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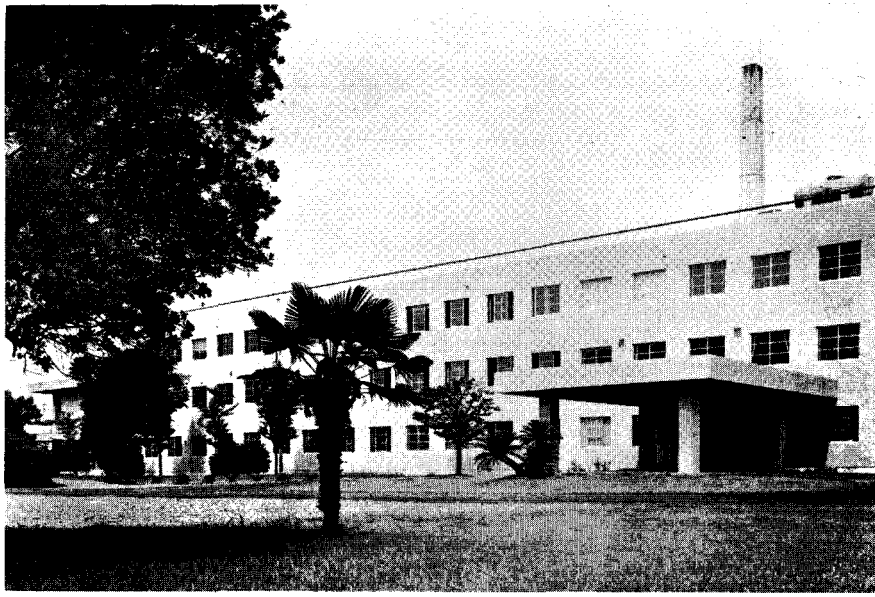

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GENERAL STATEMENT

During this fiscal year there was no significant progress toward the completion of our Institute except for the establishment of an additional laboratory in the Department of Population Genetics. We still want to add three departments, namely for molecular genetics, biophysics, and fine structure.

A budget for our main building was not allocated this year. However we shall be able to complete the remaining one-third of the building by the end of 1967.

This year Dr. T. H. Yosida received the award given by the Genetics Society of Japan for his paper on the relation between chromosomal alteration and development of tumors.

To our regret, death removed from our ranks Dr. Yô Takenaka, head of the Department of Cytogenetics, who died of stomach cancer on the 18th of March, 1966. He started his scientific career after graduating from the University of Tokyo where he had majored in cytology under Prof. K. Fujii in 1927. Immediately thereafter he went to Seoul University where he taught until 1946 botany courses for junior students. After the end of World War II, he came back to Japan and became a member of our Institute established in 1949. In 1953 he became the head of the Department of Cytogenetics. His main contributions in plant cytology were studies on *Rumex, Lilium* and *Nicotiana.* Later he concentrated on *Prunus* (cherry trees) and became even well known among the citizens of Misima as an enthusiastic investigator of cherry trees. According to his opinion *Prunus yedoensis*, one of our most popular cherries, might be a hybrid between *P. lannesiana* and *P. pendula.* He also has bred several new strains. One of them, the Showa-Sakura cherry tree, is most famous. One hundred and twenty plants of this strain were planted in the Garden of the Imperial Palace and will produce beautiful flowers in two to three years. The grateful citizens of Misima are planning to erect a monument in recognition of his contributions in front of our Institute bordered by cherry trees which he loved so much.

When he was in Seoul, Dr. Takenaka was a mountaineer and a traveler. Almost all the high mountains were climbed by him. He has written a book entitled " The Mountains and Landscape of Korea (1938)." He traveled twice to Inner Mongolia as leader of scientific expeditions.

Dr. Takenaka will not be forgotten by his pupils at the Seoul University as a teacher and also by his colleagues at the National Institute of Genetics for his endeavors in the early years of its establishment and development.

Hitoshi Kihara

STAFF

(At the End of 1966)

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KIHARA, Hitoshi, D. Sc., Member of Japan Academy, Emeritus Professor of Kyoto University

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WADA, Bungo, Emeritus Professor of Tokyo University

PROJECTS OF RESEARCH FOR 1966

Department of Morphological Genetics

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Morphological studies of *Oryza* (KIHARA and KATAYAMA)
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Department of Biochemical Genetics

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Studies on a gene for retarded moulting (*rm*) in the silkworm (TSUJITA)
Studies on the chromogranule formation in larval hypodermal cells of the silkworm (TSUJITA and SAKURAI)
Analysis of genetic action on cell differentiation in higher organisms (TSUJITA and NAWA)
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Genetical and biochemical studies of human serum proteins (OGAWA)
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Enzyme regulation in cultured organ of morning glory (ENDO)

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RESEARCHES CARRIED OUT IN 1966

I. CYTOGENETICS

Induction of Plasma Cell Neoplasms in BALB/c Mice, Their Karyotypes and γ-Globulin Specificity[1)]

Tosihide H. YOSIDA, Hirotami T. IMAI, Kazuo MORIWAKI and Shunsuke MIGITA[2)]

13 plasma cell tumors induced by Dr. M. Potter of National Cancer Institute, Bethesda, U.S.A., in BALB/c mice were characterized by near-tetraploid stemline cells, except for one tumor which had a hyperdiploid karyotype (Yosida *et al* 1964 and 1966, this Ann. Rep. **14** and **16**). Almost all tumors were observed through many transplant generations from 10th to 70th. In order to ascertain the chromosomal condition of primary plasma cell tumors, we have studied 5 primary plasma cell neoplasms induced in this laboratory. Among 35 BALB/c mice injected with complete Freund adjuvant five developed plasma cell tumors. They were named MSPC-1 to MSPC-5. The range of chromosome number distribution, modal chromosome number, ratio of cells at diploid(s), triploid(1.5s), tetraploid(2s) and octoploid(4s) level, specificity of γ-globulin in serum and urine in all five tumors are given in Table 1. In all those neoplasms no marker chromosomes were observed in the primary state.

Table 1. Karyological and biochemical characteristics of 5 plasma cell neoplasms (MSPC)

Name of tumors	Range of chrom. no.	Mode	% of polyploid cells*				No. of cells observed	Specific protein	
			1s	1.5s	2s	4s		Serum	Urine
MSPC-1	38-81	40	92	0	8	0	50	γ-A	—
MSPC-2	39-93	86	14	8	78	0	50	γ-F	—
MSPC-3	35-94	39	24	2	64	0	50	γ-F	λ-chain
MSPC-4	39-168	78	12	2	84	2	50	—	—
MSPC-5	35-94	44	72	4	24	0	50	γ-A	—

* 1s, 1.5s, 2s and 4s denote respectively near-di-, near-tri-, near-tetra- and near-octoploid chromosome numbers.

[1)] This work was supported by a research grant from the National Cancer Institute (CA 07798-03), Public Health Service, U.S.A.

[2)] Virus Institute, Kyoto University, Kyoto.

Change of Ploidy in Plasma Cell Tumors (MSPC) in Early Transplant Generations[1)]

Hirotami T. IMAI, Tosihide H. YOSIDA and Kazuo MORIWAKI

In mouse plasma cell neoplasm MSPC-1, the majority of cells (92 per cent) were characterized by having near-diploid chromosome number (s-range) in the primary state. Among them, 68 per cent cells showed exactly diploid chromosome number (40). The tumor was transplanted to 3 mice, in 2 of them successfully. But the development of the transplanted tumors was very slow. About four months after inoculation, a small tumor was recognized in the site of inoculation. The chromosome number in most cells of the tumor thus developed was reduced to 39. In the second transplant generation, 3 mice were successfully transplanted. Among them, one mouse (2a) was killed 29 days after inoculation and its tumor cells were examined. 48 per cent of them were characterized by having near-tetraploid chromosome (2s) number. The mode was at 78 chromosomes. In another mouse (2b) which was killed 43 days after transplantation, cells at 2s level were increased to 66 per cent. The mode, however, was decreased to 76 chromosomes. In the remaining mouse (2c) cells at 2s level increased to 70 per cent, while the mode was reduced to 73 chromosomes.

