allium scorodoprasum* var. *viviparum* and *Vicia faba* in the spring of 1953. These experiments yielded somewhat larger tumors in concentrations from about 0.1 to 0.01% of this substance than those obtained from the same concentrations of colchicine. But concentrations of this substance over 0.1% showed some necrotic effect, while colchicine of the same concentrations produced only small tumors.

The dwarf-tumor tests in seedlings were carried out three times with various *Brassica* and *Raphanus* species in the spring of 1953. The seeds of these species were soaked in solutions of different concentrations of

"Substance F" and also in colchine solution of the same concentrations for 28 and 43 hours. The seedlings from the treated seeds were counted.

The number of dwarfed seedlings carrying tumors caused by "Substance F" treatment, in all the species and concentrations, was somewhat smaller than that obtained from colchine treatment.

Onion root tips treated for 24 hours with this substance in 0.05% concentration showed some cells with beautiful figures of C-pair chromosomes, which suggest the occurrence of tetraploid sets of chromosomes besides perhaps octoploid sets. Furthermore, some nuclei showing irregular and abnormal mitosis similar to those found in colchicine treatment were found.

In the autumn of 1953, seeds of *Melandrium*, *Brassica* and *Raphanus* were treated by various concentrations of this substance. The proportion of polyploid individuals among the plants germinating from these seeds may be determined next spring, and this will give us a key to evaluate the efficacy of "Substance F" for this purpose.

PAPERS AND BOOKS WRITTEN BY THE MEMBERS OF THE STAFF

FUJII, T. 1953. Morphologically different twins among the offspring of a wheat hybrid. Jap. Jour. Genet. 28: 105–109.

FURUSATO, K. 1953. Defective growth in *Citrus* due to chromosome irregularity in stock. Kankitsu 5 (5): 48–50.

——— 1953. Studies on the polyembryony in *Citrus*. Jap. Jour. Genet. 28: 165–166.

GOTOH, K. 1953. Genetic studies on eggplant (*Solanum melongena* L.) I. Regression analysis of quantitative gene action. Genetica 26 (5/6): 445–452.

——— 1953. Genetic studies on eggplant (*Solanum melongena* L.) II. The heritability of some quantitative characters and estimation of minimum number of genes. Genetica 26 (5/6): 453–467.

——— 1953. Inter-racial differences in a barley variety "Hosogara No. 2". Jap. Jour. Genet. 28 (4): 166.

——— 1953. The significance of natural selection in breeding. Iden 7 (5): 45–48. 7 (6): 44–47.

——— 1953. Inter-racial differences in a barley variety "Hosogara No. 2". (Abstract) Jap. Jour. Genet. 28(4):166.

ISHIHARA, T. 1953. Some observations on transplantability in two mouse tumors. Jap. Jour. Zool. 12:397–403.

KOMAI, T. 1953. Three Japanese pedigrees of typical brachydactyly. Jour. Hered. 44:79–85.

——— 1953. Composition of wild population in the lycaenid butterfly *Neozephyrus taxila*. Amer. Nat. 833:87–95.

KOMAI, T., A. S. AÉ. 1953. Genetic studies of the pierid butterfly *Colias hyale poliographus*. Genetics 38:65–72.

KOMAI, T., K. KISHIMOTO and Y, OZAKI. 1953. Genetic studies of human microcephaly. (Preliminary report). Proc. Jap. Acad. 29:219–223.

KOMAI, T., Y. OZAKI and W. INOKUMA. 1953. A Japanese kindred of hyperphalangism of thumbs and duplication of thumbs and big-toes. Folia Hered. Path. 2:307–312, lpl.

KOMAI, T., and R. TAKAHASHI. 1953. Translation of Th. Dobzhansky's "Genetics and Origin of Species" Third edition. 1–348. Baifûkan Tokyo.

MATSUMURA, S. 1953. Origin of Wheat. 1–124. Iwasaki Shôten Tokyo.

——— 1953. "Artificial mutation" "Genome theory" in Current Theory of Genetics. 181–241, 243–286. Hokuryukan Tokyo.

——— 1953. Improvement of sugar beet by means of triploidy. 1–134. Sciencesha Tokyo.

——— 1953. Chromosome analysis of the Dinkel genome in the offspring of a pentaploid wheat hybrid IV. gene-analysis. Rep. Kihara Inst. Biol. Res. 6: 38–48.

