NATIONAL INSTITUTE OF GENETICS

(JAPAN)

ANNUAL REPORT

No. 3.

(1 9 5 2)

PUBLISHED BY
THE NATIONAL INSTITUTE OF GENETICS
(MISIMA, SIZUOKA-KEN, JAPAN)
1 9 5 3

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Contents

GENERAL STATEMENT

During the current year, the Institute acquired considerable additions to its equipment and improvement of its property. A new two-storey concrete building, 180.520-tubo (1 tsubo=6 ft. square) in floor dimentions, was completed to house the following; a library, a reading room, a librarian's office, two chemical laboratories, and an optical laboratory on the first floor, and on the ground floor an electron-microscope room and an X-ray apparatus room each with a vestibule, four constant temperature rooms for culture of *Drosophila* and silkworms, a set of constant temperature rooms regulated at −5∼0, 0∼5, 5∼10, 15∼20, 20∼25, 25∼30°C, respectively, and a room for a super-sonic wave apparatus and an ultra-short wave apparatus. Another new 26.46-tubo building is a plantron which is the first to be constructed in this country. Also, a large 46-tubo glass house was constructed primarily for research on tobacco plants. These increased facilities are giving great impetus to our research work.

The library has been expanded by acquisition of current numbers of periodicals and new reprints. Especially, Dr. GOLDSCHMIDT has continued to send his reprints and journals which help to keep the library up to date.

T. Ito resigned and his place was filled by T. IINO. T. H. YOSIDA was transferred from the Faculty of Science of Hokkaido University to the Institute. Dr. Flora Alice LILIENFELD, who had been connected with the Japan Monopoly Corporation, concurrently became a research associate of the Institute. M. TSUJITA was awarded the Sericological Prize for his study on cell inclusions, especially mitochondria, of the silkworm.

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

From the Scientific Research Fund of the Ministry of Education: to K. OGUMA and coworkers (including T. KOMAI and K. TUTIKAWA), for: Breeding and preservation of strains of rats and mice useful for medical research purposes,—¥800,000.

T. KOMAI and coworkers (including K. SAKAI and M. KIMURA), for: Researches in population genetics,—¥510,000.

Y. TANAKA and coworkers (including M. TSUJITA), for: Fundamental and applied genetics of the silkworm,—¥350,000.

T. KOMAI and coworkers, for: Genetic and psychological studies on human microcephaly,—¥50,000.

From the Scientific Experiment Fund of the Ministry of Education: to S. MATSUMURA and coworkers, for: Breeding of triploid sugar-beet strains,—¥100,000.

M. TSUJITA, for: Researches on genetics and selection of abnormal eggs of silkworm,—¥50,000.

The Institute was provided by the Ministry of Education with an extra-budgetary fund amounting to ¥7,080,000 for the construction of the plantron.

Among a number of visitors were Dr. K. RAMIAH of FAO of UN., Prof. H. H. PLOUGH of the AEC in Washington, D.C. and Dr. D.J. MCDONALD of ABCC, Hirosima.

ABSTRACT OF DIARY IN 1952

February 9. Tenth meeting of Misima Geneticists' Club.

February 21. Sixth meeting of the Board of Councillors.

March 29. Eleventh meeting of Misima Geneticists' Club.

April 25. 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.

May 31. Twelfth meeting of Misima Geneticists' Club.

June 26. Board meeting of Association for the Propagation of the Knowledge of Genetics.

July 22 to 29. Summer course in biology for teachers in local high schools.

August 4. Seventh meeting of the Board of Councillors.

August 23. Report on the trip to Pakistan and Afghanistan by Mr. O. SUZUKA.

October 25. Phage Symposium.

November 8. Thirteenth meeting of Misima Geneticists' Club.

November 21. Meeting of directors of research institutes under the jurisdiction of the Ministry of Education.

December 19. Meeting for interim reports on fundamental studies for the improvement of tobacco plants.

STAFF

Department and Laboratory Heads

Kan OGUMA, D. Agr., Director, and Head of the Second Department

Yoshimaro TANAKA, D. Agr., D. Sc., Head of the First Department

Taku KOMAI, D. Sc., Head of the Third Department

Part-time Staff

Hitoshi KIHARA, D. Sc., Professor of Kyoto University

Yosito SINOTÔ, D. Sc., Professor of Tokyo University

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Akira MIYAZAWA

Bungo SAKAGUCHI

Tôru ENDÔ

Kiyoshi TUTIKAWA

Tetuo IINO

Seizô TSUDA

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Department of Administration

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Masao MIYAZAWA, Head of Finance Section
Naomi MATSUBARA
Hiroko NAKANO
Junzô KADOWAKI
Clerks, Typist, Telephone operators, Chauffeurs, Field laborers, Janitors, etc.—14.

Misima Branch of *Hatano Tobacco Experiment Station*
Masao TANAKA, Head
Flora Alice LILIENFELD
Seiji IMAI
Assistants—3.

Whole-Japan Association of Poultry Genetics
Kan OGUMA, President
Yoshimaro TANAKA, Vice-President and Director of Researches
Také NAKAMURA, Managing Director

Association for Propagation of the Knowledge of Genetics
Kan OGUMA, President
Yô TAKENAKA, Managing Director
Seiji MATSUMURA, Managing Director

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Yusuké SUMIKI, Professor of Tokyo University
Yûshi UCHIMURA, Professor of Tokyo University
Yasuké YAMAGUTI, Professor of Ibaraki University

RESEARCH PROGRAM FOR 1952

Tanaka Laboratory

Unstable genes in silkworm—TANAKA
Genetics of dominant "retarded" strain of silkworm—TANAKA
Genetics of recessive "retarded" strain of silkworm—TANAKA
Photoperiodic effect on the diapause of the wild silkworm *Antheraea pernyi*, and the genetics of diapause—TANAKA
Lethal genes in the silkworm—TANAKA
Hereditary malformations in the silkworm—TANAKA
Linkage of "small egg" strains—TANAKA
Breeding and preservation of pure strains of rats and mice for medical purposes—OGUMA and TUTIKAWA
Genetics of the differences among strains of mice in respect to susceptibility to tumors—TUTIKAWA
Lethal genes in rats and mice—TUTIKAWA

Matsumura Laboratory

Radio-genetic studies on wheat—MATSUMURA and FUJII
Genom in *Agropyrum*, a close relative of *Triticum*—MATSUMURA
Nullisomic plants found among the progeny of pentaploid hybrid wheat—MATSUMURA
Breeding of a strain of wheat resistant to rust—MATSUMURA and HIRATSUKA (Tokyo Univ. of Education)
Breeding of a triploid sugar beet—MATSUMURA et al.
Origin of the plants of the genus *Lycoris* and their distribution—KIHARA and LILIENFELD
Genetic studies on the variations induced by ultra-short waves and super-sonic waves—MATSUMURA and FUJII
Mutation in *Nicotiana* induced by X-ray irradiation—MATSUMURA, KIHARA and FUJII

Furusato Laboratory

Breeding of varieties of *Citrus*—FURUSATO and MIYAZAWA

Biochemical genetics on flower colors—ENDO and MIYAZAWA
Polyploidy and grafting—FURUSATO
Sterility in cultivated plants—MIYAZAWA
Biochemical studies on plant pigments—ENDÔ

Oguma Laboratory

Origin of sex-chromosomes—OGUMA
Phylogeny of plants based on karyotypes—TAKENAKA and SINOTÔ
Genetics of right- and left-handedness in plant organs—KIMURA and KIHARA
Theoretical studies on problems of population genetics—KIMURA
Theoretical studies on gene action—KIMURA
Experimental studies on polygene system—KIMURA
Theoretical studies on crossing over—KIMURA

Takenaka Laboratory

Origin of differentiation of sex in higher plants—TAKENAKA
Collection and preservation of varieties of useful plants—TAKENAKA
Cytogenetics of some fungi—TAKENAKA and SINOTÔ
Karyological studies on natural polyploidy—TAKENAKA
Karyological studies on polyploids of cultivated plants—TAKENAKA
Cytogenetic studies on interspecific hybrids in *Nicotiana*—TAKENAKA and SUSUKI
Heterokaryon in *Aspergillus*—TSUDA

Komai Laboratory

Genetics of human microcephaly—KOMAI, KISHIMOTO (Nagoya Univ.) and OZAKI (Inst. Publ. Heath)
Population genetics of the lady beetle, *Harmonia*—KOMAI
Population genetics of the land snail, *Bradybaena*—KOMAI
Theoretical and experimental studies on genes—KOMAI
Experimental studies on the mechanism of aberrant mitoses—YOSIDA
Experimental studies on the cytology and biochemistry of tumors—YOSIDA
Studies on culture of normal and tumorous tissues—YOSIDA

Sakai Laboratory

Genetics of fruit crops—SAKAI, GOTOH and SUZUKI
Competition between individual plants of different genetic constitution—SAKAI and SUZUKI
Theoretical and experimental studies on selection in plant breeding—

SAKAI

Genetics of quantitative characters in tobacco plants—SAKAI, GOTOH and IYAMA

Genetic studies on flowering plants—GOTOH

Tsujita Laboratory

Lethal eggs and sterility in silkworm—TSUJITA and SAKAGUCHI

Virus infecting silk-producing insects—TSUJITA and SAKAGUCHI

Manifestation of gene effects—TSUJITA and SAKAGUCHI

Developmental genetics of the silkworm—TSUJITA and SAKAGUCHI

Minute structures of chromosomes—TSUJITA and SAKAGUCHI

Cytogenetics of silkworms, especially polyploids—TSUJITA and SAKAGUCHI

Mitochondria in insect cells—TSUJITA and SAKAGUCHI

Research Students and Research Subjects

Toshihiko OKUBO: Genetics of silkworm and poultry

Tosiaki SUZUKI: Induced mutation

Kyôzô WATANABE: Genetics of protozoa

Yasuo SUZUKI: Population genetics in cultivated plants

Etuo GOTÔ: Microtechnics

Seiji TEZUKA: Silkworm genetics

Hirosi EBIHARA: Breeding of tobacco plants

Sinya IYAMA: Breeding of tobacco plants

RESEARCHES CARRIED OUT IN 1952

A. HUMAN GENETICS

(Report by Taku KOMAI)

1. A Pedigree of Hyperphalangy in Thumbs and Polydactyly in Hands and Feet

A kindred having an abnormality of the hands and feet has been discovered near Sibukawa, Gumma-ken. All the affected individuals have three-jointed thumbs, and some of them also bear extra thumbs and bifurcated big-toes. This abnormality seems to have originated as a new mutant in a woman who was the mother of the present generation, and has appeared in ten of her descendants. The trait is inherited as a simple dominant with nearly perfect penetrance, but varying in expressivity and specificity to some extent.

2. Genetics of Microcephaly

The writer has continued, with the cooperation of Prof. K. Kishimoto of Nagoya University and Dr. Y. Ozaki of the Institute of Public Health, his study on human microcephaly. So far, 119 cases belonging to 61 sibships have been accumulated. A recessive gene is apparently responsible for the great majority of these cases. Eight sibships contain more abnormals than could be expected on the postulate of a recessive gene with probability lower than 0.05, suggesting that some other genetic or non-genetic cause is at work. Excluding these cases, the incidence of the recessive gene for microcephaly in the population has been estimated by means of Dahlberg's formula:

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

where q denotes the incidence, k the rate of cousin marriages among the parents of the affected individuals and c the rate of cousin marriages in the whole population. In our material k is 0.54, while for c, the value 0.06 found previously by the ABCC group was used, giving the value 0.0036 for q. This value comes very close to the values found by other authors for the genes for some rare abnormalities in man, such as albinism, amaurotic idiocy, congenital total color-blindness and ichthyosis congenita. Based on this value of q, the rate of mutation of the recessive gene for microcephaly was computed by means of the formula:

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

where m stands for the mutation rate, f the fertility of the abnormal individuals in comparison with that of normal individuals, and α for the coefficient of inbreeding in Wright's sense. By using 0.05 for f, and 0.005 for α, the value 3.0×10^{-5} has been found for m. This value of m comes close to the known values for the mutation rates of the genes responsible for certain rare congenital abnormalities in man, such as hemophilia, albinism, retinoblastoma, etc.

B. GENETICS OF SOME MAMMALS

1. *Problem of the Origin of the Tortoiseshell Male Cat and its Sterility*

(Report by Taku KOMAI)

Records of tortoiseshell males with statements of coat colors of their parents and litter mates have been collected. This is to test the validity of the writer's new hypothesis on the origin of this kind of males presented in No. 2 of the Annual Report of the Institute. A popular article by the writer on this subject on the Yomiuri Newspaper was highly contributive to this attempt. Thus the writer has been able to obtain to date records of as many as fifty cats whose identity as tortoiseshell males seems certain. Many of these specimens were examined by the writer, or by other biologists provided with a sufficient knowledge of the diagnosis of this kind of cat. The rest have been judged as fairly authentic by color sketches prepared by the owners of the cats. Since there is hardly any way of getting cytological proof either for or against the hypothesis, the writer has to seek indirect evidence in the data of coat colors of parents and litter mates of such a male. A fairly convincing proof against the hypothesis might be obtained if it is found that more than one tortoiseshell male were born of the same mother. So far, all the reports of such occurrence have been found to be false, due to the wrong diagnosis of the coat color. Moreover, all the available reports on coat colors of parents and litter mates, except for a few doubtful ones, conform with this hypothesis.

2. *Studies on an Apparently New Mutant, "Alopecia Periodica" found in the Mouse*

(Report by Kiyosi TUTIKAWA)

"Alopecia periodica", (*ap*) is a recessive spontaneous mutant which appeared in 1951, in a sib-mated stock of *Mus musculus* maintained in our Institute. In the homozygous condition, *ap* produces several lesions in

the integument which are at first sight apparently unrelated to each other.

The homozygous young of this strain may already be distinguished from the normal sibs at birth, and more easily on the 3rd–4th days by the shorter whiskers and also by the fact that the first coat is very short and sparse. At the age of from 13 to 15 days, the abnormal young start shedding their coat hair. The shedding is usually complete in 20–24 days except for some regions, around the nose, toes, and tail which have a few hairs left. After the loss of this first coat, the skin remains soft, smooth, and of a pinkish colour, until the next coat begins to grow.

Then a peculiar type of hair regeneration sets in, with a gradient in an antero-posterior direction. While the coat in the posterior region of the body is still incomplete, a gradual thinning recurs in the anterior region. Thus, the loss and regeneration of hair is repeated with about ten-day intervals throughout life, although the loss and regeneration of the hair in later generations does not recur so regularly as in the earlier stages, and the same individual may show regions belonging to two or even three subsequent hair generations simultaneously.

During the first coat growth, the skin appears dry and scaly. In some severe cases the scales form rigid plates all over the body, and the animal's life is endangered. The scaly skin often forms a number of rings on the tail, and the distal part of the tail may become necrotic.

The developing coat of the *ap* mouse in all hair generations apparently consists of guard hairs, awls, auchenes, and zig-zags which are, however, histologically abnormal. After the second or more generations of the hair cycle, especially the black fur becomes modified into silver-coloured. This modification of the black tone may be under the control of a modifying gene. The vibrissae of the adult *ap* mouse are short. The eyelids may be thickened, possibly due at least in part to shortening and reduction in the number of eyelashes. The teeth and nails are not affected. Both sexes of *ap* are fully fertile, but in females the ability to suckle their young is sub-normal.

C. KARYOLOGY OF TUMOR CELLS

(Report by Tosihide H. YOSIDA)

1. Karyological Study on the Tumor Cells of Takeda Sarcome in the Rat

Karyological studies on ascites tumor in rats have been carried out by MAKINO, YOSIDA, KANÔ and TANAKA (1948–1953). Makino has found in Yoshida sarcome the presence of strain cells which are characterized by having about 40 chromosomes, including large V–, small V– and small J–shaped elements.

The Takeda sarcome used in the present study is transplantable ascites tumor found in the rat (TAKEDA 1952). This paper deals with my cytological observations on the tumor developed in one animal.

(1). Frequency of tetraploid cells: This rat died of the tumor on the seventh day after transplantation. 127 metaphase plates of the tumor cells were observed through the life span of this animal. 106 (83.4%) among them were tetraploid, 14 (11.0%) were diploid, and the remaining 7 cells (5.5%) were hyperploid. It is evident from this observation that most of the cells constituting this tumor are tetraploid and they form the strain cells of this tumor.

In order to see whether these tetraploid cells normally multiply with regular mitoses or not, the relative frequency of cells of the ordinary division type, the aberrant type and the disintegration type (cf. MAKINO & YOSIDA 1951) were examined throughout the life span of the tumor animal.

Table 1. Frequency of the tetraploid cells occurring in Takeda sarcome.

	Diploid ($\pm 2n$)	Tetraploid ($\pm 4n$)	Hyperploid ($> \pm 4n$)	Total
Frequency	106	14	7	127
%	83.4	11.0	5.5	99.9

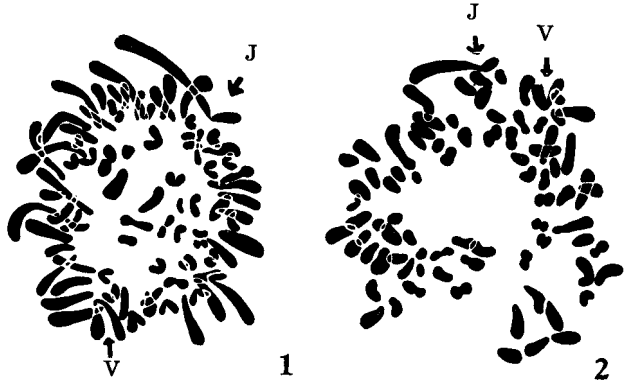
Table 2. Frequency of the cells of different types occurring in tumor cells.

Types	Division type	Aberrant type	Disintegration type	Total
Frequency	57	20	29	106
%	53.7	18.6	27.3	99.6

Table 3. Chromosome numbers of tumor cells in Takeda sarcome.