Tumor 2a, one of the second transplant generation, was again successfully transplanted to three other mice (3a, 3b and 3c). Since this generation, two tumor lines, one with diploid and another near-tetraploid chromosomes, were established separately. The diploid line of MSPC-1, however, easily changed to tetraploid condition in the course of transplantations.

In the case of MSPC-3 plasma cell tumor, 14 and 34 per cent cells were at diploid level in the primary solid and ascites tumors, respectively. In the first transplant generation of the solid tumor frequency of diploid cells was reduced to 6 per cent. Frequency of near-diploid cells in another MSPC-5 line which was characterized by 44 modal chromosomes was 72 per cent in the primary tumor, but in the first transplant generation cells at near-diploid level were remarkably decreased to about 9 per cent, and in the second transplant generation no cells at diploid level could be observed in the tumor cell population.

Based on the above results, we conclude that plasma cells can develop to malignancy in diploid condition, but they easily change to tetraploid condition in the course of cell multiplication.

[1)] This work was supported by a research grant from the National Cancer Institute (CA 07798-03), Public Health Service, U.S.A.

Comparative Study of Karyotypes in Sublines Producing and Non-Producing γ-Globulin in Mouse Plasma Cell Tumors[1)]

Tosihide H. YOSIDA, Michael POTTER and Hirotami T. IMAI

Chromosomes of γ-globulin producing (positive) and non-producing (negative) sublines in three mouse plasma cell tumors, RPC-6A, RPC-20 and MOPC-70, were compared in order to ascertain whether a karyotypic difference could be found between them. All negative lines were derived from their positive parental lines in the course of serial transplantations. The RPC-6 positive and negative lines were examined at the 56th and the 73rd transplant generations, respectively. In RPC-20 line, they were at the 77th and 75th transplant generations, respectively, while, in the MOPC-70A positive line cells at the 12th transplant generation were observed. 70A·10A and 70A·10E positive lines which were established by 10 cell transplantations of the 70A line were also observed. They were at the 69th and 59th transplant generations, respectively. In the γ-globulin negative line, the 82nd transplant generation was used (Table 1). As the table shows, karyotypes of all γ-globulin non-producing (negative) sublines were remarkably different from those of their parental positive lines.

In the RPC-20 line the change of karyotypes in the negative line from that of the positive parental line was clear. In the positive line, 77 and 76 chromosomes, among them as markers one submetacentric (SM), one metacentric (M) and one minute (m), were observed most frequently. The long telocentric chromosomes (TC) with secondary constriction near the centromere were another marker in this line. They have the appearance of SAT-chromosomes. In the negative subline, however, 75 and 74 chromosomes, among them one SM-, one M- and two m-markers, were usually observed. In the karyotype of this subline only one TC-element was found. One new minute marker found in the negative line was similar to the SAT-shaped element of TC-chromosome. Based on the above investigations, it is suggested that the karyotypes of the negative line had developed by breakage at the secondary constriction of the TC-chromosome included in the positive line karyotype, and the SAT-like element with centromere which resulted from the breakage remained in the negative line karyotype as a new minute, and then some telocentric chromosome was lost, producing the negative line karyotype from that of the positive line.

[1)] This work was supported by a research grant from the National Cancer Institute (CA 07798-03), Public Health Service, U.S.A.

Table 1. Karyotypes in protein producing (positive) and non-producing (negative) sublines in mouse plasma cell tumors

Tumor line	Protein	Chromosome no. (Mode)	Marker chromosome*	γ-Globulin	Transplant generation
RPC-6A	Positive	64–176 (68)	1M	γ-A serum	56
〃	Negative	53–145 (73)	2M, 2SM	None	70
RPC-20	Positive	67–154 (77)	1M, 1SM, 2TC, 1m	Lambda chain	69
〃	Negative	40–145 (75)	1M, 1SM, 1TC, 2m	None	70
MOPC-70A	Positive	37–128 (73)	None	γ-F serum, excess kappa	12
MOPC-70A·10A	〃	57–68 (66)	〃	〃	69
MOPC-70A·10E	〃	63–70 (68)	〃	〃	59
MOPC-70A	Negative	68–86 (77)	2L, 1m	None	82

* M=metacentric; SM=submetacentric; L=extremely long telocentric; TC=telocentric with secondary constriction near the centromere; m=minute.

The relation of karyotype change between positive and negative sublines in the other two lines (RPC-6A and MOPC-70A) was not clearly recognized, because the difference between their karyotypes was too complicated.

Karyotypes of Mouse Plasma Cell Tumor MOPC-31B before and after *in Vitro* Cultivation[1]

Tosihide H. YOSIDA, Hirotami T. IMAI, Yujiro NAMBA[2], Toru MASUDA[2], and Sunsuke MIGITA[2]

Mouse plasma cell tumor MOPC-31B which was obtained from Dr. M. Potter of the National Cancer Institute, Bethesda, U.S.A., was characterized by producing γ-F globulin and excess kappa chain. Number of chromosomes of the tumor cells at the 61st transplant generation ranged from 76 to 81 showing the highest frequency at 80 (54 per cent). Among 50 cells 90 per cent had one long telocentric and one minute as marker chromosomes, while only 8 per cent cells showed one metacentric marker in addition to the above two. These tumor cells were cultivated *in vitro*

[1] This work was supported by a research grant from the National Cancer Institute (CA 07798-03), Public Health Service, U.S.A.

[2] Virus Institute, Kyoto University, Kyoto.

by Y. Namba, one of the authors. After 10 culture generations, chromosomes in 50 tumor cells were again analysed. Among them 64 per cent cells had three marker chromosomes (one long telocentric, one metacentric and one minute) which were observed rarely in the parental ascites form. On the other hand, cells with two markers commonly observed in ascites tumor were never found in the cells of the culture adapted line, although they produced γ-F globulin and kappa chain as well as the ascites form.

Comparative Study of Mouse Leukemias Developed by Treatment with Chemicals and Radiation[1)]

Tosihide H. YOSIDA, Reiko TSURUTA and Yoshinori KURITA

In order to find a relation between the chromosomal condition of mouse leukemias and the source of carcinogenic agents, we have used methylchoranthren, DMBA, and γ-radiation for the induction of leukemias. In the present experiments chromosomes of 28 leukemias were observed. Among them 11 leukemias were induced by treatment with methylchoranthren given to adult RF-strain mice, 3 by treatment with DMBA of adult RF strain mice, 2 by DMBA given to newborn RF strain mice, 2 by DMBA given to newborn Swiss albino (SWM) mice, 4 by treatment with γ-rays of adult C57BL mice, and one by treatment with DMBA and γ-rays of an RF adult mouse. Five spontaneous leukemias of RF-strain mice were also observed. The chromosomes of all leukemias developed primarily were observed in various organs, such as bone marrow, spleen, thymus and lymphnodes. The results of observations are summarized as follows:

1) Leukemias induced by chemicals and γ-radiation showed a mode of chromosome numbers varying from 39 to 48.

2) The modal chromosome number was different by the organ examined. Frequency (per cent) of cells with diploid 40 as modal chromosome number to those with chromosomes over and under 40 as the mode differs markedly by the organ examined; namely, in bone marrow, spleen, thymus, and lymphnodes it was 76.2, 66.7, 23.6 and 53.3 per cent, respectively. Cells with chromosome numbers outside of 40, mostly had 41 chromosomes.

3) Among leukemias developed by treatment with DMBA three deve-

1) This work was supported by a Grant in Aid for Foundamental Scientific Research from the Ministry of Education in Japan (No. 94002, 1966), and by a research grant from the National Cancer Institute (CA 07798-03), Public Health Service, U.S.A.

loped by injection of the chemical at the adult stage, and other four developed by injection of the same drug to newborn mice. The former showed normal diploid chromosome numbers in all organs examined, while in the latter deviating chromosome numbers from normal karyotype were found in many organs examined.

A clear relationship between chromosome alteration and development of mouse leukemias by various carcinogenic agents could not be found at present. The study will be continued.