MATSUMURA, S. and T. FUJII. 1953. Studies on mutation in *Nicotiana* induced by X-rays (Abstract) Jap. Jour. Breed. 3(1):54.

——— ——— 1953. X-ray induced mutations in einkorn wheat (Abstract) Jap. Jour. Genet. 28:175.

MATSUMURA, S. and A. MOCHIZUKI. 1953. Improvement of sugar beet by means

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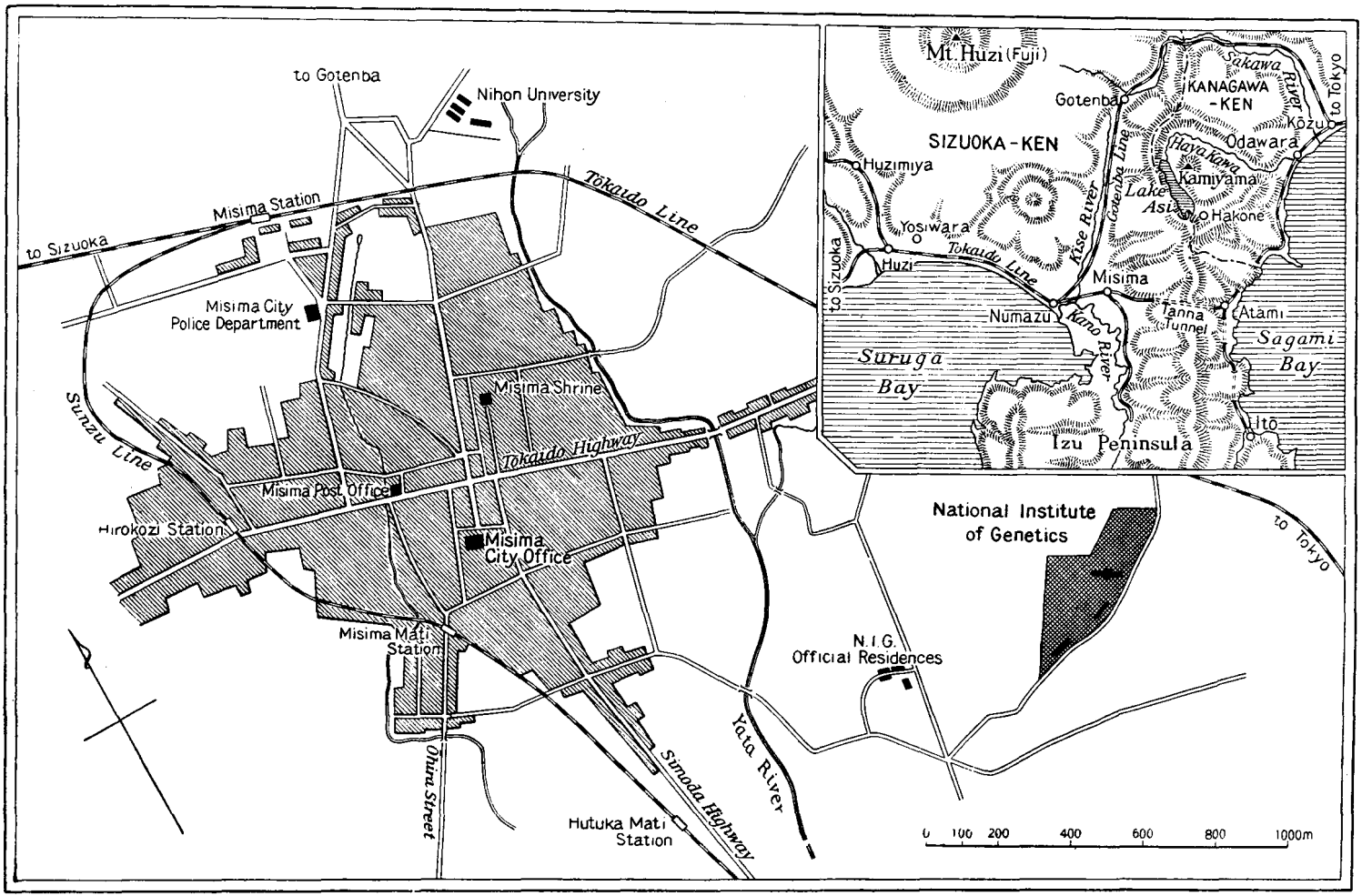
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