No. of chrom.	No. of obs.	No. of chrom.	No. of obs.
104	1	73	1
89	2	69	1
86	1	48	1
85	1	43	1
84	4	40	2
83	1	38	3
82	1	37	1
80	2	36	2
76	1	Total	26

As seen in the table, the frequency of cells of the division type, aberrant type and disintegration type was 53.7, 18.6 and 27.3 per cent respectively. It has been considered by the authors (MAKINO & YOSIDA 1951) that the cells of the division type multiply by active mitoses in the peritoneal cavity of the host. The above observations show that many tetraploid cells in the Takeda sarcome actively multiply by regular mitosis.



Figs. 1-2. Chromosomes of the tumor cells in the Takeda sarcome. 1 and 2, showing 86 and 82 chromosomes respectively. J = large J-shaped chromosomes, V = large V-shaped chromosomes.

(2). Chromosome numbers in the tumor cells : Of importance is the number of chromosomes of these tumor cells. The results of my observations are tabulated in Table 3. The chromosomes were counted on sketches of good metaphase figures. The cells having ca. 84 chromosomes were most commonly represented. From the above data, it can be concluded that the strain cells of the Takeda sarcome are characterized by having ca. 84 chromosomes.

(3). Karyotype of strain cells : The karyotype of strain cells has been analyzed from seven metaphase plates containing 76, 80, 82, 83, 84, 84 and 86 chromosomes, respectively. In five of these cells there is a conspicuous J-shaped element besides the large or small V-shaped elements which are regularly found in Yoshida sarcome cells.

Thus, the strain cells of the Takeda sarcome not only show a marked difference in karyotype from the normal somatic cells, but also from the cells in other tumors such as Yoshida, MTK or Hirosaki sarcomes.

2. *Karyological Study on Cells of the "Quinone Carcinome" in Mouse*

The "quinone carcinome" is a carcinome which was induced in the mouse epidermis by Prof. TAKIZAWA (1946) of Chiba University, by paint-

ing benzol solution of p-quinone on the skin. Observations carried out by the author on the chromosomes in the cells of this tumor have revealed the following facts:—

(1). Frequency of polyploid cells: The data on the frequencies of diploid, tetraploid and hyperploid cells occurring in the tumor tissue are summarized in Table 1. It is noticeable that the tetraploid cells occur with a frequency as high as 77 per cent. These tetraploid cells multiply by normal mitoses. The diploid cells, which occur with a frequency of about 5 per cent, often show abnormal mitotic behavior. From the above

Table 1. Frequency of tetraploid cells occurring in the quinone carcinome.

	Diploid ($\pm 2n$)	Tetraploid ($\pm 4n$)	Hyperploid ($> \pm 4n$)	Total
Frequency	4	60	14	78
%	5	77	18	100

Table 2. Chromosome numbers and karyotypes of the tumor cells.

No. of chrom.	Karyotypes					
	Rod	Small *V*	Medium *V*	Large *V*	Trabant	Dot
80	77	2	1	—	—	—
80	79	—	1	—	—	—
79	71	4	1	2	1	—
79	75	2	1	—	1	—
78	72	4	1	—	1	—
77	70	2	2	2	—	1
76	71	1	1	—	1	2
76	69	2	2	2	1	—
76	72	1	3	—	—	—
76	68	6	—	2	—	—
75	70	3	1	—	1	—
75	61	10	—	2	1	1
75	67	1	1	1	2	3
75	74	—	1	—	—	—
74	67	1	3	—	1	2
69	65	1	1	2	—	—
68	59	2	1	5	1	—
48	46	2	—	—	—	—
34	32	1	1	—	—	—

data, it can be surmised that the tetraploid cells are the strain cells of the quinone carcinome.

(2). Chromosome numbers of the tumor cells: The chromosome numbers determined on good metaphase figures are presented in Table 2. The number shows a wide range of variation from 34 to 80, cells having about 75 chromosomes being most frequent.

(3). Karyotype analysis of the tumor cells: In the germ cells of the mouse, MAKINO (1941) has observed 40 chromosomes, all of a simple rod-shaped type. The author has confirmed the correctness of this finding by the squash technique. A comparison of the chromosomes in germ cells with those in tumor cells discloses a marked difference, especially with respect to the chromosome number, and in the occurrence of *V*-shaped elements in the latter. The *V*-shaped elements which are usually found in the tumor cells can be classified into three types; large, medium and

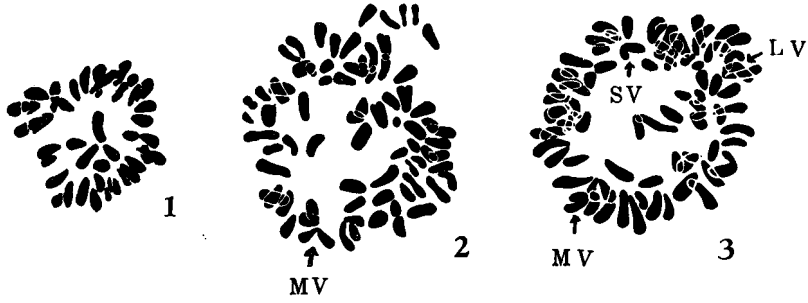


Fig. 1. Chromosome of the spermatogonial metaphase in the mouse; $2n=40$. Figs. 2-3, tumor cells showing 76 and 77 chromosomes respectively. SV=small V-shaped chromosome, MV=medium V-shaped chromosomes, LV=large V-shaped chromosome.

small. The number of these elements is shown in Table 2. The small *V* shows a wide range of variation in number. The small *V's* are probably only small rod-shaped chromosomes temporarily transformed. The large *V*-shaped chromosomes seem to be double elements derived from two long rod-like chromosomes attached by kinetochores. Such chromosomes occur in about half the observed cells. Giant chromosomes, with three or four arms, are formed possibly by attachment of the kinetochores of three or four long chromosomes; such chromosomes are observed only rarely in the tumor cells. *V*-shaped chromosomes of middle size may be observed in almost all tumor cells, numbering usually one and rarely two or three. These chromosomes seem to have a submedian fiber attachment. I have no idea of the origin of these middle-sized *V*-shaped chromosomes. They seem to be among the essential components of the chromosome complex

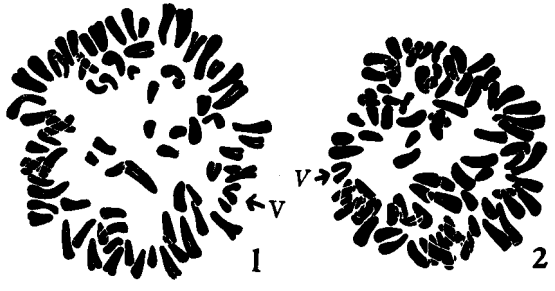
of the strain cells of this tumor. Also, small dot-like chromosomes, as well as chromosomes with trabants, may be found. The origin of these elements is not clear for the present.

In short, there are definite strain cells characterized by tetraploidy and by having middle-sized *V*-shaped chromosome in their nuclear complements.

3. *Karyological Study on the Tumor Cells of the Ehrlich Ascites Carcinome in Mouse*

Although several papers on the chromosome complex of Ehrlich ascite tumor of the mouse have appeared, no precise report of the chromosome morphology has been published. The present observation has been performed on the cells in this tumor developed on mice received from Prof. T. MIYAZI of Osaka University.

(1). Frequency of tetraploid cells: LEVAN & HAUSCHKA (1952) have observed the chromosome complex of tumor cells in the Ehrlich carcinome, and pointed out that cells in this tumor show a very wide range of variations in chromosome number, with the mode at the tetraploid number ($2n=4x=80$). The author's reexamination has revealed the results presented in Table 1. The tetraploid cells form 96 per cent of the total showing that the strain cells ("germ-line" in Levan & Hauschka's terminology) are of a tetraploid nature.



Figs. 1-2. Chromosomes of tumor cells in the Ehrlich ascites carcinome. 1, 79 chromosomes, 2, 80 chromosomes. V=V-shaped chromosomes.

(2). Chromosome number of the tumor cells: The chromosome numbers counted in 24 metaphase plates are shown in Table 2. The numbers show a wide range of variation, the cells having about 80 chromosomes being most numerous.

(3). Chromosome analysis of the tumor cells: The strain cells are characterized by having a tetraploid chromosome set. The chromosome complex of these cells is characterized by the presence of a *V*-shaped

chromosome. One (rarely two) of this element has been observed in almost all tumor cells (Table 2). It has an appearance very similar to the middle-sized *V*-shaped chromosome in the Takizawa quinone carcinome in the mouse. Exceptionally, small or large *V*-shaped chromosomes, like those seen in the quinone carcinome are also found. They seem to represent merely a temporary abnormality, as in the case of the quinone carcimome.

(4). Frequency of normal mitosis in the tetraploid cells: Relative frequencies of the division-type (cells undergoing regular mitosis), the aberrant-type and the disintegration-type mitoses occurring in the tetraploid

Table 1. Frequency of tetraploid cells occurring in the Ehrlich ascites carcinome.

	Diploid ($\pm 2n$)	Tetraploid ($\pm 4n$)	Hyperploid ($> \pm 4n$)
Frequency	8	303	6
%	2.5	95.6	1.9

Table 2. Chromosome numbers of the tumor cells, and the number of *V*-shaped chromosomes occurring in these cells.

No. of chrom.	No. of *V* chrom.	No. of chrom.	No. of *V* chrom.
132	2	77	1
94	1	77	—
89	?	74(4)	1
89	1	73	1
86	1	72	1
84	1	68	1
80(3)	1	67	1
79	1	58	—
78(2)	1	51	—

Remarks: Figures in parenthesis denote the number of observed cells.

Table 3. Frequencies of division-, aberrant- and disintegration-types occurring in tumor cells.

Types	Division	Aberrant	Disintegration	Total
Frequency	271	76	47	396
%	68	20	12	100

cells were observed daily in a tumor mouse throughout its life span (12 days in this case). The results obtained are given in Table 3. The total frequency of the division-type cells was 68 per cent, showing that most of the strain cells multiply with regular mitoses.

From these results, it can be concluded that the strain cells in the Ehrlich carcinome have a tetraploid nature, with 80 chromosomes or thereabouts, actively multiply by regular mitoses, and are characterized by the presence of a *V*-shaped element in the chromosome constitution.

1. Is There Any Cytoplasmic Effect on Unstable Genes in the Silkworm?

(Report by Yoshimaro TANAKA)

As has already been reported (TANAKA, 1951, 1952), there are several subtypes, or distribution types as they may be called, with regard to the number of spots (the multilunar and multistar markings) and protuberances (in the knobbed strain). These subtypes are evidently under the control of modifiers. The major genes, *L*, *ms* and *K* are constant to a considerable degree, while the modifiers are highly unstable, so that one subtype may be shifted to another in the course of several generations of selection. The term "unstable genes" used to designate such modifiers has a somewhat different sense from the term "mutable genes". If selection to both the plus and minus directions is effective in the case of a mutable gene, I would call it an unstable gene.

The cause of unstability of the modifiers in question is not yet known. To see if the cytoplasm has any phenotypic effect on the mode of distribution of *L*- and *ms*- spots, several reciprocal crosses between the normal and some multilunar and multistar subtypes were made, and the F_1 offspring were examined for the characters.

A) Reciprocal Crossing in the Multilunar Strains

(a) Reciprocal crossing between L_{4-10} subtype and the normal ($+^L$)

L_{4-10} is a subtype in which the brown spots are most numerous, i. e. they are present on the dorsal side of every larval segment. This type is the most stable among all the multilunar subtypes.

The variations among F_1 phenotypes were nearly alike in the reciprocals. No L_{4-10} larvae appeared, as was expected from the previous experiments, the larvae belonging to L_{4-8}, $L_{4\cdot5\cdot6\cdot7\cdot8}$ and $L_{4\cdot5\cdot6\cdot8}$ being most numerous. These subtypes altogether made 98.7% of the F_1 in $L_{4-10}♀\times+^L♂$, and 97.2% in the reciprocal cross, while the numbers of subtypes were 4 in the former and 7 in the latter. The L_{4-10} strain used in this crossing was somewhat peculier; it segregated multilunars and multistars and non-multilunars in a ratio of 1:1 in the F_1 of $L_{4-10}♀\times+^L♂$, and 2.4:1 in the

reciprocal cross. Whether this difference is merely accidental or not, must be ascertained in the future.

(b) Reciprocal crossing between $L_{5\cdot6\cdot8}$ and the normal ($+^{L}$)

The reciprocals again gave similar results. About 79% and 72% of the F_1 were multilunars in the cross $L_{5\cdot\dot{6}\cdot8}$♀ × $+^{L}$♂ and in the reciprocal respectively. The $L_{5\cdot6\cdot8}$ and $L_{5\cdot\dot{6}\cdot8}$ subtypes together made up respectively 98.3 and 86.0% of all multilunars. One thing to be mentioned here is that the larvae provided with brown spots on segment 4 were as numerous as 15 (14%) when $L_{5\cdot6\cdot8}$ was taken as the female parent, while only a single larva had spots on this segment in the reciprocal cross.

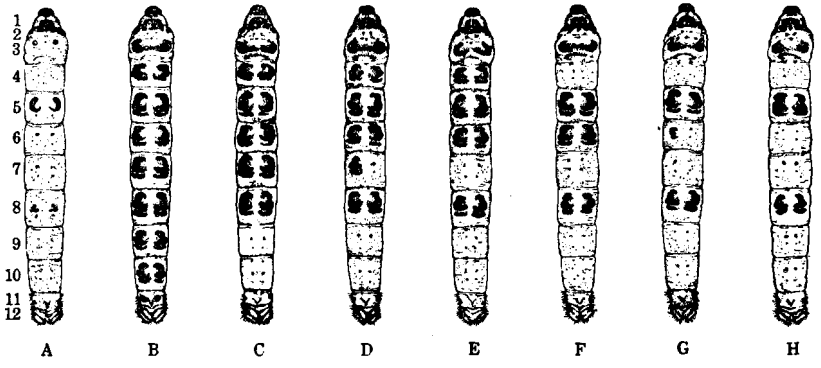


Fig. 1. Normal marking (A) and multilunar subtypes (B–H)

A: Normal; numerals designate consecutive segment numbers from 1st thoracic to 9th abdominal segments

B: L_{4-10} C: L_{4-8} D: $L_{4\cdot5\cdot6\cdot7\cdot8}$ E: $L_{4\cdot5\cdot6\cdot8}$ F: $L_{5\cdot6\cdot8}$
G: $L_{5\cdot\dot{6}\cdot8}$ H: $L_{5\cdot8}$

(c) Reciprocal crossing between $L_{5\cdot8}$ and the normal ($+^{L}$)

The normals used in this experiment were taken from the same batch as those used in (a). The result was practically the same as in the preceding experiment, namely, $L_{5\cdot8}$ made up only 3.2% of the F_1 of the cross $L_{5\cdot8}$♀ × $+^{L}$♂, and 3.6% of the F_1 of $+^{L}$♀ × $L_{5\cdot8}$♂, while the $L_{4\cdot5\cdot6\cdot8}$ subtype was reckoned as 61.5% and 46.6% respectively. There were observed 161 (86.1%) and 253 (75.5%) larvae provided with one or two brown spots on segment 4, in spite of the fact that they had been entirely absent in the multilunar parent. This may be accounted for by assuming that the modifier controlling the spots on segment 4 was introduced by the $+^{L}$ parent.

B) Reciprocal Crossing with a Multistar Strain

The multistar strain used was ms_{6-10}, and the normal $+^{ms}$ $+^{p}$ (p 22). As the multistar character is recessive to normal, the F_1's were largely

normal. The phenotypically normal marking occurred in 87.5% of the F_2 of the cross *ms* ♀×+^{ms}♂, and in 81.4% of the F_2 of the reciprocal cross, the rest being typical *ms* larvae. Possibly these normals include real normals (+^{ms}) and ms_8 individuals. These two types are phenotypically indistinguishable, as both are provided with a pair of star spots on segment 8. Distinction can be made only by breeding: real normals give only normal offspring, while ms_8 produces various *ms*-subtypes, beside ms_8, in later generations.

The variability in numbers of star spots was somewhat lower in the offspring of the cross *ms*♀×+^{ms}♂ than that in the offspring of the other cross.

C) Conclusion

In all the crossing experiments described above, the variability of the characteristic spots in F_1 or F_2 progeny was always higher in the case when the normal was the female parent than in its reciprocal cross. The data are still insufficient for deciding whether the difference is statistically significant or not.

2. *Spontaneous Recurrent Mutations of a Modifier of the Multistar Marking*

(Report by Yoshimaro TANAKA)

In batch No. 512p41, a single larva with a star spot on one side of segment 4 appeared. This was the first case of a larva with any star spot on the 4th segment in this pedigree through more than 30 generations.

Since then, a number of larvae of the same type were observed in each of the five subsequent generations, except 521ms11, as shown in Table 1.

Table 1. Spontaneous mutants appeared consecutively

Spot \ Batch No.	512p41	513p41	521ms11	522ms11	523ms11
($\overset{\circ}{4}$)	**238**	**105**	**511**	**35**	56(†)
($\dot{4}$)	1(†)	5*	0	5(†)	37(†)
(4)	0	0	0	1(†)	32(†)
Totals	239	110	511	41	125

* Laid no eggs

† Died in the larval or pupal stage

($\overset{\circ}{4}$) No star spot on segment 4

($\dot{4}$) One-sided spot on segement 4

(4) Paired spots on segment 4

The thick type shows the subtype from which the next generation was derived.

Notwithstanding the fact that each generation was derived from $(\mathring{4})$-type parents, $(\dot{4})$- and (4)-subtypes appeared repeatedly in four consecutive generations, and the frequency of mutants apparently increased gradually in later generations.

There seem to be two alternative interpretations possible in this case. First, a mutation pressure in the direction of $(\mathring{4})\rightarrow(\dot{4})\rightarrow(4)$ has gradually increased as the result of some unknown cause. Second, some polygenes causing the development of $(\mathring{4})$- and (4)-types have accidentally accumulated in this strain as a result of inbreeding. Which of these two interpretations is more appropriate cannot be decided for the present.