Alteration of Karyotypes in a Mouse Leukemia Strain DML[1)]

Reiko TSURUTA and Tosihide H. YOSIDA

An ascites leukemia strain DML developed in an RF-strain mouse by treatment with DMBA had 40 chromosomes in the primary tumor. All chromosomes were rod-shaped like those of the normal karyotype of the mouse. The chromosome number was reduced to 39 at the first transplant generation and was again reduced to 37 (mode) and 38 in the 5th transplant generation. Cells with 37 chromosomes were characterized by having two submetacentric elements, one large and one small, and those with 38 chromosomes had one small submetacentric chromosome. From the karyological analysis it is suggested that cells with 38 chromosomes have developed from those with 37 chromosomes by breakage of the large submetacentric element at the centromere. After the 11th transplant generation cells with 38 chromosomes were observed most frequently in the cell population, and this condition was maintained until the present 20th transplant generation.

In the 6th transplant generation the solid tumor developed at the site of inoculation was accompanied by an ascites tumor in the peritoneal cavity. The solid and ascites type tumors were transplanted separately and two tumor lines were established. Karyotypes of the solid type tumor did not change from those of the original ascites line. In mice bearing solid type tumors, bone marrow, splean and mesentery lymphnodes were examined karyologically. In these organs cells with typical DML karyotypes were usually observed. This result means that the DML-cells transplanted subcutaneously invaded easily those organs.

[1)] This work was supported by a Grant in Aid for Foundamental Scientific Research from the Ministry of Education in Japan (No. 94002, 1966), and by a research grant from the National Cancer Institute (CA 07798-03), Public Health Service, U.S.A.

Changes in Aggregate-Forming Activity of Cells in Carcinogenesis[1)]

Yukiaki KURODA

Dissociated cells from 10-day embryonic chick liver and heart were cultured in monolayer in standard culture medium. After 2 days of cultivation the cells were infected with Rous sarcoma viruses (RSV) for 50 minutes at 38°C, washed and cultured for another two or five days in monolayer. The cells were collected by treatment with trypsin and cell suspensions containing each 3×10^6 cells in 3 ml culture medium were rotated on a gyratory shaker by the standard procedure. After 24 hours of rotation the aggregation patterns of RSV-infected cells were compared with those of non-infected control cells which had been cultured for corresponding day number in monolayer.

Aggregates formed from RSV-infected liver cells showed an increase in average diameter in comparison with those from non-infected liver cells. RSV-infected heart cells also formed larger aggregates than those from non-infected heart cells. These results indicate that embryonic chick cells transformed by infection with Rous sarcoma virus may have altered their surface properties functioning in mutual cohesiveness in aggregate formation. The increase in adhesiveness in transformed cells coincides with the previous findings of the piling-up behavior of RSV-infected transformed cells and loss of contact inhibition of neoplastic cells.

A malignant tumor that appeared spontaneously in mammary glands of the dd_1 mouse was dissociated by treatment with trypsin. Cell suspensions containing each 3×10^6 cells in 3 ml culture medium were rotated by standard procedure. Aggregation patterns obtained from rotation cultures of mammary tumor cells were compared with those from control cultures of normal mammary gland cells of the mouse.

Mammary tumor cells formed after 24-hour rotation some large aggregates 0.5 mm in diameter and many small aggregates. This was in clear contrast with the complete absence of such aggregates in 24-hour control cultures of normal mammary gland cells.

It has been reported that the embryonic cells showed age-dependent changes in aggregation patterns and that cells maintained in monolayer cultures showed a decline in aggregate-forming activity. Changes in aggregation patterns shown in the neoplastic transformed cells may have some relation to changes accompanying differentiation which take place in the cells.

[1)] This work was supported by a Grant in Aid for Foundamental Scientific Research from the Ministry of Education in Japan.

Difference in Aggregate-Forming Activity between Normal and Malignant Cells[1)]

Yukiaki KURODA

Human cervical carcinoma, strain HeLa cells, were intermingled with a variety of normal cells from chick embryos and tested in rotation cul tures for their selective sorting-out property in co-aggregates with normal cells from tissues of various embryonic origins. It was previously found that cells of different histogenetic identities (heterotypic), when co-aggregates, tended to become sorted out into distinct, type-specific groupings, while cells with similar histogenetic functions (isotypic), though from genetically remote animals, remained interspersed within composite aggregates and formed chimaeric, mosaic tissues (Moscona, 1957).

When HeLa cells were intermixed with epidermal cells from 9-day embryonic chick dorsal skin and rotated for 24 hours, spherical or oval aggregates were formed. Internally, the cells were found grouped according to types; the aggregates consisted of a central distinct region of chick epidermal cells and of an outer region formed solely by HeLa cells. HeLa cells intermixed with 7-day embryonic chick liver cells of endodermal origin formed large aggregates with rough surface, which consisted solely of HeLa cells. Chick liver cells in the mixed cell suspension formed aggregates of spherical shape, separated from HeLa-aggregates.

When HeLa cells were intermingled with dermal cells from 9-day embryonic chick dorsal skin or mesoblast cells from 6-day embryonic chick limb-bud, aggregates formed after rotation for 24 hours consisted of chimaeric structures of HeLa cells and either of chick cells of mesodermal origin. In the aggregates HeLa cells and chick cells were interspersed and closely associated with each other.

The fact that HeLa cells became sorted out with embryonic chick cells of ectodermal and endodermal origin and formed chimaeric tissues interspersed by chick cells of mesodermal origin, suggests that HeLa cells might have originated from some mesodermal tissue of human cervix and might have maintain their original property after a long period of cultivation. Selective affinity of HeLa cells for a specific type of normal cells found in the present experiment may explain the selective mechanism by which the original neoplastic cells metastasize to some specific types of tissues or organs, though in topographically remote sites of the animal body.

[1)] This work was supported by a Grant in Aid for Foundamental Scientific Research from the Ministry of Education in Japan.

II. PHYSIOLOGICAL AND DEVELOPMENTAL GENETICS

Behavior of Nuclei in Germinating Pollen Grains of Wheat, Rice and Maize

Hitoshi KIHARA and Tadaaki HORI

Our microscopical studies on the behavior of the tube nucleus and two sperm nuclei in germinating pollen grains were carried out in three representative species of cereals, namely, *Triticum aestivum*, *Oryza officinalis* and *Zea mays*.

The germination of pollen grains was observed on self-pollinated stigmas. For staining of the tube nucleus and the male nuclei, acetocarmine solution was used. This was easy for wheat pollen grains, but very difficult for those of rice and maize. However if we strongly heat the pollen grains mounted in acetocarmine over the flame of an alcohol lamp, the trinucleate condition can be clearly seen in all three materials.

Normal pollen grains of all three materials contain one tube nucleus and two sperm nuclei. In general all three species follow the same pattern of behavior of the three nuclei, i.e., two sperm nuclei enter the pollen tube and the tube nucleus follows. This regular sequence in the movement of pollen nuclei is rarely disturbed.

Germination of the pollen grains on the stigma starts after 3–5 minutes. In wheat, the tube nucleus remains frequently in the pollen grain, while it almost always emigrates in rice and maize.

It is likely that male gametes are transported passively by the cytoplasmic stream to the pollen tube during germination, since they lie nearer to the germ pore than to the tube nucleus. It is suggested that an autonomous movement of male gametes may act as an auxiliary agent in transportation. The tube nucleus seems to be intimately connected with the cytoplasm and is located far from the germ pore. This may be the main reason why the tube nucleus enters the pollen tube later than the sperm nuclei.

Photoperiodic Response of Various *Oryza* Species. IX

Tadao C. KATAYAMA

One of the factors influencing photoperiodic sensitivity is the so-called accumulation effect. The accumulation effect is shown by the photoperiodic effectiveness of short day treatment interrupted by long day condition. Accumulation effect of strains of *O. sativa*, *O. sativa* var. *spontanea*, *O.*

perennis, *O. glaberrima*, *O. breviligulata* and *O. stapfii* was analyzed this year. A combination of 12^h30^m light+11^h30^m dark periods was used as short day condition, whereas natural day length from June to August was used as long day treatment.