3. *Studies on the Semi-allelic E-series in the Silkworm*
(a) *On the relation between E^{H} and E^{Kp}*

(Report by Mitsuo TSUJITA)

As a result of heterozygosity of the E^{H} gene, $E^{H}/+$, extra-semilunar patterns appear on the 4th abdominal segment (Text-fig. A, B), while with heterozygosity of E^{Kp}, $E^{kp}/+$, extra-abdominal legs are formed on the 5th segment (Text-fig. C, D). The larvae homozygous for E^{Kp} have extra-semilunar patterns on the 6th segment, besides the above-mentioned extra abdominal legs. The larvae with the genotype E^{H}/E^{Kp} have a combination of the characters peculiar to E^{H} and E^{Kp}. It has been believed that these genes, as well as more than ten others, form a multiple-allelic series, and that no recombination occurs among them. The writer (1952), however, has found cases of recombination of E^{H} and E^{Kp}. The results of the experiments carried out during the past two years are summarized in the following:—

From the cross wild $(+)\times E^{H}/E^{Kp}$, a few $E^{H}E^{Kp}/++$ larvae invariably appear with the recombination value 0.7%. The larva with this genotype represents a new type characterized by having extra abdominal legs on the first and second abdominal segments, as well as extra semilunar patterns on the first abdominal segment (Text-fig. E, F). Thus, the genes E^{H} and E^{Kp} furnish a good example of the so-called position pseudo-allelism (Lewis 1952).

As reported last year, a small number of E^{H} and E^{Kp} larvae appeared in the cross $+\times E^{H}E^{Kp}/++$, with the recombination value also of 0.7%. From the reciprocal cross a very small number of individuals of the genotype $E^{H}/+$ and $E^{Kp}/+$ segregated. The fact that crossing-over occurs in the female suggests that there is some chromosomal aberration at the E locus of chromosome VI. In the sib-mating of $E^{H}E^{Kp}/++$, three types of individuals, i. e. $E^{H}E^{Kp}/E^{H}E^{Kp}$, $E^{H}E^{Kp}/++$ and $+$, segregated in the ratio of 1 : 2 : 1.

All of the F_1 individuals from $E^H E^{Kp}/E^H E^{Kp} \times +$ or from the reciprocal cross have extra abdominal legs on the first and second abdominal segments and extra semilunar patterns on the first abdominal segment.

The larvae homozygous for the genes $E^H E^{Kp}$ have supernumerary patterns on the 4th and 6th abdominal segments, and two pairs of abdominal legs

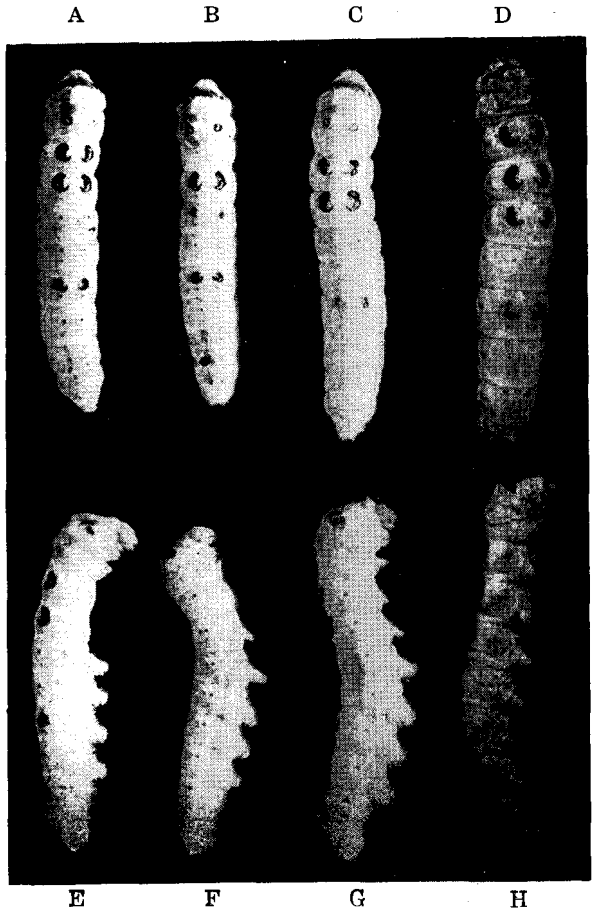


Fig. Dorsal and lateral views of larvae with the genotypes, $H/+$, $Kp/+$, $HKp/++$ and HKp/Hkp.
A: $H/+$, C: $Kp/+$, E: $HKp/++$, G: HKp/HKp (dorsal view)
B: $H/+$, D: $Kp/+$, F: $HKp/++$, H: HKp/HKp (lateral view)

on the 4th and 5th segments. The latter are so well developed that in newly hatched larvae they look like normal abdominal legs, although in the full-grown larvae they are usually smaller than normal legs (Text-figs G, H), because of the retardation of their growth. The claws of the

abdominal legs look almost normal, but they have little or no grasping power. The homozygous individuals are weaker than the heterozygotes, and the adult moths almost lack the ability to copulate; their legs are apparently paralyzed, and keep their proper posture with great difficulty. This behavior is similar to that of moths anaesthetized by DDT. Dissection of such moths has shown deformity in their posterior ganglia.

It is clear, from the facts mentioned above, that E^{H} and E^{Kp} are not real allelic genes. In order to make clear which of these genes occupies the terminal position in the chromosome, the recombination values between E^{H} and between E^{Kp} and *Nc* have been estimated; *Nc* is known to be at the locus 1.4 (Itikawa '48). However, I could not obtain clear-cut results, mainly because of the interference of gene *Nc* with the penetrance of E^{H} or E^{Kp}.

Under favorable conditions, for example at a temperature of 23–25°C. and humidity 75–78% in an ordinary rearing room, the eggs with the genotype $E^{H}E^{Kp}/++$, when treated with diluted hydrochloric acid, show a high hatching rate. Under less favorable conditions, for example at the high temperature of 27°C. and dry air with humidity 50–60% in thermostat, the same eggs show very low hatchability. Accordingly, in the F_1 of the cross $E^{H}E^{Kp}/++ \times ++$, the larvae heterozygous for $E^{H}E^{Kp}$ are much fewer in proportion than the normal larvae. We are now planning to apply this interesting finding to practical breeding.

(*b*) *On the relation between* E^{Ms} *and* E^{Mc}

(Report by Mitsuo TSUJITA and Bungo SAKAGUCHI)

The ancestor of the strain E^{Ms} was discovered as a mutant among the hybrids between Japanese No. 8. and Chinese No. 107. This mutant has duplicated star patterns on the 7th and 8th segments, or on the 8th and 9th segments; some individuals may have the patterns on all the 7th, 8th and 9th segments. The character is dominant. Embryos homozygous for this gene have supernumerary legs on the 10th and 11th segments (Text-fig. A), and they die in later embryonic stages or immediately after hatching. However, the penetrance of this gene for the supernumerary legs is low. The individuals hetrozygous for this gene have lower vitality than the normal ones, and they often die within the egg shell.

E^{Mc} is also dominant, and the larvae heterozygous for it are characterized by having duplicated crescent patterns on the dorsal side of the 5th and 6th segments, and lacking the star markings on the 8th segment. Embryos homozygous for this gene also die in a later stage. They are provided with thoracic setae on all segments, and are incapable of blastokinesis (Text-fig. B).

E^{Ns} develops neither star patterns on the 8th segment nor does it show any lethal effect.

E^{Ms} and E^{Mc} show a clear distinction from each other in the development

of the embryo. In the sib-mating of $E^{Ms}/+$ or from the cross $E^{Ms}\times$ normal or normal $\times E^{Ms}$, a very few larvae with the phenotype E^{Mc} or E^{Ns} appeared. The number of these unexpected types which have appeared during the last three years from 1950 to 1952, is as follows:

Table 1. Record* of appearance of E^{Mc} and E^{Ns} individuals in the batches of E^{Ms} or from the cross between E^{Ms} and the wild type

Rearing year and season	Mating	No.	+	E^{Ms}	E^{Ns}	E^{Mc}	Sum	$\frac{E^{Mc}+E^{Ns}}{\text{Sum}}\times 100$	Appearance rate of E^{Mc}
'50 Spring	$Ms\times +$	2	141	50	1	2	194	1.5%	1.0%
	$+\times Ms$	3	151	89	2	1	243	1.2	0.4
	〃	5	18	50	1	1	70	2.9	1.4
'50 Summer	$Ms\times Ms$	2	94	17		1	112		0.9
	〃	3S4	15	201		1	217		0.5
	〃	4S3	109	183		1	293		0.3
	$Ms\times +$	1	163	75		1	239		0.4
	〃	2	324	124		1	449		0.2
'51 Autumn	$Ms\times +$	3	87	167		1	255		0.4
	$+\times Ms$	2	251	101	1	1	354	0.6	0.3
'52 Summer	$+\times Ms$	2	49	32	2	2	85	4.7	2.4

* This is the record of the batches which produced the E^{Mc} individuals among many E^{Ms} batches reared in the last three years.

As shown in the table, the rate of appearance of E^{Ns} or E^{Mc} individuals varies from lot to lot.

E^{Ms} appears in the batches of E^{Mc} very rarely. Out of the many batches of E^{Mc} reared during last three years, 1950 to 1952, one E^{Ms} larva was obtained as shown in Table 2:

Table 2. Appearance of a single individual with the gene E^{Ms} in a batch of E^{Mc} from the cross $+\times E^{Mc}$

Rearing year and season	Mating	No.	+	E^{Ms}	E^{Mc}	Sum	Rate of E^{Ms} %
'52 Summer	$+\times Mc$	3	118	1	229	348	0.3

It is thus evident that the so-called *E*-series occupies not a point locus, but a section of chromosome of some extent, which has probably a complicated structure. Possibly this section consists of several genes with similar effects arranged in close sequence at the end of chromosome VI. According to this view, it seems valid to assume that E^{Mc} is derived from E^{Ms} by recombination, perhaps also with some position effect.

The question remains, however, as to why a change in the reverse direction, i. e. from E^{Mc} to E^{Ms}, is more difficult to obtain. This may be due to the fact that individuals heterozygous for this gene have lower

vitality than the normal individuals, and often die in the egg or in early larval stages.

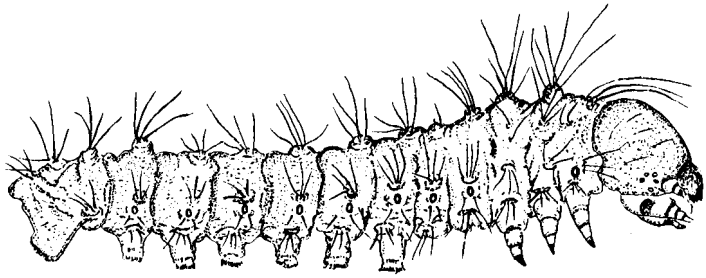


Fig. 1. Embryo homozygous for E^{Ms}

Fig. 2. Embryo homozygous for E^{Mc}

4. *Maternal Inheritance of "Lethal Yellow"*

(Report by Mitsuo TSUJITA)

The gene "*lem*^{l}" is recessive[1]. It has been known that embryos homozygous for this gene develop normally to black-colored young larvae and die immediately after the 1st moulting. In the cross $+/lem^{l} \times +/lem^{l}$ or $+/lem^{l} \times lem/lem^{l}$ the yellow lethal larvae can be recognized in the 2nd instar[2]. In the cross $lem/lem^{l} \times +/lem^{l}$ or $lem/lem^{l} \times lem/lem^{l}$, however, all homozygous lem^{l}/lem^{l} individuals die within the eggs which show through the semi-translucent shell the yellowish-brown dying larva. The maternal inheritance of "yellow lethal" is shown schematically in figure 1.

The yellow-colored lem^{l}/lem^{l} larvae immediately after the 1st moulting (E in fig. 1) cannot chew up mulberry leaves, because of incomplete hardening and imperfect differentiation of the mandibular cuticle, and starve

1. The designation "lem^{l}" corresponds to "ly" in my previous report (1951).
2. The three genes, +, *lem* and lem^{l} form a multi-allelic series with the dominant-recessive relations $+ > lem > lem^{l}$.

to death (UMEYA and TSUJITA 1951). The young *lem*^{l}/*lem*^{l} larvae developed from eggs laid by a *lem*/*lem*^{l} female moth (I in fig. 3) cannot even chew through the chorion. These larve, when liberated from the eggs by cutting

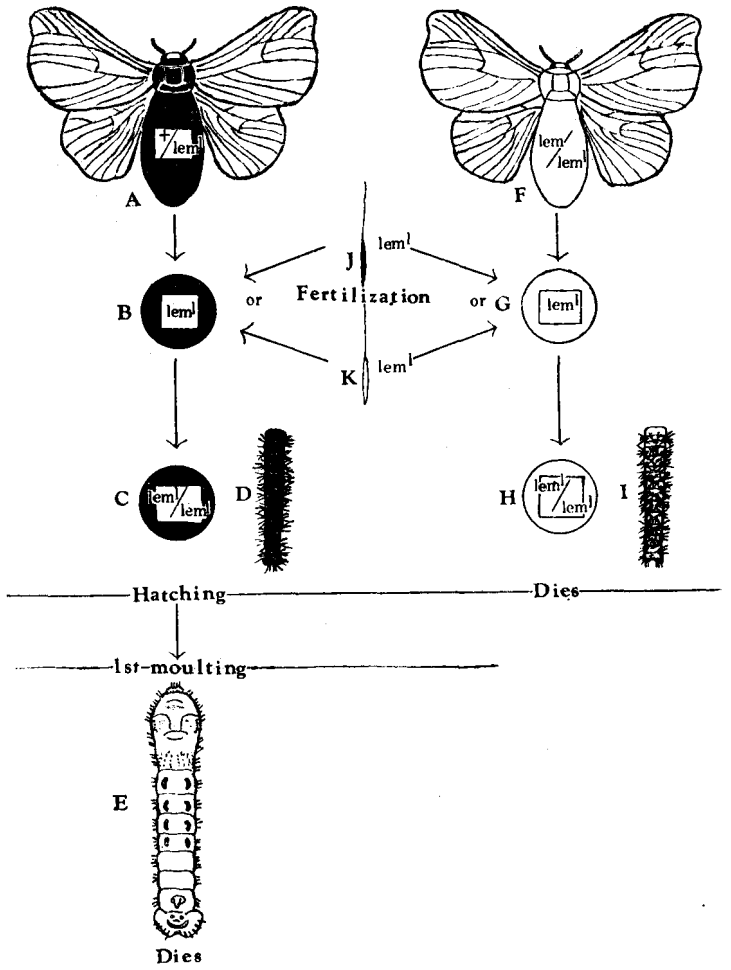


Fig. 3.

Fig. 3. Schematic representation of the maternal inheritance of "yellow lethal". On the left side: A, +/*lem*^{l} ♀ ; B, unfertilized *lem*^{l} egg laid by +/*lem*^{l} ♀ ; C, egg fertilized by a *lem*^{l} sperm; D, black young *lem*^{l}/*lem*^{l} larva; E, lethal yellow larva directly after 1st mouting. On the right side: F, *lem*/*lem*^{l} ♀ ; G, unfertilized *lem*^{l} egg laid by a *lem*/*lem*^{l} ♀ ; H, egg fertilized by a *lem*^{l} sperm; I, yellow young larva dying inside the egg. The two *lem*^{l} sperms produced by +/*lem*^{l} ♂ and *lem*/*lem*^{l} ♂ are shown in the middle. Black and white background indicates the difference between the ooplams derived from the two kinds of mothers and the difference between the sperm plasm derived from the two kinds of fathers (+/*lem*^{l} and *lem*/*lem*^{l}).

through the shell, show a normal appearance, and move about actively, but cannot feed on mulberry leaves and consequently starve to death. It is assumed that the cause of the death of the young larvae within the eggs is the same as in the case of the moulted lethal larvae, namely, incomplete hardening and imperfect differentiation of the cuticular layer of the hypodermis, especially that of the mandible.

The maternal inheritance of "yellow lethal" may be due to the presence or absence of some diffusible chemical substance, such as a precursor of pigment, in the ooplasm derived from the mother. It seems probable that there is some intimate relation between the production of the black (melanin) and yellow pigments, and between the formation of melanin and the hardening of the cuticular layer of the hypodermis. The young normal lem^l/lem^l larvae developed from the eggs of the $+/lem^l$ female (genotype w_1/w_1) are black, while the lem^l/lem^l larvae developed from the white eggs of the lem/lem^l female are distinctly yellow. This fact suggests that the formation of pigments such as melanin and xanthopterin-B is somehow related to the tryptophane metabolism.

In order to clarify this relation, biochemical experiments were carried out. The results presented in the next report were obtained last year.

Table 3. Comparison between 'lethal yellow' and 'red' larvae

Organs	Lethal yellow larvae	Red young larvae
Head color	yellowish brown	brown
Mandible	light yellowish-brown, brownish at the apical end	dark brown, deep dark brown at the apical end
Maxilla	yellow	dark brown
Ocelli	dark brown	dark brown
Labrum	yellowish brown	dark brown
Body surface	light yellowish brown, lst seg. yellow	reddish brown
Thoracic legs & abdominal legs	yellow	dark brown
Claws	light yellow, slightly brownish	dark brown
Stigmata	stigma ring and entrance to trachae yellowish brown	stigma ring black, entrance to trachae blackish brown
Setae	whitish	light brown

The lem^l/lem^l eggs (H in fig. 1) may be called "Lethal yellow eggs", They resemble the "red" strain not only in the external egg features in the pigmentation period, but also in the color of the young larva (I in fig. 1) inside the chorion, as shown in the table.

5. *Genetical and Biochemical Studies of Yellow Lethal Larvae*

(Report by Mitsuo TSUJITA and Bungo SAKAGUCHI)

Materials and methods:—Embryos of the normal (+) strain, larvae of the lethal yellow directly after the first moulting (designated as lem^l), embryos of the same strain dead within the egg (designated as lem^le) and w_1/w_1 larvae from white eggs (designated as $lem^le \cdot w^1$) were used as materials. The embryos or larvae were extracted in 80% methanol, 10–15% acetic acid, 10–20% alkali, or in other solvents. Paper-chromatographical analysis of the extracts obtained by centrifuging or by sucking filtration were used for the detection of pigments and amino acids.

As solvents, buthanol-acetic acid, phenol, sodium citrate and pyridine were used. Fluorescence analysis of pigments by ultra-violet rays, and amino acid detection by means of ninhydrin reaction were carried out.

All treatments, extraction, spreading and drying, were carried out in a dark room. Synthetic leucopterin, xanthopterin, isoxanthopterin and riboflavin were used as controls.

Experimental results:—Yellow pigments contained in the lem^l have a distinct spot near Rf 0.40 like the yellow pigment contained in the hypodermis of the lemon larva. When subjected to photolysis, this coloring matter has almost the same Rf value as xanthopterin, and turns blue. Moreover, its fluorescent color changes according to the change in pH value. From these facts it can be safely concluded that this pigment is xanthopterin-B.

Next, eggs of each strain in a later stage of incubation were dried, and 0.3 g. of the dry matter were extracted in 5 ml of 10% acetic acid or in 80% ethanol. The extracts, condensed to about 0.5 ml by the depression method, were examined to determine the relative amounts of isoxanthopterin and xanthopterin-B. The relative amounts of isoxanthoperin in these strains have been shown to be $+ > lem > lem^l > lem^le \cdot w_1$. The relative amounts of xanteopterin-B tended to be the reverse, i.e. $+ < lem < lem^l < lem^le \cdot w_1$.

The amount of xanthopterin-B is apparently greater in the lem^l/lem^l embryos developed in the white eggs (w_1). This fact indicates that the presence of kynurenin has some relation to the formation of xanthopterin-B.

A substance of unknown nature has been recognized in the eggs of lem^l and lem^le. This substance produces a faint bluish fluorescence near Rf 0.67.

In order to detect the various kinds of amino acids and their relative amounts in each material. 0.3 g. of eggs in the later incubation period were examined by the two-dimensional paper chromatogram method. About twenty different amino acids were detected, including tyrosine, proline, glutamic acid, glutamine, aspartic acid, cystenic acid, methionine, arginin, taurine and β-alanin. These amino acids showed differences in their relative amounts from strain to strain. Above all, the methionine reaction was rather weak in + and *lem* eggs, and very strong in *lem*^{l} and *lem*^{l}*e* eggs, especially in the latter. The significance of this finding is not clear as yet.

It is inferred, from the results described above, that the important factors which determine the differences among the materials. +, *lem*, *lem*^{l} and *lem*^{l}*e*, are the difference in relative quantities of xanthopterin-B, isoxanthopterin and substances related to these pigments.

We are planning to pursue further biochemical studies in these strains.

6. *Trisomic Silkworm Produced by Treatment with BHC Powder*

(Report by Mitsuo TSUJITA)

In a previous report (Annual Report No. 1 1950) the production of trisomic silkworms ($p^{S}/p^{M}/+^{P}$) by treatment with BHC powder was recorded. The trisomic nature of the six individuals obtained in this experiment has been deduced from the facts that in the F_1 of their cross (carried out in both directions) with wild, segregation occurred so as to produced four kinds of larval patterns, namely, wild, striped, moricaud and striped-moricaud, and also from the observation of a trivalent metaphase figure of the first spermatocyte division.

There remains, however, the question, why the segregation of the four types occurred in a ratio nearly conforming to 1 : 1 : 1 : 1, as shown in Table 1.

If disjunction of the chromosome in the trivalent had been normal, the striped larvae ($p^{S}/+^{P}$ and $p^{S}/+^{P}/+^{P}$) and the moricaud larvae ($p^{M}/+^{P}$ and $p^{M}/+^{P}/+^{P}$) should have been twice as numerous as either the striped-moricaud or the wild. This is not the case with the results shown in the table.

Another interpretation might be considered as plausible, namely, that a chromosomal fragment containing the locus p^{S} or p^{M} had been translocated to another chromosome. Further experiment, however, has shown that this is highly improbable.

In order to solve this problem, possible linkages between p^{S} and *Y*, or between p^{M} and *Y*, in the chromosomes of the strain in question were tested. Two strains with different genetic constitutions were obtained,

Table 1. Segregation in the cross $p^S/p^M/+^P \times +^P$ and its reciprocal

Mating type	No.	Wild	Striped	Moricaud	Striped Moricaud	Total
$p^S/p^M/+^P \times +^P$	1	109	81	91	100	381
	2	134	114	149	120	517
	3	117	74	114	96	401
	4	108	84	143	119	454
	5	110	84	102	94	390
	6	47	43	95	49	198
	7	93	108	85	72	358
	Total	718	588	743	650	2699
$+^P \times p^S/p^M/+^P$	8	92	104	87	95	378
	9	108	87	117	97	409
	10	129	107	124	118	478
	Total	329	298	328	310	1265

$p^S Y/p^M y/+^P y$ and $p^S y/p^M y/+^P y$. The difference between these two strains consists only in the location of Y on the chromosome p^S or p^M. From the experimental results presented in Table 2, complete linkage in the female has been confirmed either between p^S and Y, or between p^M and Y or between $+^P$ and y (Tables 2–3).

Table 2. Segregation in the cross $p^S Y/p^M y/+^P y \times +^P y$

No.	$+^P y$	$p^S Y$	$p^M y$	$p^S p^M Y$	Total
1	86	81	98	85	350
2	86	88	92	91	357
3	96	113	79	67	357
4	86	80	73	86	325
5	109	93	76	63	341
6	62	70	73	69	274
Total	525	525	491	461	2002

Table 3. Segregation in the cross $+^P y \times p^S y/p^M Y/+^P y$

No.	$+^P y$	$p^S Y$	$p^M Y$	$p^S p^M Y$	Total
1	137	112	103	111	463
2	101	102	98	105	406
3	125	121	118	123	487
Total	363	335	319	339	1356

The recombination value is very similar in the male either between $p^S Y$, $p^M y$, and $+^P y$, for the individuals with the genotype $p^S Y/p^M y/+^P y$ or between $p^S y$, $p^M Y$, and $+^P y$ for the individuals, with the genotype $p^S y/p^M Y/+^P y$, being 1.56 in the former and 1.77% in the latter (Tables 4–5).

Table 4. Segregation in the cross $+^P y \times p^S Y/p^M y/+^P y$

No.	$+^P$		p^S		p^M		$p^S p^M$		Total	Recombination value
	y	Y	y	Y	y	Y	y	Y		
1	78	0	1	79	80	1	0	71	310	0.65
2	63	0	3	65	70	2	0	75	278	1.80
3	83	1	1	70	76	3	1	67	302	1.99
4	76	1	3	70	62	1	0	64	277	1.81
Total	300	2	8	284	288	7	1	277	1167	1.56

Table 5. Segregation in the cross $+^P y \times p^S y/p^M Y/+^P y$

No.	$+^P$		p^S		p^M		$p^S p^M$		Total	Recombination value
	y	Y	y	Y	y	Y	y	Y		
1	130	3	105	2	5	115	0	109	469	2.13
2	101	1	112	2	3	102	0	108	429	1.40
Total	231	4	217	4	8	217	0	217	898	1.77

These values are much lower than the normal recombination value between p^S and Y, namely, 25.6%.

The recombination value in F_1 either between p^S and Y, in the individuals with the genotype $p^S Y/+^P y$, or between p^M and Y in the individuals $p^M Y/+^P y$, was examined in the cross between the trisomic and wild. Complete linkage was found both between p^S and Y and between p^M and Y (Tables 6–7).

Table 6. Segregation in the cross $p^S Y \times +^P$

No.	$p^S Y$	$+^P y$	Total
1	205	198	403
2	114	95	209
3	156	161	317
Total	475	454	929

Table 7. Segregation in the cross $p^M Y \times +^P$

No.	$p^M Y$	$+^P y$	Total
1	216	208	424
2	183	205	388
3	205	218	423
Total	604	631	1235

In the two reciprocal crosses the recombination value was 1.67 and 1.80% respectively (Tables 8-9).

Table 8. Segregation in the cross $+^P y \times p^S Y$

No.	$p^S Y$	$p^S y$	$+^P Y$	$+^P y$	Total	Recombination value
1	162	4	3	189	358	1.96
2	183	3	5	192	383	2.09
3	220	2	2	189	413	0.97
Total	565	9	10	570	1154	1.67

Table 9. Segregation in the cross $+^P y \times p^M Y$

No.	$p^M Y$	$p^M y$	$+^P y$	$+^P Y$	Total	Recombination value
1	156	5	175	3	339	2.36
2	183	4	199	2	388	1.55
3	149	8	180	2	339	2.95
4	209	1	197	2	409	0.73
5	115	0	136	2	253	0.79
6	180	7	210	3	400	2.50
7	179	5	227	2	413	1.69
Total	1171	30	1324	16	2541	1.80

The recombination value thus obtained is similar to that found for the trivalent; it is considerably lower than the value in normal bivalents.

From cross $p^S Y/+^P y \times p^M Y/+^P y$, individuals with the genotype $p^S Y/p^M Y$ were obtained. In the F_1 hybrid wild $(+^P) \times p^S Y/p^M Y$ or $p^S Y/p^M Y \times$ wild $(+^P)$ only p^S and p^M individuals segregated, as shown in Tables 10-11.

Table 10. $p^S Y/p^M Y \times +^P$

No.	$p^S Y$	$p^M Y$	Total
1	218	225	443
2	89	83	172
3	115	121	236
Total	422	429	851

Table 11. $+^P \times p^S Y/p^M Y$

$p^S Y$	$p^M Y$	Total
86	75	161

From the experimental results presented above, it can safely be concluded that the strains in question are true trisomics. The observation that nearly a 1 : 1 : 1 : 1 ratio was obtained for wild : striped: moricaud : striped-moricaud can be explained as follows:—The segregation of the trivalent chromosomes, $p^S/p^M/+^P$, can occur in the three different ways, $p^S p^M \leftrightarrow +^P$, $p^S +^P \leftrightarrow p^M$ and $p^M +^P \leftrightarrow p^S$. If the segregation mentioned first occurred more frequently than the other two, and nearly twice as often as the last, the experimental results can be understood. It is possible that there is an intimate connection between the abnormal segregation of the trivalent chromosomes and the factor inhibiting the crossing over between $p^S - Y$ or $p^M - Y$.

7. *Genetical and Biochemical Studies in the Eri-silkworm*

a. *On the nature of the pigments in the epidermal tissues of the Eri-silkworm*

(Report by Bungo SAKAGUCHI)

Genetical and biochemical examinations of the yellow pigments contained in the larval epidermal cells of the yellow strain were made, as follows:—(1) The pigments contained in the integument in young larvae and the pigments in the hypodermal cells of the head of full-grown larvae were analyzed by means of paper-chromatography. Petroleum ether extracts of the integument and the hypodermal cells of the heads were prepared. The extracts were observed with filter paper (Tôyô No. 50, 45–50 cm long and 15–18 cm wide) and a linear chromatograph, about 10 cm in length, was formed on the filter paper on the level of about 10 cm.

The solvent was washed first in sulphuric acid, and then in distilled water. Carotinoid pigments from the yellow cocoon of the silkworm were used as controls.

The analysis has shown the Rf value for light reddish-orange yellow to be 0.3, for orange-yellow pigment 0.7 and for yellow pigment 0.95. These spots are often indistinct, as the sample is diffusible.

To confirm the results thus obtained, column chromatography was used. The absorbents were deactivated calcium carbonate and deactivated alu-

mina (Merck);* the former was used for the upper fraction and the latter for the lower fraction in the absorption tube.

The pigment of the sample was extracted by petroleum ether into an absorption tube; then the chromatogram was taken by petroleum ether. The chromatogram showed various yellow zones at the calcium carbonate fraction, and an orange yellow zone at the alumina fraction. After desolved from each of these colored zones by carbon disulfide, these pigments were reacted by conc. H_2SO_4, conc. HCl, Fe_2Cl_6 and $ZnCl_2$. These results were presented by the specific coloring reaction of carotinoid pigments.

It seems clear from these results that the pigments were composed of xanthophyll and carotin, which belong to the carotinoid pigments.

(2) After the yellow pigments from the integuments of young larvae had been nearly completely extracted into distilled water or diluted acetic acid (10–20%), the extract was analyzed by paper chromatography; a buthanol-acetic-water mixture (in the ratio 4 : 1 : 1) and a pyridine 0.1% ammonia mixture (in the ratio 75 : 15) were used as solvents. After the development of the paper chromatogram, the paper was dried and examined for fluorescence by ultra-violet rays. As a control of this experiment, synthetic leucopterin, xanthopterin, isoxanthopterin and riboflavin were used. The results of this analysis are shown in the following table:

Table. Results of paper-chromatographical analysis of pigments soluble in water or in dilute acetic acid

Name of substance	Leucopterin	Isoxanthopterin		Riboflavin	Xanthopterin
Rf value	0.10	0.23		0.35	0.38
Buthanol-acetic acid (solvent) skin & head	◍ Blue	◎ Purple		○ Yellow	◉ Sky-blue
Rf value	0.11	0.23	0.70	0.91	
Pyridine (solvent) skin & head	◍ Pale blue	◎Dilute purple	◎ Purple	○ Yellow	

The table shows that the hypodermal cells of Eri-silkworm larvae contain leucopterin, isoxanthopterin, riboflavin and xanthopterin, and that, when buthanol-acetic acid is used as solvent, the spot of isoxanthopterin appears at a single point at Rf 0.23, but when pyridine is used as solvent, spots appear at two points, at Rf 0.23 and 0.70. These observations suggest that the coloring matter is composed of two kinds of pigments, of which one is perhaps isoxanthopterin and the other a derivative of iso-

* oxide of aluminium

xanthopterin.

Comparing these results obtained in the Eri-silkworm with the results obtained in the silkworm, little difference is found for pterin pigments, but a considerable difference is apparent for carotinoid pigments. Thus, in some (but not all) strains of silkworm, the carotinoid pigments contained in mulberry leaves are able to pass through the mid-gut epithelium. Moreover, the pigments contained in the blood can pass through the silk gland epithelium in some of these strains. However, carotinoid pigments have never been found to pass through the epidermal cells of the silkworm.

In the strain of Eri-silkworm used in this experiment, it has been confirmed that carotinoid pigments can pass through the mid-gut epithelium into the blood, and that the pigments in the blood cannot pass through the silk-gland cells, while they can pass through the epidermal cells.

Thus, there is a marked difference between the silkworm and the Eri-silkworm in the permeability of the tissue epithelia to carotinoid pigments. Since the permeability of the epithelial cells is probably controlled by gene action, the facts found in this experiment suggest some interesting problems regarding the permeability of the epithelia of various organs to pigments.

E. CYTOLOGY OF SOME INSECTS

(Report by Tosihide H. YOSIDA)

1. *Cytological Study on the Racial Hybrids of Philosamia cynthia (Lepidoptera, Saturnidae)*

Philosamia cynthia is classified into three subspecies, *P.c. Walkeri*, *P.c. Pryeri* and *P. c. ricini*. *Walkeri* is distributed in China, Manchuria and Korea, *Pryeri* in Japan and *ricini* in the East Indies; Walkeri has some local races. The present paper deals with cytological investigations of the hybrids among these subspecies and races. The materials were derived from the following sources:—*Walkeri* from Manchuria (designated as M) and Korea (C), *Pryeri* from Kyushu (K) and *ricini* from stock which had been kept in Formosa (E).

The chromosome numbers in M and C of *Walkeri* are $2n=26$ and $n=13$, and the chromosomes of these races are nearly identical in general appearance. K and E have the chromosome number: $2n=28$, $n=14$, and there is little difference between the subspecies in the shape of the chromosomes.

The hybrid C×M shows a karyotype $2n=26$, $n=13$, and cannot be distinguished from that of either race C or M of *Walkeri*. All the hybrids C×K, C×E, E×M, and M×E show similar karyotypes consisting of $2n=27$, $n=13$ (=12 bivalents+1 trivalent). The hybrid M×K shows a

peculiar tetrad formation, namely, 2n=27, n=12 (=10 bivalents+1 trivalents+1 tetravalent).

From observations of the tetrad chromosomes of the F_1 hybrids, it has been surmised that the races having the chromosome set $2n=28$, $n=14$ were derived from a race having $2n=26$, $n=13$ by duplication of one of the chromosomes. Also, M and K have a reciprocal translocation between non-homologous chromosomes. Thus, the differentiation of the three subspecies of *Philosamia cynthia* is due to duplication and translocation of some chromosomes. The geographic races C and M of *Walkeri* have been differentiated presumably by the presence or absence of this reciprocal translocation.

2. *Multiple Sex-chromosome Mechanism in Rhaphidopalpa femoralis* (*Coleoptera, Chrysomelidae*)

The chromosome number of *Raphidopalpa femoralis* has been determined by the author (1952). The present paper deals with the sex chromosome mechanism in this insect. The chromosomes in the first spermatocyte are 29 in number. Among the second spermatocytes there are two kinds of cells, one having 30 and the other 29 chromosomes. The chromosome set in the first spermatocyte consists of a very large V-shaped chromosome and 28 ordinary V- or rod-shaped chromosomes. The large V is a trivalent consisting of one small and two large elements, as is clearly seen in the side view of the metaphase of the first division. At anaphase the large components usually migrate to one pole, while the small component goes to the opposite pole. In the second spermatocyte there are observed two kinds of cells as expected, one having 30 monads and the other having 29 monads. The former cell contains two large elements and 28 ordinary elements, and the latter one small element and 28 ordinary elements. It is obvious that the two large components are X_1 and X_2, the small one is Y, and the 28 ordinary elements are autosomes.

The chromosomes of insects belonging to Chrysomeridae have been observed by many workers, such as HENKING, NOWLIN, SMITH, STEVENS, WIEMAN and YOSIDA. Most of the species of this family have haploid chromosome numbers from 10 to 17 (cf. " A review of the chromosome numbers in animals " by MAKINO 1950). *Rhaphidopalpa femoralis*, however, has 29 chromosomes in the haploid nucleus, suggesting tetraploidy. In this connection it is noticeable that the X-chromosome in this species is duplicated. The author (1944-1952) has found, by comparison of the chromosome sets in some species of Coleoptera and Hemiptera, that the ratio of the total chromatin volume of the sex chromosomes to that of the autosomes is nearly constant. The present study in *Rhaphidopalpa* gives another instance confirming this general rule.