W0027, a strain of *O. glaberrima*, treated by 15S (15 short days followed by long day condition) headed 3.7 days earlier than the control plot; plants treated by 5S+5L+10S (5 short days plus 5 long days plus 10 short days, followed by long day condition) headed 0.3 day earlier than the control plot. This result indicates that the interposed 5 long days cancelled out the effect of the first short day treatment. However, plants treated by 5S+5L+15S headed 26.0 days earlier than the control plot. Thus, post-treatment by a longer period of short days could bring about recovery from the cancelling effects of the interposed long day condition. On the other hand, plants treated by 10S+5L+5S headed 16.0 days earlier than the control plot. In this case the effect of a long initial period of short days could not be cancelled out by an interposed long day condition. From these results, the following conclusions are drawn. Even if the total number of given short days was the same, the longer was the initial short day treatment, the larger was the accumulation effect. In other words, complete photoperiodic induction is easily achieved by an early continuous short day treatment.

In order to clarify the differences between the accumulation effects in various strains or species, the heading dates of each strain treated in the same way were compared. Having received 15 short days as a total, four plots, i.e., 1) 15S, 2) 10S+5L+5S, 3) 5S+5L+10S and 4) 5S+5L+5S+5L+5S, were compared. For example Kyoto Asahi, a Japanese cultivated variety of *O. sativa*, headed respectively 17.7 days, 17.7 days, 15.3 days and 14.7 days earlier than the control plot in 1), 2), 3) and 4) (5 per cent l.s.d.=1.06). Even in combined treatment, the effect of pre-treatment was not cancelled out.

W0106, an Indian strain of *O. sativa* var. *spontanea*, headed 23.3 days, 26.7 days, 19.0 days and 1.3 days earlier than the control plot, in the above 1), 2), 3) and 4), respectively (5 per cent l.s.d.=4.84). In this strain, the effect of pre-treatment was almost completely cancelled by the combined treatment as the result in plot 4) shows. It is clear that the accumulation effect varies widely by the strain. Moreover, the lower was the latitude from where the strain was obtained, the smaller was the accumulation effect. This finding indicates adaptation to low latitudes, where the period of effective short days for photoperiodic induction is longer than in higher latitudes.

Anatomical Studies on Interior Root Found in Root of Rice Plant

Tadao C. KATAYAMA

Frequently in rice root, a lateral root grows into the cortex and elongates downward when the aerenchymatous tissue is strongly developed. Such root can be called "interior root." Interior roots are often recognized at the base of seminal and adventitious roots, but are never found in lateral roots. In many cases lateral roots, differentiated in mature plants or initiated from old main roots, remain as interior roots. They can be divided into the following six groups according to their development and shape. 1) Interior root elongates into the middle part of the cortex of the main root but not into exodermis; it bends there and extends downward. 2) It pushes into exodermis of the main root and remains there. 3) It reaches to the exodermis of the main root and further extends downward along it. 4) It extends downward and then pushes into the exodermis of the main root. 5) It elongates straight and pushes into the exodermis of the main root, extending there downward. Shape of the main root is consequently changed. 6) It breaks the exodermis of the main root and extends downward. In all cases, the tip of an interior root never breaks out through the exodermis of the main root.

Interior roots differ from normal lateral roots in anatomical structure, especially that of a small central cylinder. In general, they have been found in old roots or in basal parts of a root. Formation of interior roots may be caused by the following factors; decreased cell divisions, and hardening of cell membranes in old plants.

A Genetic Study on Skeleton-Length in Japanese Quail

Iwahiro ISOGAI, Takatada KAWAHARA and Kan-Ichi SAKAI

Eighty pairs, each of one male and one female bird, all selected at random from a quail population maintained for three generations as a closed flock in the National Institute of Genetics were propagated. Seven hundred birds from those pairs were killed and their bone-samples were stained with alizarin-red. The length was recorded of skull, cervical vertebrae, thoracic vertebrae, synsacrum plus caudal vertebrae, humerus, ulna, metacarpus, femur, tibia and tarsometatarsus.

By the analysis of variance and covariance, it was found that bones of a female were always larger than those of a male except for skull length which was not different. Heritability of bone length of posterior extremities (femur, tibia and tarsometatarsus) was 0.84 on the average, while the bones of anterior extremities (humerus, ulna and metacarpus) had

the little lower value of 0.68. Lower heritabilities were found in trunk bone length (thoracic vertebrae, synsacrum and caudal vertebrae) with the value of 0.38, cervical vertebrae with the value of 0.26, while the lowest value was obtained with the skull, the heritability being 0.21. Genetic correlations among different bones were generally higher than phenotypic correlations. Comparison among trunk bones, anterior and posterior extremities showed that genetic correlations between the two latter were very high with $r_g=0.87$. The trunk tended to be more or less highly correlated with posterior than with the anterior extremities. Bones within each body part were highly correlated with each other in comparison with correlations between different body parts.

Genetic Changes in Body Weight Caused by Competition in Chickens

Tohru FUJISHIMA

Three breeds of domestic fowl, White Leghorn, Rhode Island Red and Barred Plymouth Rock, were crossed by each other following the scheme of 3×3 diallel crosses, and the progeny were used to investigate the effects of competition on growth, and to divide the body weight, when competition occurred, into the components of body weight and competitive ability.

At one week of age, the males and females obtained from those crosses were divided into three paternal strain groups, three maternal strain groups and two mixed groups involving three different paternal strains. Each paternal and maternal strain group contained 27 birds comprising 9 birds of each of three different dams in a paternal strain and three different sires in a maternal strain respectively, while each mixed group contained a total of 27 birds consisting of 3 birds of each of three different dams in every paternal strain.

Therefore, it is probable that competition may occur among different maternal strains in a paternal strain group, among different paternal strains in a maternal strain group, and among both combined in a mixed group. The experiments were conducted under restricted and full feed conditions in the mixed groups, but only under restricted conditions in the paternal and maternal strain groups. The same amount of feed consumed by the full-fed group in the previous week on a per bird basis was fed to the restricted group every week during the experimental period. Under these experimental conditions, the birds were reared until 6 weeks of age and weighed every week.

The models for estimating the genetic parameters were as follows;

$$Yij=M+Si+Dj+(SD)ij+Eij$$

where Yij=progeny mean of the cross of sire breed i and dam breed j. And, in paternal strain groups,

$$Si=si\ ,\qquad Dj=dj+Cdj\ ,\qquad (SD)ij=(sd)ij+Cdij$$

In maternal strain groups,

$$Si=si+Csi\ ,\qquad Dj=dj\ ,\qquad (SD)ij=(sd)ij+Csij\ ,$$

and in a mixed strain group,

$$Si=si+Csi,\qquad Dj=dj+Cdj\ ,\qquad (SD)ij=(sd)ij+C(sd)ij\ ,$$

where

si =effect of body weight of sire breed i,
Csi =effect of competitive ability of sire breed i,
dj =effect of body weight of dam breed j,
Cdj =effect of competitive ability of dam breed j,
$(sd)ij$=effect of interaction between si and dj,
$Cdij$=effect of competitive ability of dam strain j within i paternal strain group,
$Csij$ =effect of competitive ability of sire strain i within j maternal strain groups,
$C(sd)ij$=effect of interaction between Csi and Cdj,

Thus, in male progeny,

$$si=Ai+Li\ ,\quad dj=Aj+Lj+Mj\ ,\quad Csi=Aci+Lci\ ,\quad Cdj=Acj+Lcj+Mcj\ ,$$

and in female progeny,

$$si=Ai+Li\ ,\quad dj=Aj+Mj\ ,\quad Csi=Aci+Lci\ ,\quad Cdj=Acj+Mcj\ ,$$

where

Ai =cumulative additive effect of the autosomal genes for the body weight of breed i,
Li =cumulative additive effect of the sex-linked genes for the body weight of breed i,
Mj =average maternal effect of body weight of breed j,
Aci =cumulative additive effect of the autosomal genes of competitive ability for breed i,
Lci =cumulative additive effect of the sex-linked genes for the competitive ability of breed i,
Mcj=average maternal effect of competitive ability of breed j,

$$\sum_i Ai=\sum_j Mj=\sum_i Li=\sum_i Aci=\sum_i Lci=\sum_j Mcj=0$$

Table 1. Variance components and heritabilities for body weight.

		2 weeks of age		6 weeks of age	
		Male	Female	Male	Female
Paternal strain group	σ_S^2	2.76	27.97	481.40	268.44
	σ_D^2	5.61	3.11	441.19	133.82
	σ_{SD}^2	9.35	17.01	617.69	740.46
	σ_E^2	69.16	75.17	1,806.07	1,785.62
	h_S^2	0.06	0.45	0.29	0.18
Mixed group	σ_S^2	2.82	18.78	101.96	486.45
	σ_D^2	11.30	2.44	601.98	76.93
	σ_{SD}^2	25.20	13.32	1,484.45	637.53
	σ_E^2	38.10	49.29	2,209.88	1,108.35
	h_S^2	0.07	0.45	0.05	0.42

Table 2. Autosomal, sex-linked, and maternal effects of body weight and competitive ability.

		Body weight			Competitive ability		
		W.L.	R.I.R.	B.P.R.	W.L.	R.I.R.	B.P.R.
Autosomal effect	A	−20.9	+98.1	−77.2	Ac − 1.7	−78.4	+80.1
Sex-linked effect	L	+68.4	−81.2	+12.8	Lc +13.0	+52.7	−65.7
Maternal effect	M	−31.7	−61.2	+92.9	Mc +35.8	+33.9	−69.7

W.L.: White Leghorn, R.I.R.: Rhode Island Red, B.P.R.: Barred Plymouth Rock

The results of the present experiment, as shown in Tables 1 and 2, indicate that when competition occurs in a population, the body weight of birds is genetically changed according to their competitive abilities, that is, although at 2 weeks of age when no competition yet occurs, the heritabilities for body weight in paternal strain groups are equal to those in mixed groups, while at 6 weeks of age, in mixed groups in which competition occurs they are considerably different from those in paternal strain groups, and the heritability for body weight at 6 weeks of age changes from 0.29 to 0.05 in males and 0.18 to 0.42 in females.

It is found from Table 2 that the autosomal and maternal effects on competitive ability are compensatory with each other, and the effects on competitive ability are compensatory with those on body weight.

A Genetical Study on Organ Formation in *Nicotiana tabacum* L.

Seiichiro HIGUCHI and Kan-Ichi SAKAI

This study aimed at finding the genetical basis of organ formation in *Nicotiana tabacum* L. Ten varieties were used for the study: Hicks, Connecticut Broad Leaf, Coker 139, Coker 316, Coker 319, Ibusuki, Daruma, Nicotin-free tobacco, T. I. 448A and Ambalema. Five plants selected at random from each variety were investigated for the size of vegetative and reproductive organs. Genetic correlations among them are given in Table 1.

Table 1. Genetic correlation coefficients between the dimensions of vegetative and reproductive parts of *Nicotiana tabacum* L.

		Short filament	Long filament	Style	Corolla diameter	Corolla tube	Calyx	Stipule			Leaf	
								Upper	Intermediate	Lower	Upper	Intermediate
	Long filament	0.94										
	Style	0.58	0.60									
	Corolla diameter	0.69	0.63	0.77								
	Corolla tube	0.59	0.65	0.90	0.85							
	Calyx	0.30	0.22	0.58	0.72	0.63						
Stipule	Upper	−0.01	0.08	0.18	0.16	−0.11	0.69					
Stipule	Intermediate	−0.16	−0.02	0.32	0.26	0.19	0.56	0.96				
Stipule	Lower	0.03	0.17	0.55	0.33	0.54	0.20	0.56	0.87			
Leaf	Upper	0.26	0.46	0.64	0.19	0.62	−0.14	−0.11	0.19	0.58		
Leaf	Intermediate	0.09	0.30	0.38	0.01	0.39	−0.32	−0.22	−0.13	0.29	0.87	
Leaf	Lower	−0.02	0.09	0.37	0.30	0.48	−0.28	−0.33	−0.12	0.40	0.68	0.86

It is found from Table 1 that the dimensions of flower parts, *i.e.* filaments, style and corolla, are mutually highly correlated, but not with those of stipules and leaves. Calyx is moderately correlated with either flower organs or stipules. Stipules are highly correlated with each other but not with leaves, while leaves are highly correlated among themselves. Thus, according to size, organs of a tobacco plant may be divided into

three groups: 1) flower parts, *i.e.* stamens, pistil and corolla, 2) stipules and 3) leaves. No conclusion can be drawn at present for the calyx. Within each group, genetic correlations were relatively high, whereas those between different groups tended to be low. In addition, there was a tendency to high correlation between adjacent parts. No definite tendency could be seen in environmental correlations.

Major Gene and Polygenes Governing the Rachis Deficiency in Rice

Kikuo WASANO and Kan-Ichi SAKAI

The present study aims at finding the role of polygenes affecting a major gene-controlled character in rice. The character under consideration is the rachis deficiency controlled by a recessive *sp*-gene. The nature

Table 1. Effect of major gene, *sp*, and polygenes affecting the *sp* phenotype, on some quantitative characters in rice

	Character	sp^+	*sp*			*sp* effect (M)	Polygene effect (P)	Ratio (P)/(M)
			L	H	m			
	Expressivity of *sp* gene	0	45.00	56.65	50.83	50.83**	5.83**	0.11
Weight of	Plant	40.45	42.16	35.66	38.91	− 1.54	−3.25**	2.11
Weight of	Panicles	18.36	6.32	3.99	5.16	−13.20**	−1.17**	0.09
Weight of	Straw	22.41	35.83	31.67	33.75	11.34**	−2.08	−0.18
Number of	Tillers	11.23	12.41	11.98	12.19	0.96**	−0.22**	−0.23
Number of	Panicles	11.04	11.37	9.95	10.66	− 0.38	−0.72*	1.89
Number of	Non-bearing tillers	0.19	1.03	2.02	1.53	1.34**	0.50**	0.37
Length of	Culm	102.65	99.33	90.76	95.04	− 7.61**	−4.29**	0.56
Length of	Panicle	21.14	15.89	14.12	15.01	− 6.13**	−0.89**	0.15
Length of	1st Internode	38.15	32.85	31.48	32.17	− 5.98**	−0.69**	0.12
Length of	2nd Internode	26.21	26.43	25.97	26.20	− 0.01	−0.23	⩾10.00
Length of	3rd Internode	21.92	23.10	20.87	21.99	0.07	−1.12**	⩾10.00

sp^+ : Normal phenotype.
L : Lines selected for low expressivity.
H : Lines selected for high expressivity.
m : (H+L)/2.
**, *: Significant at 1% and 5% level, respectively.
(M) : (H+L)/2−sp^+.
(P) : (H−L)/2.
⩾10.00 means that polygene effect is very large in comparison with the effect of major gene.

of the character is described briefly in the preceding issue, Ann. Rep. **16**: 74. In the F_2 population between a wild and a *sp*-strain, 88 plants of 323 in total were rachis deficient, homozygous for the recessive *sp* locus. Of these 88 segregants, selection for high as well as low expressivity was conducted. The results are summarized in Table 1. From Table 1, we find that, 1) the major gene, *sp*, not only governs the rachis deficiency but also increases straw weight and number of immature tillers and decreases weight of panicles per plant, panicle length and length of first internode or culm. 2) There are polygenes which affect the above character in addition to the major *sp*-gene. These polygenes also affect other characters pleiotropically. The effects, however, are not always the same as those of the major gene. 3) Polygenes enhancing rachis deficiency operate toward decreasing other quantitative characters. 4) The intensity of polygenes in comparison with that of the major gene, *sp*, is found in the last colmn of Table 1. It is given in the form of a ratio of polygene effect against the effect of the major gene. Of twelve ratios, straw weight and number of tillers show negative values indicating that the effect of polygenes is opposite to that of the major gene. It is also interesting that 2nd and 3rd internodes are little affected by the *sp*-gene, whereas they are more or less affected by polygenes.