F. POPULATION GENETICS OF SOME BUTTERFLIES AND A LAND-SNAIL

(Report by Taku KOMAI)

1. *The Butterflies Colias and Neozephyrus*

The paper recording the findings of the writer and his co-worker AÉ on the genetics of the Pierid butterfly *Colias hyale poliographus* has been published in 'Genetics', 38, No. 1 (1953). The manuscript of the other paper by the writer dealing with the population genetics of the Lycaenid butterfly *Neozephyrus taxila* is ready for publication. This paper presents the writer's observations on thirteen population samples of this polymorphic butterfly obtained from twelve localities in Honshû and one in Hokkaido. The essentials of the findings have already been presented (The Annal Report, No. 2).

2. *The Land-snail Bradybaena*

During this year, as in the previous one, many specimens of the polymorphic land-snail, *Bradybaena similaris*, were obtained from a locality in Nagasaki. The sample from this locality includes the type banded-brownish in larger proportion than that from any other locality. Laboratory experiments are under way to test whether the genes for bandedness and brownish color are allelic in the strict sense, or semi-allelic. Also, it is being examined whether there is any evidence of a differential viability among the four marking- and color types of this snail.

G. GENETICS OF SOME CEREALS

1. *Irradiation Experiments with X-rays in Einkorn Wheat*

(Reported by Seiji MATSUMURA)

As to the relation between the frequency of chromosome aberrations in the PMC's of *Triticum monococcum* and the dose or wave length of X-rays, the results of experiments performed last year have been confirmed. The data are shown in Table 1. The frequency of ears with chromosome aberrations increases with X-ray dose, but not in a linear relation. It is also fairly certain that the shorter the wave length of X-rays, the higher the frequency of chromosome aberration. These facts can be explaind on the basis of the difference in the distribution of ionization within the nuclei and the chromosomes. When irradiated by hard X-rays, the majority of the resulting ionization is more scattered than in the case where soft X-rays are

used. The chromosome breaks induced by hard rays are scattered here and there on the same chromosome or on different chromosomes, so that union of broken ends of different origin, or interchange, takes place more easily than in the case with soft-ray irradiation, which produces more closely adjacent breaks, and is liable to give a high proportion of restitution.

Table 1. Relation between dosage or wave length of X-rays and frequency of chromosome aberrations in *T. monococcum*

Voltage (KVP)	Dosage (*r*)	No. of observed ears	No. of ears with aberrations				No. of aberrations (%)
			$6_{II}+2_{I}$	④+5_{II}	⑥+4_{II}*	④+④+3_{II}*	
Control	—	19	—	—	—	—	0 (0.00)
180	5,400	120	1	6	—	—	7 (5.83)
180	8,100	101	—	8	1	2	14 (13.86)
180	13,500	93	—	28	2	—	32 (34.41)
130	8,100	109	1	14	—	—	15 (13.76)
80	8,100	116	1	5	—	—	6 (5.17)

* Counted as two aberrations.

Many mutants were chlorophyll abnormalities: albinistic, yellow, virescent, striped, etc. There also appeared other mutations, such as: dwarf, twisted, short-awned, narrow-leaf, early and giant. Most of these mutants behaved like simple Mendelian recessives. Of these, the early and giant mutants could be used for breeding purposes. The mutation rate of the X_2-generation seems to depend not only upon the radiation dosage but also upon the wave length.

2. *Effect of Ultra Short Wave Irradiation on Einkorn Wheat*

(Reported by Seiji MATSUMURA and Taro FUJII)

Dormant seeds of *Triticum monococcum* were exposed to ultra short electric waves. Impulse waves were applied (wave length 10m) to remove the heat effect, at 5,100v, 10mA current and about 8cm distance. Few chromosomal irregularities were observed in the meiosis of the treated plants (S_1-generation). Among the progeny (S_2) obtained by selfing of the S_1-plants, however, there were found some dwarf plants, which were about half as tall as the normal plants and highly sterile. In the progeny (S_3) of the sister plants of these dwarfs, again some sterile dwarfs appeared, but their height and fertility were somewhat improved. Crossing of these dwarfs with *T. monococcum* and *aegilopoides* was unsuccessful. In the S_4-generation recovery of fertility and plant height advance considerably

further, and in the S_5-generation, at last, no dwarfs could be found. The mechanism of these phenomena awaits further investigation.

On the other hand, after the treatment of seeds of the same species with supersonic waves, chlorophyll mutants such as albino were observed.

3. *Cytogenetic Studies of Wheats and their Relatives*

(Reported by Seiji MATSUMURA)

Nullisomic dwarfs and tetrasomic giants in their offspring Dr. SEARS has been studying crosses between his own nullisomics in *Triticum vulgare* and the author's a~g-dwarfs. The latter are also nullisomics which were originated from the offspring of pentaploid wheat hybrids, i.e. they were deficient in a chromosome pair from the D-genome. SEARS' results have shown that Nulli-XVI corresponds with my g-dwarf. The author's crossing experiments, however, have revealed that chromosome XVI could correspond to no other than the f-chromosome, as already reported (comp. Ann. Rep. No. 2, p. 25). To solve this descrepancy between our findings, a new Nulli-XVI kindly sent by Dr. SEARS has been pollinated with the a~g-dwarfs. From the cytogenetic study of these hybrids it has been confirmed again that Nulli-XVI corresponds with the f-dwarf.

The a~g-dwarfs have been crossed with *T. compactum*, in order to decide whether or not the compact gene *C* is located in the D-genome. Also SEARS' nullisomics have been used as one of the parents in crosses which attempt to find out what chromosomes are involved in the supernumerary pair of the tetrasomic giants which were originated from the offspring of the a~g-dwarfs.

Agropyrum, a genus related to Triticum Among the hybridization experiments between *Triticum* spp. and *Agropyrum* spp., the cross between *T. vulgare* (AABBDD) and *A. glaucum* (BBEEFF) has been successful. Many *Agropyrum* species have been collected from several districts in Japan and from various foreign countries. Seeds of *A. triticeum*, an annual plant having 14 somatic chromosomes, have been treated by colchicine, to obtain autotetraploids. The author is sceptical about the hypothesis of MCFADDEN and SEARS, that *A. triticeum* should have the BB-genomes.

H. GENETICS AND CYTOLOGY OF *NICOTIANA*

1. *Cytogenetical Problems in the Genus Nicotiana*

(Report F. A. LILIENFELD)

The hybrid *N. sylvestris*×*N. tomentosiformis* and its amphidiploid were investigated. At I. M. in the P.M.C.'s of the hybrid 0–5 very loosely

connected bivalents are formed. The univalents show a pronounced tendency to ward concentrating near the poles, where most of them undergo the further meiotic development and become included, without division, into the respective daughter nuclei. A small number of univalents approach the equatorial plate; these are often divided, and the halves move toward the opposite poles. Non-reduction is not seldom. The hybrid is quite sterile, but the pollen showed a small percentage of unreduced, large, healthy grains (1–2%). Their number was very variable; at the end of the flowering period almost none at all were observed. The amphidiploid showed very good chromosome pairing, except for a rather frequent occurrence of 1–2 univalents and a single tri- or tetravalent. In spite of that it was, on the female side, completely sterile. The pollen was highly fertile (86–92% good grains), and the single amphidiploid plants showed an unchanging percentage of good pollen throughout the whole flowering season. Seedlings obtained from a pollination of the variety Dixie Bright with pollen of the amphidiploid were markedly stronger than those obtained from selfed Dixie Bright. These findings are, in general, in accord with the reports of other authors.

In order to explain the puzzling female sterility of the amphidiploid, some time ago GREENLEAF postulated several complementary genes which act, when brought together in the amphidiploid, as female-sterility factors. In a kind of *experimentum crucis*, a series of crosses have been undertaken, in the attempt to produce female-sterile *sylvestris* and *tomentosiformis* and in search of a linkage between GREENLEAF's putative sterility genes and other genes.

A number of crosses were carried out between other diploid species, with the object in view of producing new amphidiploids and testing them for their usefulness as breeding material.

2. *Irradiation Experiments with X-rays in Nicotiana*

(Reported by Seiji MATSUMURA and Taro FUJII)

Dormant seeds of *Nicotiana sylvestris* ($n=12$) and *N. tabacum* ($n=24$, Bright Yellow and Dixie Bright 101) were exposed to hard X-ray irradiation, which was applied at 180KVP, 3mA, without filter. The dosage was 5,000–50,000*r*. At more than 15,000*r* the irradiated seeds of *N. sylvestris* did not germinate at all, and at 15,000*r* the germination rate in this species was markedly reduced. In *N. tabacum*, Bright Yellow showed higher tolerance against X-rays than Dixie Bright. The higher the dosage above 15,000*r* the more delayed and uneven were the germination of treated seeds, as well as the growth of the seedlings. At the highest dosage, the germination rate of the seeds was reduced, especially in Dixie

Bright, from 78.75% in the untreated seeds to 6.00%.

N. sylvestris has usually 12_{II} in the PMC's. In most of the cases of induced chromosome aberrations found in the treated X_1-plants, single translocation ($1_{IV}+10_{II}$), seldom $11_{II}+1_I$, $11_{II}+1_I$+fr., or asynaptic configurations have been observed. In *N. tabacum* rare occurrence of $23_{II}+2_I$ and 24_{II}+fr., besides $(1\text{–}3)_{IV}+(22\text{–}18)_{II}$ caused by 1–3 translocations, has been observed. Such induced aberrations also occurred more frequently in Dixie Bright than in Bright Yellow. The frequency of chromosome aberrations, on the whole, increased in a linear relation to the X-ray dosage. In two or three inflorescences of the same plant, identical chromosome aberrations were always observed.

Among the X_1-plants from seeds irradiated at 15,000*r* and at higher dosage, various kinds of morphological abnormalities appeared, such as narrow, shrivelled and variegated leaf. These characters seem to originate from plasmic abnormalities. In the X_2-generation many recessive mutants were observed, such as small round, mottled, yellowish green, yellow petiole, etc.

From these results it is assumed that adequate dosage of X-rays for radiogenetical study on induced mutations in tobacco is 15,000–30,000*r*.

3. *Cytogenetic Studies on the Genus Nicotiana* IV.

(Report by Yô TAKENAKA)

a) Reduction divisions in hybrids between N. glauca and the N. alata group.

The three species, *N. alata* (n=9), *N. longiflora* (n=10) and *N. plumbaginifolia* (n=10), were crossed each with *N. glauca* (n=12). The crosses *N. glauca* × *N. alata* and *N. glauca* × *N. plumbaginifolia* gave some seeds, while no seed was obtained from the cross *N. glauca* × *N. longiflora*. Of the reciprocal crosses, *N. alata* × *N. glauca* gave no seed, but *N. longiflora* × *N. glauca* produced some germinating seeds.

The external characters of the hybrids which were produced from the seeds of *N. glauca* × *N. alata* and *N. glauca* × *N. plumbaginifolia* agreed with Kostoff's description of those of the same hybrids. No report of the hybrid *N. longiflora* × *N. glauca* has been published, so far as I know. The features of this hybrid were in agreement with Kostoff's description of those of the hybrid of the reciprocal cross.

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

At the first metaphase in the hybrid *N. glauca* × *N. plumbaginifolia*, 0–4 bivalents were counted with the mode at 2_{II}.

In the hybrid *N. glauca* × *N. alata*, 0–7 bivalents were observed, and the most frequent configurations found were $3_{II}+15_{I}$ and $4_{II}+13_{I}$.

In the hybrid *N. longiflora* × *N. glauca*, the number of bivalents was 1–6 and the typical configuration of the chromosome complement was $6_{II}+10_{I}$, although the frequency of this combination was low in proportion among the observed PMC's since polyvalents ranging from tri- to octavalents were often found. The chromosomes of this plant tended to be sticky at meiosis, and the divisions were more irregular than those in the above two hybrids. Such remarkable irregularities were not found in the reciprocal cross made by Kostoff, or in the hybrid *N. glauca* × *N. plumbaginifolia* according to Kostoff and Goodspeed, in which the paternal parent was taxonomically closely related to *N. longiflora*.

These irregularities may be due to the difference in the degree of affinity between the cytoplasm of the maternal plant, *N. longiflora*, and the chromosomes of the paternal plant, *N. glauca*, which was probably lower in this direction of cross than in the reciprocal direction.

The above findings about the three hybrids suggest the presence of a few partially homologous chromosomes between *N. glauca* on one side and *N. alata*, *N. plumbaginifolia* or *N. longiflora* on the other. Moreover, a few chromatids or chromosomes in diplotene and diakinesis showed end-to-end junctions, and anaphasic chromosome bridges were always observed.

b) Reduction divisions in hybrids between N. tabacum and three other species.

The external characters of the hybrids *N. tabacun* (n=24)×*N. sylvestris* (n=12), *N. tabacum* × *N. glauca* (n=12) and *N. glutinosa* (n=12) × *N. tabacum* agreed exactly with the description of Kostoff and many other previous investigators.

At first metaphase in the PMC's of *N. tabacum* × *N. sylvestris*, the most frequently observed chromosome configuration was $3_{III}+9_{II}+9_{I}$. This observation largely agrees with Kostoff's. The meiotic figure mentioned above suggests the presence of three pairing chromosomes between two *sylvestris* genomes and the *tomentosa* genome.

At first metaphase in the PMC's of *N. tabacum* × *N. glauca*, the chromosome conjugation types were $0\text{–}4_{III}$, $3\text{–}10_{II}$ and $12\text{–}24_{I}$. The former two types suggest the presence of several homologous or semi-homologous chromosomes between the *glauca* genome and the two subgenomes of *tabacum*. The chromosome behaviour at first metaphase largely agreed with the observations of Kostoff and also of Sarana, while the omission of the second division found by the present author is not pointed out by the previous investigators.

In the PMC's of the hybrid *N. glutinosa* × *N. tabacum*, 1–7 bivalents

were observed with four bivalents as the mode. This is in agreements with the observations of Kostoff and also of Müntzing. Accordingly, beside the intersubgenomatic chromosome conjugation in *N. tabacum*, one or more semihomologous chromosomes seem to occur between the *glutinosa* genome and the *tabacum* subgenomes.

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

Reciprocal crosses between *N. suaveolens* (n=16) and four other species, *N. gossei* (n=18), *N. alata* (n=9), *N. longiflora* (n=10) and *N. plumbaginifolia* (n=10), were carried out. The reciprocal crosses between *N. suaveolens* and *N. gossei* gave always many germinating seeds, while the other six crosses did not produce any seeds, with the exception of *N. suaveolens* × *N. longifolra* and *N. suaveolens* × *N. plumbaginifolia*.

In the meiosis of the PMC's of the hybrid *N. suaveolens*×*N. gossei*, the chromosome configurations most frequently found were of the following three types, $2_{III}+14_{II}$, $1_{III}+15_{II}+1_{I}$ and $16_{II}+2_{I}$. Besides, several polyvalents were occasionally observed, and many chromosome bridges were seen at first anaphase. This suggests the presence of some inversions or translocations between the chromosomes of *N. suaveolens* and those of *N. gossei*.

The study of meiosis in the PMC's of the two hybrids, *N. suaveolens*× *N. longiflora* and *N. suaveolens* × *N. plumbaginifolia*, agreed very well with Kostoff's observations, the number of bivalents in the former being 0–3 and in the latter 0–4. Since the chromosome configuration $1_{II}+24_{I}$ was mostly observed in the above two hybrids, it seems that one pair of chromosomes with long homologous parts in common is present among the chromosomes of *N. suaveolens* and *N. longiflora* or *N. plumbaginifolia*.

In the hybrid *N. suaveolens* × *N. longiflora*, the cells in the tapetum tissue are considerably enlarged at an early stage of meiosis, and almost all PMC's have degenerated and disintegrated befere the end of the reduction division, although the cause of this change is entirely unknown.

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

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

Genetic analysis of the mid-rib proportion and the shape of leaf in the tobacco plant was conducted on the hybrids between the varieties White Stem Orinoco and Holmes of *Nicotiana tabacum* L. It has been shown in the 1951 experiment that the leaves in White Stem Orinoco are rather elongate and slender and have a high mid-rib proportion, while Holmes

has rather round leaves and low mid-rib proportion. Means and variances of these characters have been determined in the P_1, P_2, F_1, F_2, and two backcross populations, and are presented in the table.

	Mid-rib proportion**		Leaf shape	
	Mean (%)	Variance	Mean (%)	Variance
White Stem Orinoco	38.35	3.4410	38.74	10.1410
Holmes	25.69	2.7093	63.56	14.2227
F_1*	29.90	—	63.85	—
$F_1 \times$ W.S.O.	33.88	7.6176	47.82	47.4962
$F_1 \times$ Holmes	28.10	7.4638	58.75	57.4716
F_2	30.40	14.3868	53.20	85.7460

*) The variance of F_1 hybrid has not been determined because of an accidental fault in the course of cultivation.

**) The mid-rib proportion in the table was measured in fresh leaves, since it has been shown in the 1951 experiment that the correlation of the characters between fresh and dry leaf is extraordinarily high.

On the basis of such determination, the variance due to additive genetic effect (D), that due to dominance effect (H), and that due to environmental effect (E) were calculated, and the heritability and the number of effective factors were estimated for each character. The partitioning of such components of variance was made according to Mather.

	Mid-rib proportion	Leaf shape
D	27.3844	133.0484
H	-9.4856	28.5040
E	3.0660	12.0958
Heritability in the broad sense	0.787	0.859
Heritability in the narrow sense	—	0.776
Number of effective factors	—	1.15

The H value for the mid-rib proportion in the table is found to be negative, suggesting a possible occurrence of linkage between effective factors, or else, of some unknown influence of circumstances. It seems necessary, therefore, to make further experiments with the F_3 population before we come to any definite conclusion with regard to heritability in the narrow sense and the estimation of the number of effective factors for that character.

It may safely be inferred that of the genes controlling the mid-rib proportion, those for lower proportion behave as partially dominant, and of

the genes controlling leaf-shape, the genes for round leaves show complete dominance over those for elongate leaves.