Estimation of Genetic Parameters in *Chamaecyparis* Forests

Kan-Ichi SAKAI, Shigesuke HAYASHI and
Hiromasa MUKAIDE

Hinoki, *Chamaecyparis obtusa Sieb. et Zucc.*, is one of the most important tree species in Japan. They are propagated only from seed. The present study deals with investigations on inter-tree competition and estimation of genetic, environmental and competitional parameters in several forests of the species.

The forests were artificially planted and were 20 to 60 years old.

The adopted method of study will be published in detail in the coming issues of Silvae Genetica (1967). The results are presented in Table 1.

It is found from Table 1 that competition apparently occurs in *Chamaecyparis* forests. It is of interest to find that an amount of genetic variation for growth (h^2) appears to be inversely correlated with the index of competitive stress (c^2). This might suggest, though it is quite speculative at present, that in this species the genotypic growth is negatively correlated with competitive ability.

Table 1. Genetic, environmental and competitional variance, heritability (h^2), index of competitive stress (c^2) and correlation coefficient between adjoining two trees ($r_{i,\,i+1}$)

	Strain				
	Okayama (1)	Okayama (2)	Gifu (1)	Gifu (2)	Ōita (A)
Number of trees	200	200	207	191	246
Age	27	22	45	60	26
G	8.02±0.27	−2.35±1.61	6.83±0.12	11.82±6.57	−0.26±2.56
E	0.05±0.01	2.30±0.49	1.20±0.04	2.30±2.20	7.03±1.05
C	——	3.62±1.31	0.20±0.09	0.88±5.05	4.20±1.74
h^2	0.99	0	0.83	0.79	0
c^2	——	0.61	0.02	0.06	0.37
$r_{(i,\,i+1)}$	+0.150	−0.190	−0.038	−0.122	−0.004

Developmental Genetic Study of Panicle Formation in Rice

Shin-ya IYAMA

Two populations of rice variety Norin No. 8, one irradiated by 20,000 r X-rays and the other as untreated control, were maintained by one parent-one offspring method for this investigation. At the fifth generation, 25 lines were derived from the control and 35 lines from the X-rayed population. Ten plants from each line were chosen at random, and the length

Table 1. Mean lengths of the various parts of culm and panicle in the control and the X-rayed population

Character	Mean length (cm)	
	Control	X-rayed
1. Third culm internode	16.758	16.368
2. Second culm internode	19.968	19.616
3. First culm internode	31.581	30.205
4. Panicle	18.245	17.555
5. Second rachilla	6.610	6.463
6. Third rachilla	7.015	6.812
7. Fourth rachilla	7.199	6.963
8. First rachis internode	2.473	2.376
9. Second rachis internode	1.316	1.297
10. Third rachis internode	1.818	1.734

of vaious parts of culm and panicle were measured. The culm internodes were numbered starting from the top and the parts within panicles starting from the base. The first rachilla was not dealt with because of its occasional absence. Analyses of variance and covariance were made and the genetic variances and covariances as well as the genetic correlations among those characters were computed. The characters investigated and the mean values of the two populations are given in Table 1.

Following results were obtained: 1) There were no significant differences between the means of the two populations as to all characters investigated. 2) Genetic variances of all but the second and the third panicle internodes were increased by the X-ray irradiation. 3) Genetic correlations among various parts in the control and the X-rayed population were computed as shown in Tables 2 and 3. Three groups of characters

Table 2. Genetic correlations among various parts of culm and panicle in control population

Character*	1	2	3	4	5	6	7	8	9	10
1	1.000	0.990	0.317	−0.177	−0.048	−0.040	0.141	−0.072	0.259	0.321
2	0.990	1.000	0.533	0.059	0.127	0.233	0.357	0.195	0.542	0.803
3	0.317	0.533	1.000	0.220	0.248	0.320	0.331	0.330	0.500	0.391
4	−0.177	0.059	0.220	1.000	0.890	0.934	0.915	0.920	0.048	0.428
5	−0.048	0.127	0.248	0.890	1.000	0.963	0.942	0.869	0.059	0.442
6	−0.040	0.233	0.320	0.934	0.963	1.000	0.977	0.975	0.264	0.655
7	0.141	0.357	0.331	0.915	0.942	0.977	1.000	0.944	0.305	0.678
8	−0.072	0.195	0.330	0.920	0.869	0.975	0.944	1.000	0.394	0.565
9	0.259	0.542	0.500	0.048	0.059	0.264	0.305	0.394	1.000	0.883
10	0.321	0.803	0.391	0.428	0.442	0.655	0.678	0.565	0.883	1.000

* Same as in Table 1.

were found as enclosed with broken lines, the genetic correlations between the two belonging to the same group being very high, while those between the two belonging to the different ones very low.

It was concluded from the results that 1) the variety itself had certain amount of genetic variation in the characters investigated. 2) Mutations induced by the X-ray irradiation occurred in both positive and negative directions, having no final effect on the population means. 3) From the genetic correlations (Tables 2 and 3), the genes concerning to the formation of various parts investigated in panicle and culm were divided into three groups, one is controlling the culm internode lengths, a second

Table 3. Genetic correlations among various parts of culm and panicle in X-rayed population

Character*	1	2	3	4	5	6	7	8	9	10
1	1.000	0.689	0.802	0.539	0.524	0.476	0.513	0.176	0.151	0.346
2	0.689	1.000	0.783	0.701	0.589	0.596	0.712	0.525	0.170	0.420
3	0.802	0.783	1.000	0.848	0.748	0.782	0.837	0.561	0.147	0.472
4	0.539	0.701	0.848	1.000	0.884	0.935	0.960	0.797	0.051	0.460
5	0.524	0.589	0.748	0.884	1.000	0.974	0.906	0.658	0.118	0 548
6	0.476	0.596	0.782	0.935	0.974	1.000	0.970	0.747	0.140	0.579
7	0.513	0.712	0.837	0.960	0.906	0.970	1.000	0.804	0.079	0.559
8	0.176	0.525	0.561	0.797	0.658	0.747	0.804	1.000	0.317	0.634
9	0.151	0.170	0.147	0.051	0.118	0.140	0.079	0.317	1.000	0.718
10	0.346	0.420	0.472	0.460	0.548	0.579	0.559	0.634	0.718	1.000

* Same as in Table 1.

controlling the lengths of panicle, the lower rachillas of panicle and the first rachis internode, and a third responsible for the lower internode lengths of panicle. 4) In addition, it was suggested from the genetic correlations of the X-rayed population that most new mutations would have been induced more or less pleiotropically for all the characters in the same direction, making correlations between groups in the X-rayed population higher than those in the control population.

A Developmental Genetic Study in Rice

M. S. BALAL and Kan-Ichi SAKAI

Organ formation in rice was investigated from the standpoint of statistical-genetics. Materials used were 99 lines selected at random from the Norin No. 8 variety established since 1959 and the same number of X-irradiated lines of the same variety. Characters investigated were length of panicle, first, second and third leaves counted from the top and first, second, third and fourth culm internodes. Analysis of variance between and within lines showed that both populations of 99 lines each contained significant genetic variation, indicating that in an established rice variety a certain amount of genetic variation was retained, on the one hand, and that the genetic variance in the X-rayed population was larger than in the control population, on the other hand. Estimation of genetic correlation and degree of pleiotropic effect of polygenes (see Sakai and Suzuki 1964, in Rad. Bot. **4**: 141~151) suggested that organs of

rice could be divided into two developmental groups. One is the upper organ group which includes panicle, first and second leaves and first culm internode, which show high positive correlation among each other. The second is the basal organ group which includes third and fourth culm internodes which are highly positively correlated. The remaining two organs, third leaf and second culm internode, are moderately correlated with the organs of either upper or basal group. In general, genetic correlation between neighbouring organs, regardless whether they were leaves, panicle or culm internodes, tended to be higher than that among remote organs.