The total and genetic correlation coefficients between mid-rib proportion and leaf shape were obtained for the F_2 population, as shown below:

Total correlation coefficient.....—0.52*
Genetic correlation coefficient.....—0.57

*) This value exceeds the 1% level of significance.

It has thus been found that the correlation coefficient obtained for the F_2 population agrees very well with that obtained from the variety trials conducted in the preceding year.

It may be concluded from the findings given above, that the number of effective factors for the leaf shape is rather small, and that the heritability in the narrow sense as well as in the broad sense, as to the leaf shape and that in the broad sense as to the mid-rib proportion are so high in the present cross, that the individual selection during the breeding procedure will be quite effective for getting tobacco strains with round leaves and low mid-rib proportion.

I. GENETICS OF *SOLANUM* AND *CAPSICIUM*

1. *Genetic Studies on Eggplant* (*Solanum melongena* L.)

(Report by Kanji GOTOH.)

a. Regression analysis of quantitative gene action.

The majority of economic characters in the eggplant seem to be polygenic in inheritance. The experiment reported here was designed with the purpose of determining the mathematical models fitted for analysis of some of the quantitative characters in this plant, and clarifying the dominance relations of their genes. By constant parent regression analysis following Griffing's method, the model of gene actions on the shape and weight of the fruit, as well as of those on the period from seeding to flowering, was found, and the dominance relations of genes for the above characters were examined. Moreover, the dominance relations of the genes for bunchiness and hairiness, which are the characteristics of the variety Burma, were also examined.

Seven varieties of eggplant and 18 of their F_1 hybrids were used as material.

The results obtained may be summarized as follows:

1. There are arithmetically cumulative actions with negative dominance with respect to the genes for shape, and the genes for the period from seeding to flowering. The degree of dominance of the genes for shape

is very slight. The degree of dominance is highly variable for the genes for the length of the period between seeding and flowering; in some crosses even overdominance has been observed.

2. In the case of the genes for weight, a more adequate model of gene action is logarithmic.
3. The genes for bunchiness show no dominance so far as the present experiment is concerned, and the genes for hairiness show negative dominance.

b. The heritability of some quantitative characters and estimation of the minimum number of genes.

The present investigation has been undertaken to estimate the heritability of some of the quantitative characters in the eggplant, and the minimum number of the genes governing these characters. The characters studied were fruit shape, fruit weight, period from seeding to flowering, fruit yield and fruit number per plant, bunchiness and hairiness. Six eggplant varieties and five of their F_1 and F_2 hybrids were used for this study. The planting was made according to randomized block arrangement with three replications. The heritability obtained belongs to the category in the broad sense, and the environmental variance is represented by the variance of F_1. The estimation of the minimum number of genes concerned was made according to Wright's formula.

Fruit shape, fruit weight, the length of the period from seeding to flowering, fruit yield and fruit number per plant have heritability averaging 89.3, 88.6, 69.6, 10.0 and 4.1 per cent respectively.

In the two crosses, Burma × Sendai-naga No. 1 and Turuboso-sen-nari × Black beauty, the fruit yield and fruit number have given a negative genotypic variance. The heritability of these characters is generally low, and highly variable according to crosses. It may be concluded that the yield per plant in the F_1 generation is greatly influenced by both environmental condition and experimental errors. The largest minimum number of genes concerned with fruit shape, fruit weight and the period from seeding to flowering is estimated as 5, 9 and 4 respectively. It should be noted, however, that the minimum number of genes thus estimated is highly variable among the crosses, and that the estimated number of genes for fruit weight is proportional to the differences between the parents.

The vigor in the F_2 for fruit yield and fruit number declines to 73.8～74.0 per cent of that of the F_1, taking the average of the five crosses.

In the cross, Burma × Sendai-naga No. 1, the heritability of bunchiness and that of hairiness is 80 and 70.5 per cent, and the minimum number of genes concerned is 3 and 4 respectively.

The phenotypic correlation between the shape and fruit weight in four crosses, as well as among four characters, namely, length of period from

seeding to flowering, fruit shape, bunchiness and hairiness, in the Burma × Sendai-naga No. 1 are generally low. Thus, it may safely be surmised that the genes governing these quantitative characters are segregating independently of one another.

The pigmentation on the anther and the pigmentation of the lower part of the calyx, which is a characteristic of the varieties Burma, Black beauty and Florida high bush, are due to a pleiotropic effect of one and the same major gene. In the Florida high bush × Sendai-naga No. 1 cross, association between the pigmentation and the three characters, namely, fruit shape, fruit weight and the period from seeding to flowering, has been confirmed.

2. *Inheritance of Fruit Color in Capsicum*

(Report by Toru ENDÔ)

By crossing of the two varieties, Goshiki and Takanotsume, of the pepper, *Capsicum*, the intensity of the color due to chlorophyll in the succeeding generation and the extension of the colored area due to anthocyanin of the fruits were determined; they were found to be distributed in the manner shown in the table.

	The intensity of the color due to chlorophyll						The extension of colored area due to anthocyanin				
Grade / Generation	1 pale	2	3	4	5 deep	Total	0	1	2	3	Total
P_1 (Takanotsume)	—	—	—	10	17	27	6	—	—	—	6
P_2 (Goshiki)	27	21	—	—	—	39	—	—	—	39	39
F_1 ($P_2 \times P_1$)	—	—	15	31	—	46	—	3	36	7	46
B_1 ($F_1 \times P_1$)	—	—	13	97	32	142	17*	106	20	1	144
B_2 ($F_1 \times P_2$)	14	26	29	41	1	111	—	10	34	66	110
F_2	7	20	24	53	17	121	7*	44	44	24	119

* Including fruit which contained a trace of anthocyanin.

From this result, the number of responsible genes was estimated by the formula $n=(\overline{P_1}-\overline{P_2})^2/8(V_{F_2}-V_{F_1})$. It has been found to be 1.39 for the genes for chlorophyll color, and 1.18 for the genes for anthocyanin color. It is, accordingly, to be inferred that there are one or two partially dominant genes controlling the chlorophyll character and one gene of the same sort for the anthocyanin character.

The anthocyanin of the pepper has been found by the writer, using paper chromatographic analysis, to be the glycoside of delphinidin. This

anthocyanin apparently has a tendency to be produced only in the part exposed to the sun, while the carotenoid is produced first in the same part and later extends to the other sides of the fruits.

3. *On Genes Controlling Quantitative Characters in Capsicum annuum* L.

(Report by Akira MIYAZAWA)

Two varieties of *C. annuum*, Taka-no-tsume and Chinese, which differ distinctly in quantitative characters, were crossed, and the number of genes controlling fruit- and leaf-characters and the correlations among some of them were analysed. The experiment was conducted according to the complete randomized block design with three replications on the P_1, P_2, F_1 and F_2 populations. Measurements of characters were always made after they had ceased to grow. The characters measured were weight, length and width of fruit, length and width of leaf, length of petiole and number of seeds per fruit. Estimations of the number of genes were made according to the CASTLE-WRIGHT formula. The genetic correlation was calculated between two of these characters.

The results obtained by such analyses are presented in the table.

Table. Showing the number of genes controlling the characters of *Capsicum*, and correlation coefficient between some of these characters.

Number of genes								
Wt. of fruit	Lg. of fruit	Wd. of fruit	Lg. of leaf	Wd. of leaf	Lg. of petiole	No. of seeds	Lg. F./ Wd. F.	Lg. L./ Wd. L.
52.24	0.79	9.52	1.59	7.92	0.04	6.51	1.11	5.63

Correlation Coefficient				
Wt. F. vs. No. S.	Lg. F. vs. Lg. L.	Wd. F. vs. Wd. L.	Lg. L. vs. Lg. P.	Lg. F./Wd. F. vs. Lg. L./Wd. L.
0.86**	0.81**	0.73**	0.82**	0.20*

*) Exceeds the 5% level of probability.

**) Exceeds the 1% level of probability.

+) Abbreviations··Wt.: Weight; Lg.: Length; Wd.: Width; No.: Number; F.: Fruit; L.: Leaf; S.: Seeds; P.: Petiole.

It is found that the number of genes controlling width in any of the organs seems to be higher than that controlling length in the corresponding organs. It is also found that a rather high correlation exists between weight of fruit and number of seeds per fruit. Between similar characters

of different organs such as leaf and petiole, or fruit and leaf, high correlation is observed. The fact that the number of genes controlling length in each of the three organs is very small suggests a possible pleiotropic effect of the genes. The correlation between the length/width ratio of fruit and that of the leaf is low.

J. GENETICS OF SOME FLOWERING PLANTS

1. Cytogenetic Studies on Sex in Cannabis sativa L. II. Meiosis in Diploid, Triploid and Tetraploid Males.

(Report by Yô TAKENAKA)

Diploid males: In the meiosis of the pollen mother cells of the diploid males, one unequal pair was clearly distinguished among ten bivalents. The larger member of this pair is the largest of all the chromosomes. It is, however, difficult to distinguish the larger member in size and shape from a member of another large but equal pair.

Triploid males: The meiosis of male plants, which had been determined to be triploid ($2n=30$) by chromosome counts on the root tips, was observed. The chromosome complement of this plant had been assumed to be 27a+X+X+Y from the chromosome complements of their parents, 36+4X and 18+X+Y. The chromosome conjugation at first metaphase showed very many trivalents and a few univalents :—

0	univalents	among	10	PMC's
1	,,	,,	34	,,
2	,,	,,	22	,,
3	,,	,,	17	,,
4	,,	,,	3	,,

The chromosome configuration $9_{III}+1_{II}+1_{I}$ was the most frequent, and the configurations $8_{III}+2_{II}+2_{I}$ and $7_{III}+3_{II}+3_{I}$ came next. Often PMC's with only trivalent were found. The V-shaped trivalents in each PMC numbered from one to four, of which two were very large. One, composed of equal members, was probably an autosome complex. The other, composed of three unequal members, seemed to represent the sex-chromosome complex. In the latter, the chromosome situated at one end was equal in size to, or larger than, the largest chromosome of the autosome complex, and the two others, one at the other end and the other in the middle, were slightly smaller. Therefore, the configuration of this chromosome complex may be considered as Y−X−X. Triploid intersexes also showed the same configuration as far as the sex chromosomes are concerned, viz., Y−X−X.

Tetraploid males: In the meiosis of the PMC's of tetraploid male

plants, induced by colchicine treatment, about ten chromatid groups were found in the pachytene stage, whereas in diakinesis about twenty chromosomes were observed. Most nuclear plates in the first meiotic division showed 18-20 gemini besides occasionally a quadrivalent or a trivalent accompanied by a univalent. These trivalents or quadrivalent chromosomes represent mostly the sex-chromosome complexes. The sex-chromosome complexes are classifiable into four types ; Y−Y, X−Y, Y−X−X−Y and Y−X−Y, with the frequency 77, 29, 26 and 8, respectively, apart from a few other types. Among the four types of sex chromosome complexes, both Y−Y and Y−X−X−Y types would give only pollen containing X and Y, while the X−Y type would give pollen with two X's or two Y's, in addition to that mentioned above, and the Y−X−Y type is expected to produce four kinds of pollen grains, namely, those having two X's, two Y's, two Y's and one X, and only one X, respectively. However, such pollen grains should form only a fraction of the total pollen. Accordingly, a cross between tetraploid females (36a+4X) and tetraploid males (36a+2X+2Y) would produce many tetraploid plants having the constitution 36a+3X+Y, as pointed out by Warmke and Davidson.

2. *Pigment Combination of Viola tricolor*

(Report by Toru ENDÔ)

Determination of pigment combination of ten varieties of the Swiss giant pansy was made by means of paper chromatography. The results obtained are shown in the table.

Name of Variety	Flavonoid		Anthocyanin					Carotenoid				
	a	b	a	b	c	d	e	a	b	c	d	e
Pure White	##	##	−	−	−	−	−	−	−	−	−	−
Coronation Gold	+	##	−	−	−	−	−	+	+	+	−	−
Giant Orange	+	##	−	−	−	−	−	+	+	+	+	+
Mont Blanc	+	##	−	−	−	(##)	(+)	−	−	−	−	−
Rhinegold	+	##	−	−	−	(##)	(+)	+	+	+	−	−
Raspberry Rose	##	##	+	+	##	−	±	−	−	−	−	−
Fire Beacon	##	##	+	+	+	−	±	+	+	+	−	−
Alpenglow	##	##	+	+	##	−	±	+	+	+	−	−
Lake of Thum	##	##	−	−	−	+	±	−	−	−	−	−
Berna	+	+	−	−	−	##	+	−	−	−	−	−

The anthocyanins in brackets are those which produce the color of the blotches.

Paper chromatographic analysis of hydrolysed products of anthocyanins has proved the existence in flowers of all the seven varieties of both cyanidin and delphinidin. It may be inferred from this analysis that the anthocyanins —a, —c, and —e are glycosides of cyanidin, and —b and —e are glycosides of delphinidin. The Rf values as well as the color of flavonoid —b are exactly similar to those of rutin in all of the four solvents. A distribution test of crude extracts of carotenoids by petroleum ether and methanol has succeeded in classifying carotenoid —a, —b and —c as belonging to the xanthophyll-group, and —d and —e as belonging to the carotene-group.

K. IMPROVEMENT OF SOME USEFUL PLANTS

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

(Reported by Seiji MATSUMURA)

If $2x$ and $4x$ beets are planted alternately, $3x$ seeds can be obtained through natural pollination either from the $2x$ or from the $4x$ plants. Offspring from $4x$ mother plants were called, for convenience, $3x$–A, while those from $2x$ plants were designated as $3x$–B. The triploid seeds, however, are inferior to $2x$ seeds in germination rate. In order to overcome this difficulty, and to find out the best combination, various intervarietal triploid hybrids were obtained in 1951 and 1952 and examined for germination rate, as well as for other characters.

Table 1 shows the results of experiments conducted on the same scale in two experimental fields of the Japan Beet-Sugar Manufacturing Company in Obihiro and Shibetsu in Hokkaido. In these experiments various $3x$ seeds, obtained by planting of $4x$ and $2x$ beets in the ratio of 3:1, were used. In the middle of the growth period, plant height and number of leaves were examined. There was no significant difference in these characters between $3x$ hybrids and $2x$ beets (Hon-iku No. 192, the most widely grown variety), while $3x$ beets had a decidedly larger girth than $2x$ in the end of August. At the same time susceptibility of the leaves to diseases was investigated and denoted by an index. The larger index shows a higher susceptibility. One triploid combination and its reciprocal, No. 4398 (Hon-iku 398–$4x$) × 162–A and –B, were most resistant of all, while the combinations No. 4402 (Hon-iku 402–$4x$) × 399 and No. 4402 × 48 were most susceptible. In yield and sugar content the combination No. 4398 × 162 showed the best results, and this is the most promising combination. Finally, in the beginning of 1952 this combination was recommended for promotion as one of the best and called $3n$–1 by the Hokkaido Agricultural Experiment Station and Hokkaido Seed Association. This new combina-

tion variety will gradually replace in several years the common varieties throughout a considerable part of the Hokkaido beet area.

Table 1. Comparison of susceptibility to diseases, yield and sugar content in various 3x hybrids (Mean value of Obihiro and Shibetsu in 1952)

Combination	Susceptibility index (Rate)	Yield per Tan* (Rate) Kin**	Sugar % (Rate)	Sugar content per Tan* (Rate) Kin**
Hon-iku No. 192	1.970 (100)	5,069 (100)	16.85 (100)	751 (100)
4398× 162-A	1.620 (82)	5,948 (117)	16.54 (98)	862 (115)
162 ×4398-B	1.510 (77)	5,865 (116)	16.40 (97)	845 (113)
4398× 399-A	2.390 (121)	5,324 (105)	17.16 (102)	806 (107)
399 ×4398-B	2.576 (131)	5,112 (101)	16.80 (100)	766 (102)
4398× 401-A	1.981 (101)	4,995 (99)	17.55 (104)	773 (103)
4402× 399-A	2.476 (126)	5,115 (101)	16.84 (100)	780 (104)
4402× 48-A	3.077 (156)	4,952 (98)	16.92 (100)	743 (99)
48 ×4402-B	2.438 (124)	5,078 (100)	16.96 (101)	765 (102)
4048× 399-A	2.698 (137)	5,050 (100)	16.99 (101)	760 (101)
399 ×4048-B	3.317 (168)	5,213 (103)	16.62 (99)	769 (102)
4048× 192-A	2.772 (141)	5,043 (100)	16.65 (99)	732 (98)
192 ×4048-B	2.850 (145)	4,613 (91)	16.56 (98)	675 (90)
LSD 5%	0.713	570.2	0.733	99.4

* Tan=ca. 0.1 ha, % Kin=0.6 Kg.

2. *Tetraploidy in Citrus*

(Report by Kazuo FURUSATO)

Most species of *Citrus* are diploid, and have 9 gametic chromosomes. However, during his breeding studies, the author has encountered a few tetraploid plants with 36 somatic chromosomes.

These tetraploids were of spontaneous origin, artificial stimulation being out of the question.

In order to make some observations on the conditions and the ways in which tetraploids in *Citrus* arise, a number of seeds of several diploid varieties (*Citrus Unshu*, *C. natsudaidai* and *C. Aurantium*) were planted in soil, and, after germination, the seedlings were examined for tetraploids. In each of the three varieties, a few tetraploids occurred, as shown in Table 1.

The tetraploids could easily be distinguished from the diploids. Their leaves were of a darker green, the mesophyll was thicker and the stomata larger; moreover, the main root was thicker and the number of lateral roots was considerably smaller than in the diploids.

Table 1.

	No. of plants examined	No. of tetraploids	Frequency of tetraploids
Citrus Unshu	228	1	0.4%
C. Natsudaidai	1920	3	0.2%
C. Aurantium	2048	3	0.1%

On the other hand, quite like the diploids, they were very uniform, and had all the features of the mother plant.

It may safely be assumed that they were autotetraploids originated from nucellar cells.

Sometimes, the tetraploids were found in twins ; in one case both twin plants were tetraploid ; in another, one was tetra- and the other diploid.

So far, the author has found tetraploids in the following five varities (Table 2). All of them were produced from untreated seeds.

Table 2.

Species	Number of chromosome
Citrus junos	36
Poncirus trifoliata	36
Citrus Unshu	36
Citrus Natsudaidai	36
Citrus Aurantium	36

3. *Abnormal Growth of Citrus Trees Caused by Deviating Chromosome Numbers in the Grafting Stocks*

(Report by Kazuo FURUSATO)

During an inspection of *Citrus* groves, the author was shown trees which, for no obvious reason, were very poorly developed and growing very slowly. The possible effect of non-genetic factors such as parasites, management technique or soil condition, has been excluded after a thorough investigation. The surrounding specimens were all normal, healthy and well-grown trees.

The examination of the roots revealed that the lateral roots were very poorly developed. The investigation of the chromosome number in root tips showed that the grafting stocks of the underdeveloped trees were tetraploid.

In one grove there were as many as 7 such trees among 1000. They were scarcely 2/3 as high as the surrounding normal trees. Their fruits were very small and few. Therefore, they meant a considerable loss to the owners.

The differences between the normal and the underdeveloped trees can be seen from the following table.

Table 3. Comparison of 15 year old trees grafted on di- and tetraploid stocks in *Citrus Unshu*.

	Plant height		The branches of a tree (diameter)		The trunk (diameter)		Number of fruits (per year)	
	diploid	tetra-ploid	diploid	tetra-ploid	diploid	tetra-ploid	diploid	tetra-ploid
A section	2.05m	1.20m	2.65m	1.53m	9.9cm	4.9cm	187.5	40.0
B section	1.46	1.05	1.10	1.03	4.4	3.1	49.7	9.0
C section	2.30	1.50	2.80	1.72	8.5	4.8	210.0	45.0
D section	1.94	1.25	2.18	1.42	7.6	4.17	149.1	31.3

As to other features of the trees grown on tetraploid stocks, their leaves were small, curled inward, and of a light green color.

It was interesting to observe that the rind of their small fruits was of a firm texture in contrast to the loose rind of the normal fruits. The color of the rind was slightly lighter and the fruits had a normal sugar content. The firm rind is an advantageous feature with regard to storage. The author is now studying the keeping properties of stored *Citrus* fruits, and expects to be able to make some use of the firm rind texture characteristic of those fruits.

From this investigation it has become evident that tetraploid seedlings occur so frequently in some varieties of *Citrus* (e.g. *Poncirus trifoliata*) that they cause serious loss to the owners of groves who use them as grafting stocks. Fortunately, it is easy to recognize them by their very thick main roots and by a very poorly developed system of lateral roots, in selecting plants to serve as grafting stocks.

4. *Prospects of Using Triploids in Tobacco Breeding*

(Report by Kazuo FURUSATO and Akira MIYAZAWA)

Tetraploids have been produced by colchicine treatment in three varieties of tobacco, Bright Yellow, Judy's pride and Xanti. In order to obtain triploids the tetraploids were pollinated with pollen of the respective diploids.

The plants thus obtained were highly variable in plant height, number of leaves, and so forth, and their practical utility could hardly be considered.

The lack of uniformity was probably due to irregularities in chromosome distribution at meiosis of the tetraploids, with the result that in addition to the expected triploids also a number of aneuploids, i.e. hypo- and

hyper-triploids, have been produced. This was borne out by examination of the meiotic phenomena.

In the P.M.C.'s uni- and trivalents were observed, in addition to bi- and tetravalents. A similar situation could have been expected in the E.M.C.'s.

In general, triploids can be produced only when the pollen of the diploids is put on the stigmata of the tetraploids. However, in tobacco, triploids could be easily produced in both directions of the cross. Moreover, it was observed that the F_1 was more uniform when a tetraploid was the pollen provider. The author assumes that this was due to the fact that pollen grains with balanced chromosome sets are more successful in competition.

It is hardly necessary to add, that "triploids" and "tetraploids" in tobacco plant are in reality hexaploids and octoploids, respectively.

L. GENETICS OF MOULDS

1. *Appearance of Reversional Mutants in the Methionine-requiring Strain of Ustilago maydis.*

(Report by Tetsuo IINO)

Mass-cultures of a methionine-requiring mutant strain 4-24 of *Ustilago maydis* are often found to contain prototrophic cells. Such cells may be found even in the cultures originating from a sporidium which had been proved to require methionine. This prototrophic characteristic is maintained through successive generations, and the proportion of these cells to the requiring cells is increased with the age of the cultures, in spite of the fact that the growth rates in the complete media are nearly alike. This phenomenon may be more properly explained by assuming successive reversional mutations of the requiring cells, than by assuming an increase of the pre-existing prototrophic cells or some change in physiological property.

The reversional mutants were screened from the complete sporidia culture by the following procedure. The culture is centrifuged and washed 3 times in 0.9% NaCl aq. solution. It is then diluted by the minimal solution, and finally plated on to minimal agar media in petri dishes, and kept at constant 25°C. A dilution of from 5×10^5 to 5×10^6 cells per dish seems to be adequate for avoiding indistinction by residual growth and syntrophism.

The spontaneous mutation rate has been calculated from the numbers of the reverted cells at the late logarithmic period after Delblück's formula 2 (Newcombe 1948). It has been found that the strain undergoes reverse

mutation at the rate of 3.0×10^{-7} per sporidium per division in complete liquid culture. This frequency may be raised by X-ray (4.5×10^{4} *r*-dose) irradiation, or by treatment with tris-nitrogen mustard (1/200 mol, pH9.5) up to the orders of 10^{-4}.

The growth habit of these reverted mutants has been investigated in twenty clones. Sixteen of these were capable of growing on the minimal media like the wild type strain. These clones have been named "complete reversion types (CR)". Four other clones have shown a restricted growth rate on the minimal media, but when supplementary l-methionine was added, they grew normally like the wild type. These clones maintained the restricted growth habits through several subcultures, but within 3 to 8 generations, they acquired the normal growth rate even in the minimal media. These clones are distinguished from CR, and named "inferior reversion types (IR)". The restricted growth of the IR is not due to the mixture of the original mutant and CR. This is demonstrated by the unimodal distribution of the colony size after plating IR to the minimal media. The cause of the inferior reversion may be explained by assuming the following alternatives : either a sort of suppressor mutation or phenomic lag. At present it is hard to decide whether the present IR type is due to the first or the second alternative. This will be clarified in the future.

2. *Studies on Heterocaryosis in Aspergillus and Penicillium.*

(Report by Seizô TSUDA)

The author (1951) found a spontaneous heterocaryon which occurred frequently in the stock culture of *Aspergillus candidus*, and he has carried out morphological and cytogenetical investigations on this strain.

The author has succeeded in inducing heterocaryon formation between two varieties of *Aspergillus Awamori* and between two mutants of *Penicillium chrysogenum* THOM. Q176 strain. The culture medium was synthetic CZAPEK-DOK agar medium.

The heterocaryon formed by the 5-9 and the N-19 varieties of *Aspergillus Awamori* was intermediate in character between the two parental varieties, so far as the color and the form of colony were concerned.

The heterocaryon in *Penicillium chrysogenum* was formed between two mutants induced by ultra-violet irradiation. One of these, UY-3S, forms a jagged colony and produces a large amount of yellow-colored substance which is sorbicillin and penicillinic acid. The other strain, UW-1S, forms a round and colorless colony. The spores are whitish in both strains and hardly distinguishable. The conidia of the heterocaryon formed between these mutants are green, and contain yellow substance, just as in the wild type of *Penicillium chrysogenum* THOM. Q176.

The presence of the colored substance and the jaggedness of the outline

of the colony in the former strain are closely correlated, and this is also true of the correlation between the colorlessness and the smoothness of the latter strain. The heterocaryon is highly variable in the color and outline of the colony. The number of nuclei in each cell of *Penicillium* is usually more than 12, and it is more than probable that the high variability in the characters of the heterocaryon is largely due to variety in the combinations of the two parental nuclei.

M. STUDIES ON COMPETITION

1. *Competition between diploid and autotetraploid plants of barley.*

(Report by Kan-Ichi SAKAI and Yasuo SUZUKI)

Diploid and autotetraploid plants of each of two barley varieties were compared for their competitive ability. Seven plants were planted in a group so that six of them surround one plant which was to be tested with respect to its competitive ability. The spacing between any two plants was 23 cms. The design of the experiment was the split plot one with five replications in one variety and six in the other. Data have been taken for plant weight, number of culms, number and weight of heads, number and weight of seeds and sterility percentage of spikelets on an individual plant basis. Analysis of variance of the data obtained has been conducted.

Variety	Character	Mean values of seven characters of diploid vs. tetraploid plants in pure stands and in mixtures			
		Diploid surrounded by		Tetraploid surrounded by	
		diploids	tetraploids	diploids	tetraploids
Golden Melon	Plant weight	33.1	50.6	23.7	10.0
	Number of culms	11.8	15.6	6.8	3.9
	Number of heads	11.6	15.5	6.7	3.8
	Weight of heads	13.7	20.1	8.6	3.4
	Weight of seeds	11.0	16.1	6.1	2.4
	Number of seeds	196.6	270.4	91.7	35.1
	Sterility	9.3	7.2	29.7	36.6
Hosogara-No. 2	Plant weight	37.0	57.0	31.8	11.5
	Number of culms	9.4	14.0	7.4	3.5
	Number of heads	9.4	13.8	7.0	2.6
	Weight of heads	17.4	26.3	12.9	4.0
	Weight of seeds	14.2	20.8	8.0	2.6
	Number of seeds	530.4	826.4	251.0	83.4

The mean values of each quantitative character of the diploid and tetraploid plants affected by competition, as contrasted with those of pure stands, are presented in the table above.

As shown in the table, the diploid plants were always better competitors than the tetraploid plants of the same variety in both varieties. The diploid plant surrounded by six tetraploid plants of the same variety has gained 33–56 percent increment in the characters as compared with those in the pure stand. The sterility percentage of spikelets of tetraploid plants was distinctly increased by the surrounding effect of the diploids.

It may be concluded accordingly that the autotetraploid plants of the barley varieties are considerably handicapped by severe competition with diploid plants, if they are among a population of the parental diploids, at least under such circumstances as in the present experiments. Thus it seems certain that a few autotetraploid plants spontaneously arisen in a diploid barley population have but small chance to supplant the parental diploids by competition with the latter, unless some change in environmental conditions takes place.

2. *Competition between an Artificially Synthesized Amphidiploid and its Ancestral Species of Abelmoschus.*

(Report by Kan-Ichi SAKAI and Yasuo SUZUKI)

Abelmoschus glutino-textilis ($2n = 192$) is an artificial amphidiploid species between *A. esculentus* ($2n=124$) and *A. Manihot* ($2n=68$), originally synthesized by Prof. F. Kagawa in Kyoto University. We have made an experimental study on the relative competitive ability of the original and synthesized species. The experiment was conducted by the split-plot design with four replications. The planting of individuals was made hexagonally, so that six plants surround one plant which was to be tested with respect to its competitive ability. The spacing between any two of the plants was 24 cms. The characters examined were plant weight, plant height, number and weight of fruits and number and weight of seeds on an individual plant basis. Statistical tests of the data obtained were conducted by analysis of variance.

The mean values of all six characters of the three species in both pure stands and mixtures are presented in the table.

Inspection of the mean values of the characters in the table leads one to conclude that the amphidiploid *Abelmoschus glutino-textilis* is the best competitor and that one of the parental species *A. Manihot* is the worst competitor of the three species examined.

All the characters, except plant height, of *A. glutino-textilis* in the mixture with *A- Manihot*, reached twice or even more than three times as much as those in pure stands. Even with *A. esculentus*, the increment of

Character	Average values of plant weight, plant height, number and weight of fruits and number and weight of seeds on an individual plant basis in:								
	Manihot surrounded by			*esculentus* surrounded by			*glutino-textilis* surrounded by		
	*M**	*e**	*g**	*e**	*M**	*g**	*g**	*M**	*e**
Plant weight (g)	55.3	21.8	14.6	154.3	320.5	63.7	150.3	332.5	274.7
Plant height (cm)	43.5	34.2	29.7	107.6	92.7	97.9	161.4	165.6	166.0
Number of fruits	3.5	1.2	0.7	2.7	4.9	1.1	3.1	7.1	5.9
Weight of fruits(g)	3.9	1.4	1.1	18.7	38.5	6.9	6.1	20.8	18.3
Number of seeds	107.9	27.0	25.9	199.4	382.9	57.8	42.4	148.4	126.8
Weight of seeds (g)	2.3	0.4	0.4	12.7	21.4	3.4	2.1	7.0	6.2

* *M*, *e* and *g* stand for *Abelmoschus Manihot*, *A. esculentus* and *A. glutino-textilis* respectively.

A. glutino-textilis gained in the mixture was distinctly large.

From the results described above, it may be concluded that the amphidiploid species is a good competitor against both its parental species so far as the present plant species and the conditions of the present experiment are concerned.

3. *Intervarietal Difference in Competitive Ability in Hordeum sativum* L. and *Oryza sativa* L.

(Report by Kan-Ichi SAKAI and Kanji GOTOH)

In this experiment the competitive abilities of twelve varieties of barley were compared, as well as those of five varieties of rice-plants.

(1) *Hordeum sativum* L.

Twelve varieties of barley in pure stands and in mixtures of all possible combinations of two varieties in each were planted according to a simple lattice design with four replications. The characters examined were the number of culms and the weight of grains per plant. Analysis of variance of the data obtained has proved that the variation among varieties in pure stands as well as in mixtures was statistically significant. Further analysis of variance was conducted on the increment or decrement of characters which was solely due to competition between different varieties. This analysis has proved that the varieties differed significantly in their competitive ability. These analyses have enabled the writers to rank according to competitive ability, the twelve varieties of barley into grades from the lowest to the highest, taking the number of culms and weight of grains as indicators of the ability. The rankings in regard to the two characters are in fair agreement with each other.

(2) *Oryza sativa* L.

In 1951, six varieties of rice plant, *Oryza sativa* L. were examined for their competitive ability (cf. 1951 issue of this Report). Further studies were attempted on five other varieties by using the three of the six varieties of the 1951 experiment, as testers. Experiments were conducted by the split-plot method, with three replications. Analysis of the variances in plant height, plant weight, as well as in the number of panicles has revealed that the varieties differed significantly for all of the four characters, but that the variation due to the competitive effect was significant only at the 5 percent level of probability as to plant weight and weight of panicles, while it was almost significant with respect to the number of panicles. Comparison of the mean values of increment or decrement in the five varieties has enabled the writers to classify them according to their competitive ability.

We seem to be justified for the present to conclude from the two experiments described above, as well as the one already reported, that varieties within one and the same species may significantly differ with respect to their competitive ability.

4. *Competition between the Upland-Rice Plant and the "Red-Rice" Plant.*

(Report by Kan-Ichi SAKAI and Yasuo SUZUKI)

It is known that the so-called "red rice" often contaminates a population of upland rice and spoils the crop. "Red-rice" is one of the primitive varieties of rice belonging to the subspecies *Indica* of *Oryza sativa* L. Our interest has been directed to finding out if the red rice has any selective advantage in a mixed population with the commercial rice plants. This is the first report dealing with the relative competitive ability between the red-rice variety and the commercial variety of upland-rice.

Young plants of the Nōrin No. 21 variety of upland rice, and those of red rice grown from seeds obtained in Saitama-ken, were individually planted in the plots. The planting was made in such a manner that a group of seven plants form a regular hexagon with one plant in the center. Arrangement of the experimental plots was made according to the split-plot design with three replications.

The characters examined were the height and weight of plants, the number and weight of panicles, and the weight and number of grains on an individual plant basis. The analysis of the data thus obtained has proved that the variation due to interaction between competition and variety was highly significant. The mean values of each character in every treatment in the experiment may be seen in the following table.

Number of surrounders of another variety	Upland rice			Red rice			Standard error
	0	3	6	0	3	6	
Plant height (cm)	82.3	79.9	73.2	75.8	83.8	82.6	3.32
Plant weight (g)	13.4	10.3	8.2	7.2	9.5	9.4	0.97
Number of panicles	3.84	2.53	2.38	4.11	5.05	5.25	0.45
Weight of panicles (g)	6.33	4.45	3.08	3.10	4.38	4.67	0.59
Weight of grains (g)	5.60	3.90	2.78	2.67	3.76	4.26	0.66
Number of grains	231.1	139.5	94.7	145.5	215.1	213.8	35.7

It is apparent from the table that the upland-rice plant surrounded by red-rice plants suffered considerable decreases for all the characters examined, and the reverse is true for the case of the red-rice plant surrounded by the upland-rice plants. It is concluded from this finding that the red-rice variety is a good competitor against the upland-rice variety, and it is possible that a few plants of the red rice mingled in a population of the upland rice will propagate at an extraordinary rate, so far as the competition is concerned.

N. THEORETICAL GENETICS

1. *Theoretical Considerations on Random Drift of Allelic Genes or Chromosomes and Decrease of Heterotic Vigor in Allogamous Plants.*

(Report by Kan-Ichi SAKAI and Yasuo SUZUKI)

Concerning the production of original seeds of allogamous plants, especially of root and leaf crops, there are some problems which are of interest from the viewpoint of population genetics. The problem dealt with in this paper is that which concerns the possible degeneration of an improved variety of these crops due to selection of, and propagation from, a very small number of elite plants for many generations for seed growing. Such a procedure is customary among seedsmen in Japan. The elite plants are chosen primarily with reference to morphological characteristics of the crop only.