A selection experiment for the length of third culm internode showed that the fourth was also affected by that selection, though it had little effect on panicle and first as well as second culm internode. The application of nitrogen at the time of panicle formation yielded about 40 per cent increase in length of third and fourth culm internodes but only a 10 per cent or less increase in panicle and first culm internode. An anatomical study indicated that the third and fourth culm internodes developed in advance of panicle primordia, while the upper internodes (first and second) developed after them.

The foregoing evidences indicate that organs developing in rice are not controlled by the same genes but by genes locally controlling specific organs.

Electrophoretic Comparison of Soluble Proteins from Different Organs of Tobacco Plant

Sumiko NARISE and Kan-Ichi SAKAI

In order to inquire into the mechanism of gene control over the development of higher plants, an electrophoretic analysis was undertaken with soluble proteins from different organs of *Nicotiana tabaccum* L.. Organs investigated were stamens, pistils, corollas, calyxes, nerves and mesophylls of stipules and also those of leaves. They were separately homogenized with the 0.1 M phosphate buffer (pH 7.2) and centrifuged. The supernatants were precipitated with saturated ammonium sulfate and the precipitate was used for analysis after dialysis against distilled water. Electrophoresis was carried out in columns of polyacrylamide gel using a modified method of Orstein and Davis. Protein bands were stained with amido black. All protein bands separated were compared on the basis of their relative mobility measured as fractions to the distance the fastest band manifested. The experiment was repeated five to ten times for each organ. By the *t*-test, twenty-eight different kinds of bands in all

were identified. Of those 28 bands, 14 were found in leaves from the middle part of stem, 22 in leaves from the top, 17 in stipules, 7 in calyxes, 10 in petals, 9 in pistils and only 5 in stamens. For the purpose of comparison among organs, it seemed appropriate to take into account not only the distribution pattern of bands, but also quantitative measurements of intensity of each band. The quantitative evaluation was made by grading the intensity into six classes 0 to 5 by naked eye. Thus, the results obtained from this combined comparison were as follows: There was a great deal of similarity among leaves in the number and the concentration of proteins of identical mobility, whereas not much similarity between leaves and reproductive organs. Furthermore, the similarity between stipules and leaves was relatively high and the same tendency was shown between stipules and calyxes. These results may help to solve the puzzle of genetic control over the development of higher plants.

Analysis of Genetic Correlations between Panicle, Internode and Leaf Lengths among Mutant Strains of a Rice Variety, Norin 8

Hiroko MORISHIMA and Hiko-Ichi OKA

For a survey of genetic variations in the sizes of different organs, 33 mutant strains induced from a rice variety, Norin 8, were grown in an experimental plot, and were measured regarding the lengths at maturity of the panicle, the first to fifth (from the top) internodes, and the first to fourth leaf sheaths and leaf blades. Those strains, obtained from the National Institute of Agricultural Sciences through the kindness of Dr. T. Kawai, were normally fertile and their yields were comparable to that of Norin 8.

Genetic correlations between the measured character were computed, and to find an integrated picture of character variations, the matrix of genetic correlations was studied by the method of principal component analysis. The first component appeared to represent the general size of various organs, the vector having almost equal loadings on all the characters. The scores given by this vector showed the variation between generally tall and generally short strains.

The second component vector had positive loadings on the panicle, the upper (first and second) internodes and the upper leaves (both sheath and blade; first and second), but negative loadings on the lower internodes and lower leaves. This indicates that the strains vary between a type having well-elongated upper organs and short lower organs, and the opposite type. These plant types may be called the "upward-elongation"

and "basal-elongation" types. The former type appeared to have higher yielding potential than the latter, when the scores given by the second component vector were compared with grain yields of the strains, estimated as panicle number per plant×spikelet number per panicle×fertility ×mean grain weight.

We have formerly found from a segregating population of rice two latent phases of character association which we considered as variation axes of "genetic plant types" (genetically conditioned character association), one representing the variation between the "panicle-number" and "panicle-length" types, and the other representing the variation between the "internode-length" and "internode-number" types (Ann. Rep. **16**: 68~69). The above-mentioned "upward-elongation" and "basal-elongation" types may be compared with the "internode-length" and "internode-number" types, respectively, as the former would have fewer but longer elongated-internodes than the latter. The occurrence of this plant-type variation in different materials seems to suggest that it might result from a general trend of the developmental system to respond to genetic variations.

The third component vector seemed to distinguish between different kinds of organs, *i.e.*, internode, leaf sheath and leaf blade. There may be a phase of correlation at which organs of the same kind are correlated more intimately than those of different kinds. The first to third components thus extracted could represent a 86 per cent portion of the total multi-dimensional variation.

Analysis of Growth Curves for Panicle and Internode Elongation in Mutant Strains of a Rice Variety, Norin 8

Hiroko MORISHIMA and Hiko-Ichi OKA

The growth and development of plants may be investigated from different viewpoints. This study is concerned with multivariate analysis of growth parameters separately obtained from different organs of rice strains. In 23 mutant strains induced from Norin 8 (a part of the materials mentioned in the previous report), the lengths of panicles and the first to fourth internodes were measured five times before and after heading at one week interval and at maturity. In each strain, growth curves of these organs were estimated by fitting the data to Robertson's equation, $\log(x/A-x)=k(t-t_{1/2})$, where x is the size at time t, A is the final size, $t_{1/2}$ is the time at which a half of the final size is reached, and k is a parameter for growth rate. The actual growth rate (dx/dt) was computed from these values.

Regarding the variations in A, $t_{1/2}-H$ (number of days between the $t_{1/2}$

time and heading time), and dx/dt of different organs, genetic variances and correlations were computed. To observe the pattern of association of these values, principal components were extracted from phenotypic correlation matrices of A, $t_{1/2}-H$ and dx/dt, respectively. Genetic contributions to the components were then estimated in terms of the regression coefficients of genetic values on the component axes by a method newly devised by Hashiguchi *et al.* (in press). From the "genetic vectors" obtained, variation patterns in the growth of those organs were estimated as follows: 1) In the final size (A), the first component (A_I) showed the variation in general size of all the five organs, and the second component (A_{II}) showed the phase of variation between the "upward-elongation" and "basal-elongation" types, in the same manner as set forth in our previous report. 2) The first component from the interval between $t_{1/2}$ time and heading time represented the general earliness of panicle and internode elongation relative to heading. 3) The first component from the growth rate (dx/dt) represented variation in the "general growth rate", while the second component represented a phase of variation at which the growth rates of panicle and the third internode were negatively associated with those of the first and second internodes.

The component scores given by these vectors were computed in each strain. Correlations between the scores indicated that 1) strains having generally long organs tended to have a generally high growth rate for those organs, and 2) strains of the "upward-elongation" type tended to have a low rate of panicle elongation and a high rate of internode elongation, and *vice versa*. It seems that slow panicle growth and fast panicle emergence (due to fast internode elongation) bring about well developed panicles, elongated upper internodes and relatively short lower internodes. Such a growth type may have high yielding potential.

Differentiation of Aggregation-Promoting Materials from Embryonic Chick Liver Cells

Yukiaki KURODA

Trypsin-dissociated cells from avian and mammalian embryos when cultured by rotation-mediated procedure tend to aggregate and form histoformative structures. Liver cells dissociated from chick embryos at the ages of 7-, 8-, 10-, 14-, 18-, and 20-days were tested for their aggregability in rotation cultures. Liver cells dissociated from 7-day embryos formed two or three large aggregates after 24 hours of cultivation. Aggregates formed from liver cells of older embryos showed a gradual decline in average diameter. Almost complete loss of aggregability was

seen in liver cells dissociated from 20-day embryos.

Mixed suspension of liver cells from both younger and older embryos produced some aggregates which had an average diameter intermediate between those found for each of these ages separately. Histologically the major part of those aggregates consisted of liver cells from younger embryos.