Let us consider a panmictic plant population with an allelic pair of genes or of chromosomes causing heterosis for a given character in the heterozygotic condition. The heterosis may concern either some productive property or resistance against some deleterious effect, but on account of its quantitative nature, it may not become an object of selection. Let a pair of genes or chromosomes be A and a with frequency p and q respectively

$(p+q=1)$. If we take N individuals at random from the population, $2N$ of A and a will be included in a small population of N individuals. The probability of the small population to have a with the frequency q_i will be given by

$$\frac{(2N)!}{(2Nq_i)!(2Np_i)!}\, p^{2Np_i}\, q^{2Nq_i}.$$

The small population thus obtained will be propagated, and selection of N elite plants from the increased population will again be carried out. This process will be repeated year after year. After n times replication of such a propagation and selection, a population involving a in the frequency q_n will be found with the probability given below :

$$\sum_{i=0}^{2N}\sum_{j=0}^{2N}\cdots\sum_{m=0}^{2N}({}_{2N}C_{2Nq_i}\, p^{2Np_i}\, q^{2Nq_i})({}_{2N}C_{2Nq_j}\, p_i^{2Np_j}\, q_i^{2Nq_j})\cdots({}_{2N}C_{2Nq_n}\, p_m^{2Np_n} q_m^{2Nq_n}).$$

This process of repeated propagation and selection of elite plants accordingly will ensue random drift of allelic genes or chromosomes. As a result

Number of generations, in each of which a given number of mother plants being selected	Number of mother plants selected					
	2	3	5	7	10	15
0	0 *0.50*	0 *0.50*	0 *0.50*	0 *0.50*	0 *0.50*	0 *0.50*
1	12.50 *0.38*	3.13 *0.42*	0.20 *0.45*	0.01 *0.46*	— *0.48*	— *0.48*
2	33.20 *0.28*	14.56 *0.35*	2.78 *0.41*	0.52 *0.43*	0.04 *0.46*	— *0.47*
3	49.79 *0.21*	27.48 *0.29*	8.28 *0.36*	2.47 *0.40*	0.41 *0.43*	0.02 *0.45*
4	62.33 *0.16*	39.20 *0.24*	15.24 *0.33*	5.88 *0.37*	1.42 *0.41*	0.11 *0.44*
5	71.75 *0.12*	49.23 *0.20*	22.59 *0.30*	10.28 *0.35*	3.16 *0.39*	0.43 *0.43*
6	78.81 *0.09*	57.66 *0.17*	29.77 *0.27*	15.21 *0.32*	5.55 *0.37*	0.60 *0.41*
7	84.11 *0.07*	64.71 *0.14*	36.50 *0.24*	20.33 *0.30*	8.43 *0.35*	1.53 *0.40*
8	88.08 *0.05*	70.59 *0.12*	42.71 *0.22*	25.44 *0.28*	11.65 *0.33*	2.76 *0.39*
9	91.06 *0.04*	75.49 *0.10*	48.37 *0.19*	30.40 *0.26*	15.07 *0.32*	4.27 *0.37*
10	93.30 *0.03*	79.58 *0.08*	53.50 *0.17*	35.14 *0.24*	18.60 *0.30*	6.00 *0.36*

* The Roman type numerals represent the probability of losing one member of the pair of allelic genes or chromosomes, and the italicized numerals, the average magnitude of heterotic vigor.

of such random drift, the average heterotic vigor in the population will decrease gradually.

Now let p and q of the initial population both be equal to 0.5, and assume that 2, 3, 5, 7, 10 or 15 elite plants are selected for various characteristics, other than the heterotic vigor in question, in each generation. Thus, the probability of losing one of the allelic genes or chromosomes, that is, the probability of becoming homozygous with respect to the allele, and the intensity of heterotic vigor have been computed for ten generations of propagation and selection of mother plants, and the values thus obtained are presented in the accompanying table. The heterotic vigor in this case has been assumed as 0 in AA or aa homozygotes and 1 in Aa heterozygotes.

The numerical values presented in the table may offer a warning against the present status of the small-scale seed growing in this country.

2. *"Stepping-Stone" Model of Population.*

(Report by Motoo KIMURA)

It is recognized by many recent investigators that population structure is one of the most important factors in speciation. For theoretical studies on the problem of speciation, models of population structure are used. Wright has designed two different models, namely, the island model and the model of continuous distribution (cf. Ann. Eugen. 15, 1951). In the present the report writer proposes a new model of population structure which may be called the "stepping-stone" model.

In this model, a whole population is subdivided into many local subgroups within each of which mating occurs at random and exchange of individuals between the groups is allowed to occur only between adjacent ones. This may represent an intermediate situation between those of the two contrasting models stated above. Fig. 1 and Fig. 2. show respectively cases of linear and area distribution. Contrary to the case of the island model, there exists a high correlation between adjacent subgroups in the stepping-stone model, so that a considerable amount of random differentiation of gene frequencies may be expected among the subgroups even if the rate of migration is appreciably high. The following is a result obtained by the study on the linear distribution.

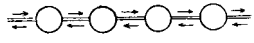


Fig. 1

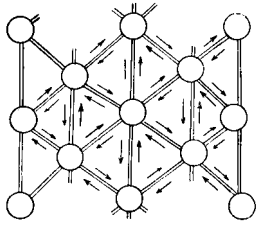


Fig. 2

If the number of breeding individuals in each subgroup is N and the net rate of exchange of individuals between two adjacent subgroups is ε, then the distribution curve of gene frequency (X) among the subgroups will be given by

$$\phi(X)=CX^{8N\varepsilon(1-r)\bar{X}-1}(1-X)^{8N\varepsilon(1-r)(1-\bar{X})-1},$$

where $\bar{X}$ is a mean gene frequency in the whole population, and C is a constant chosen such that $\int_0^1\phi(X)dX=1$. In this formula r represents a correlation coefficient between the gene frequencies of two adjacent subgroups and is a positive root of the quadratic equation;

$$\varepsilon r^2+(2-3\varepsilon)r+(4\varepsilon-2)=0.$$

Therefore if ε is small, r is approximately equal to $1-\varepsilon$.

In this case the variance of gene frequencies among subgroups becomes

$$V=\bar{X}(1-\bar{X})/[1+2N\varepsilon^2(4+\varepsilon)].$$

If $8N\varepsilon^2(1-\bar{X})$ and $8N\varepsilon^2\bar{X}$ are smaller than 1, a considerable amount of local differentiation due to random fixation of alleles will be expected. Since $2N\varepsilon$ is the number of immigrants coming from adjacent subgroups per generation, if $\bar{X}=0.5$, such conspicuous differentiation may be expected unless this number does not exceed $\sqrt{N}$. For example, if the size of the subgroup is 10,000, such a situation will be realized unless the number of the migrants is less than 100. On the other hand, in the island model the number of immigrants should be less than $\frac{1}{4}$, for the corresponding amount of differentiation to be realized.

In the stepping-stone model, if the long range dispersal of gametes is taken into consideration, it comprises the island model as a special case. Thus under certain circumstances the stepping-stone model may reflect the structure of a natural population more fully than the island model or the model of continuous distribution.

3. *Process leading to Quasi-Fixation of Alleles due to Random Fluctuation of Selective Values.*

(Report by Motoo KIMURA)

Since the pioneering works of Fisher and Wright, a considerable number of mathematical studies have been performed concerning the "genetic drift" which is brought about by random sampling of gametes in a finite population. Its genetical implications have also been discussed extensively. On the contrary, little has been worked out on the process of change in gene frequency controlled by the random fluctuation of selection intensities. In his brief account in the annual report of this institute (1952), the present writer has shown by using an approximate method of transformation that this process can be looked upon as a sort of deformed Gauss process. Recently, he has succeeded in solving completely this problem for the simplest case.

Let A and A' be a pair of alleles which are neutral on the average and lacking dominance. If the population is indefinitely large, the relative pro-

bability $\phi(x, t)$ that the frequency of gene A will be $x \sim x+dx$ in the t-th generation satisfies the following Fokker-Planck equation :

$$(1) \qquad \frac{\partial \phi(x, t)}{\partial t} = \frac{V_s}{2} \frac{\partial^2}{\partial x^2}\{x^2(1-x)^2\phi(x, t)\},$$

where V_s is the variance of the selection coefficient of gene A.

If the initial frequency of gene A in the population is x_0, the solution of (1), satisfying the boundary condition ; $\phi(0, t)=\phi(1, t)=0$, is

$$(2) \quad \phi(x, t)=\frac{1}{\sqrt{2\pi V_s t}} \exp\left\{-\frac{V_s t}{8}-\frac{\left[\log \frac{x(1-x_0)}{(1-x)x_0}\right]^2}{2V_s t}\right\} \frac{[x_0(1-x_0)]^{1/2}}{[x(1-x)]^{3/2}} \quad (t>0).$$

When $x_0=0.5$ the distribution curve given by (2) is unimodal when t is smaller than

$$\frac{4}{3V_s}$$

but becomes bimodal if the value of t exceed this amount. Therefore when t becomes very large, the curve will assume a U shape.

It may be important to investigate the process of change in terminal distributions after a sufficient number of generations has elapsed. Let us consider the terminal region of distribution, where the frequency of the gene A is very low. If $x_{\max}$ is a gene frequency giving a maximum value ($\phi_{\max}$) of the distribution curve, then

$$(3) \qquad x_{\max} \sim \frac{x_0}{1-x_0} e^{-\frac{3}{2}V_s t} \longrightarrow 0$$

and

$$(4) \qquad \phi_{\max} \sim \frac{1}{\sqrt{2\pi V_s t}} \frac{(1-x_0)^2}{x_0} e^{V_s t} \longrightarrow \infty,$$

as t becomes indefinitely large. Under such circumstances special attention must be paid to the following relations :— Let ε be any assigned gene frequency, no matter how low, if we take the number of generations (t) as sufficiently large so that

$$t \gg |\log \delta| / V_s,$$

then the probability that the frequency of the gene A in the population is lower than ε approaches $1-x_0$. Symbolically ;

$$(5) \qquad P_r\{0<x<\varepsilon\} \sim (1-x_0)-0\left(\frac{e^{-\frac{1}{8}V_s t}}{\sqrt{V_s t}}\right) \qquad (t \to \infty).$$

Nevertheless, the probability that it is lower than $x_{\max}$ approaches 0,

$$(6) \qquad P_r\{0<x<x_{\max}\} \sim 0\left(\frac{e^{-\frac{1}{2}V_s t}}{\sqrt{V_s t}}\right) \qquad (t \to \infty).$$

Similar relations hold for another terminal region where the frequency of gene A very high. As will be seen from the relation ;

$$\lim_{\varepsilon \downarrow 0} \int_{\varepsilon}^{1-\varepsilon} \phi(x, t)\, dx = 1,$$

this process cannot lead to the complete fixation or loss of alleles as in the case of the drift. But as is shown in the above formulae, (3)~(6), the class frequencies shift toward either terminus indefinitely with time. So after sufficient numbers of generations, a situation is to be realized in which an allele is *almost* fixed in the population or *almost* completely lost from it. To distinguish this from the fixation or loss in the case of the drift, the terms quasi-fixation or -loss may be proposed. In practice, this process will finally be checked by the opposing mutation pressures.

4. *On Simultaneous Distribution of Gene Frequencies in Populations.*

(Report by Motoo KIMURA)

Any attempt to obtain a comprehensive formula for the simultaneous distribution of gene frequencies in populations may encounter serious mathematical difficulties, when one assumes the existence of random fluctuations in selective values. Wright's formula for the simultaneous distribution of gene frequencies apparently does not give an answer to this problem.

For solving the problem, extension of the Fokkar-Planck equation to a multi-variate case may be useful.

Let x_i $(i=1, 2, \ldots, n)$ be the gene frequency in the i-th locus. It can be shown that the relative probability, $\phi(x_1, x_2, \ldots, x_n; t)$, concerning the simultaneous distribution of gene frequencies for the n loci in the t-th generation satisfies the following partial differential equation:—

$$(1)\qquad \frac{\partial \phi}{\partial t}=\frac{1}{2}\sum_{i=1}^{n}\frac{\partial^2}{\partial x_i^2}(V_{\delta x_i}\phi)+\sum_{i>j}\frac{\partial^2}{\partial x_i \partial x_j}(W_{\delta x_i \delta x_j}\phi)-\sum_{i=1}^{n}\frac{\partial}{\partial x_i}(M_{\delta x_i}\phi),$$

where δx_i is the rate of change of gene frequency (x_i) per generation for the i-th locus, $M_{\delta x_i}$ and $V_{\delta x_i}$ are respectively the mean and the variance of x_i, and $W_{\delta x_i \delta x_j}$ is the covariance between δx_i and δx_j. The distribution at the stationary state may be obtained by putting $\frac{\partial \phi}{\partial t}=0$.

A special example for $n=2$ will be given here. Consider a very large random breeding population of a haplont species, and assume that selection operates only in haplophase. We may put selective values and mutation rates as in Tables 1 and 2, where A, a and B, b are two pairs of alleles.

Table 1

Genotype	Selective Value
AB	1
Ab	1
aB	1
ab	$1+k$

Table 2

Mutation Rate
$A \underset{u_1}{\overset{v_1}{\rightleftarrows}} a$
$B \underset{u_2}{\overset{v_2}{\rightleftarrows}} b$

If we write the frequencies of a and b respectively as x and y, then (1) becomes

$$(2)\quad \frac{\partial\phi}{\partial t}=\frac{V_k}{2}\frac{\partial^2}{\partial x^2}[y^2x^2(1-x)^2\phi]+V_k\frac{\partial^2}{\partial x\partial y}[x^2(1-x)y^2(1-y)\phi]$$
$$+\frac{V_k}{2}\frac{\partial^2}{\partial y^2}[x^2y^2(1-y)^2\phi]-\frac{\partial}{\partial x}[(\bar{k}yx(1-x)-u_1x+v_1(1-x))\phi]$$
$$-\frac{\partial}{\partial y}[(\bar{k}xy(1-y)-u_2y+v_2(1-y))\phi],$$

where $\bar{k}$ and V_k are respectively the mean and the variance of the selection coefficient k. If mutations are non-recurrent and the *ab* genotype is selectively neutral on the average ($\bar{k}=0$), the distribution at the stationary state satisfies the following partial differential equation of parabolic type;—

$$\frac{\partial^2}{\partial x^2}[x^2y^2(1-x)^2\phi]+2\frac{\partial^2}{\partial x\partial y}[x^2y^2(1-x)(1-y)\phi]+\frac{\partial^2}{\partial y^2}[x^2y^2(1-y)^2\phi]=0,$$

which has a general solution;

$$\phi(x,y)=\frac{F\left(\frac{1-x}{1-y}\right)+\frac{G\left(\frac{1-x}{1-y}\right)}{1-x}}{x^2y^2(1-x)(1-y)}.$$

In this formula $F(\eta)$ and $G(\eta)$ are two arbitrary functions of η.

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昭和28年 9 月30日 印刷
昭和28年10月 5 日、発行

編集者 小 熊 捍

静岡県三島市谷田 1,111
發行所 国立遺伝学研究所

東京都千代田区富士見町1の10
印刷者 笠 井 康 頼

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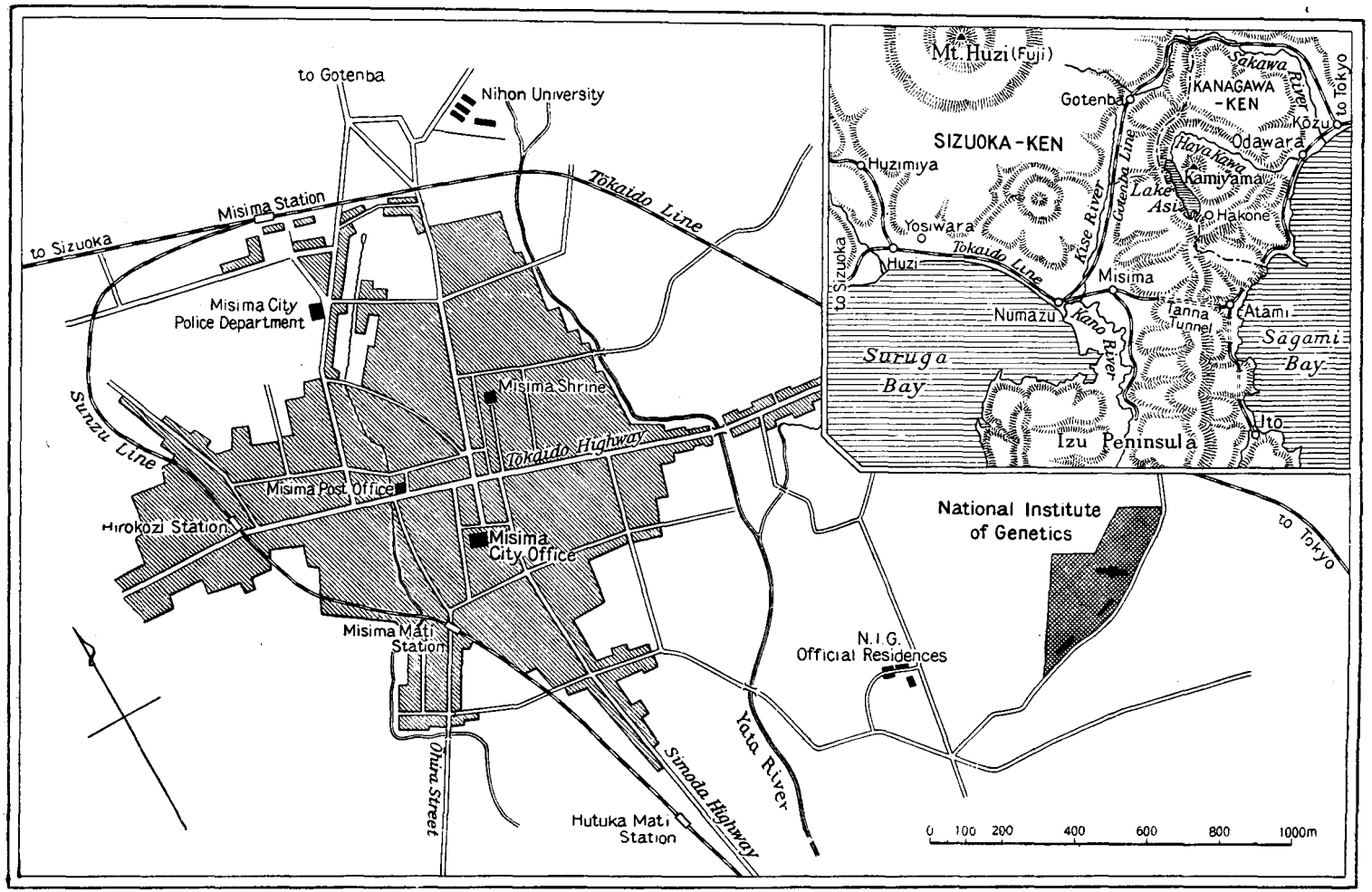
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