Cell-free supernatants were obtained from reciprocation cultures of embryonic chick liver cells, and tested at 28°C for their activity in promoting aggregation. Supernatants prepared from 7-day embryonic liver cells were considerably enhancing aggregation of 7-day embryonic liver cells, but had no effect on those from 18-day cells. Supernatants prepared from 18-day embryonic liver cells had no effect on liver cells either from 7-day or 18-day embryos.

Supernatants prepared from 7-day embryonic liver cells promoted the formation of aggregates from 18-day embryonic liver cells by rotation *at 38°C* for three or more days. The promoting activity of aggregate formation by the supernatant seems to be temperature-dependent, since *at 28°C* this supernatant had no effect on the dissociated cells from 18-day embryo. It seems likely that non-aggregation of cells from older embryos might be related to changes accompanying progressing specialization. Incubation of such specialized cells with active supernatants may modify the cells so that they produce some materials acting on cell cohesion.

Characterization of Tissue-Specific Materials with Cell-Binding Activity Obtained from Embryonic Chick Cells

Yukiaki KURODA

It has been previously found that in the process of aggregation of dissociated embryonic cells some substances released from the cells into the culture medium promoted the adhesive property of each cell. These aggregation-promoting substances were contained in cell-free supernatants prepared from a culture medium in which dissociated cells were shaken by reciprocating motion at 100 rpm for 2 hours at 38°C. Supernatants prepared from 7-day embryonic liver cells showed an activity in enhancing aggregation of 7-day embryonic liver cells at 28°C, but no effect on 7-day embryonic heart cells, whereas supernatants prepared from embryonic heart cells acted only on embryonic heart cells in forming aggregates, but had no effect on embryonic liver cells; the aggregation-promoting activity of the supernatants was tissue- or organ-specific. In the medium added with 0.5 μg/ml actinomycin D or 5 μg/ml puromycin dissociated liver cells were inhibited from forming aggregates. These results suggest

that the production of aggregation-promoting materials by the cells might be mediated by DNA-dependent RNA synthesis and protein synthesis.

Some informations on the composition of the supernatants from 7-day embryonic liver and heart cells were obtained. Both those supernatants had UV absorption spectra with a peak at 280 mμ and some amount of protein was detected by chemical determinations with slight amounts of DNA and RNA. The supernatants lost their aggregation-promoting activity by heat-treatment at 80°C for 10 minutes.

Amino acid composition of proteins contained in supernatants from 7-day embryonic liver and heart cells was compared. For the purpose of this analysis Tyrode's solution was used in which the cells were shaken on a reciprocating shaker at 38°C for 2 hours. Molar ratios of amino acid components in acid hydrolyzates of supernatant proteins are given

Table 1. Molar ratios of amino acid components in proteins in supernatants prepared from 7-day embryonic chick liver and heart cells

Amino acid	Liver cell supernatant	Heart cell supernatant
Acidic and neutral		
Alanine	0.83	0.78
Aspartic acid	1.08	1.03
Glutamic acid	1.07	1.05
Glycine	0.97	0.84
Isoleucine	0.56	0.62
Leucine	1.00	1.00
Methionine	0.03	0.18
Phenylalanine	0.37	0.39
Proline	0.55	0.51
Serine	0.63	0.49
Threonine	0.45	0.49
Tyrosine	0.21	0.27
Valine	0.77	0.81
Basic		
Ammonia	1.40	0.96
Arginine	0.60	0.61
Histidine	0.32	0.30
Lysine	1.00	1.00

Samples were hydrolyzed in 6N HCl for 40 hours at 105°C. A Hitachi KLA-2 type amino acid analyzer was used. Amino acid components are expressed in molar ratios corresponding to 1.00 mole of leucine or lysine for acidic and neutral or basic amino acids respectively.

in Table 1.

Protein in supernatants prepared from 7-day embryonic liver cells contained more glycine and serine and less isoleucine, methionine, and tyrosine than those from 7-day embryonic heart cells.

In Vitro Cultivation of Single Cells from *Drosophila melanogaster* Larvae

Yukiaki KURODA

In vitro cultivations of single cells from various imaginal discs of *D. melanogaster* larvae were carried out. Newly laid eggs of a wild strain

Table 1. Components of synthetic medium for cultivation of *Drosophila* single cells

Ingredient	mg/1000ml	Ingredient	mg/1000ml
NaCl	2,100	L-Methionine	680
KCl	1,790	L-Proline	2,620
$CaCl_2 \cdot 2H_2O$	1,100	L-Phenylalanine	100
$MgCl_2 \cdot 6H_2O$	4,180	L-Serine	1,000
$NaHCO_3$	700	L-Tyrosine	380
$NaH_2PO_4 \cdot 2H_2O$	1,240	L-Threonine	340
K_2HPO_4	2,080	L-Valine	340
Glucose	1,000	Thiamin·HCl	0.02
Trehalose	4,000	Riboflavin	0.02
Glycogen	300	Pyridoxine·HCl	0.02
L-Arginine	200	Niacin	0.02
L-Aspartic acid	120	Ca-pantothenate	0.02
L-Asparagine	630	Biotin	0.01
L-Alanine	990	Folic acid	0.02
β-Alanine	900	Choline·HCl	0.2
L-Cystine·HCl	430	Inosite	0.02
L-Glutamic acid	170	*p*-Aminobenzoic acid	0.02
L-Glutamine	1,460	Malic acid	600
L-Glycine	1,080	α-Ketoglutaric acid	350
L-Histidine	920	Succinic acid	60
L-Isoleucine	70	Fumaric acid	60
L-Leucine	140	Penicillin G potassium	5,000 units/ml
L-Lysine·HCl	430	Dihydrostreptomycin sulfate	5,000mg/ml

pH was adjusted to 6.6 with 1 N NaOH.

(Oregon-R) were collected and sterilized in 70 per cent ethyl alcohol for 10 minutes. The eggs were transferred to autoclaved food medium contained in bottles. Mature third-instar larvae (96 hours after hatching at 25°C) grown under those sterile conditions were dissected in order to isolate various imaginal discs used for cultivation. Wing discs, eye-antennal discs, leg discs, testes, and ovaries were cultured in a synthetic medium supplemented with 10 per cent calf serum in TD-7 flasks at 25°C. The components of the culture medium are shown in Table 1. Detailed procedure for cultivation will be described elsewhere.

Another attempt was made to dissociate the imaginal discs from mature larvae into single cells by treatment with trypsin. Isolated imaginal discs were incubated in 1 per cent trypsin solution for 15 minutes. After rinsing with physiological saline the discs were dispersed into single cells in the culture medium by flushing them briskly through the tip of a fine pipette. The single cell suspension obtained in this way was distributed into TD-7 flasks and incubated at 25°C. Establishment of cell lines having some distinct genetic markers and isolation of clones from a variety of tissues or organs by colony formation procedure are carried out.

III. BIOCHEMICAL GENETICS

Some Notes on the Chromogranules in Hypodermal Cells of Silkworm Larvae

Mitsuo TSUJITA

When a part of white turbid cytoplasm of the hypodermis of 5th instar larvae is placed on a seat mesh covered with a collodion film and electron-microscopically examined, a number of elliptical, oval or spindle shaped chromogranules into which the electron-rays do not completely penetrate can be observed. The chromogranules in larval skin cells of the *w-c* strain have almost uniform size ($1\times1.5\,\mu$). On the contrary, in C-108 strain those having the usual size are mixed with small ones. In some chromogranules their granule (vesicle) membrane can be clearly discriminated. Their density is 1.4~1.7 which is higher than of any other cell organelles.

In general, in the cytoplasm surrounding the chromogranules no structure interrupting intense electron rays can be observed. This seems to indicate that the chromogranules produced abundantly in the cytoplasm of larval skin cells of the normal strain are the organelles which bring about the non-transparency of larval skin.

Each mature chromogranule consists of a vesicle and a thin membrane surrounding it. In the course of development, ribosomal particles, 150~200 Å in diameter, arrange themselves in a uniform layer at the periphery of the granules. It seems that they are intimately associated with the granule membrane. Although those particles are apt to be lost after the maturation of the granule, it was often observed that most of them remain sticking to the membrane.

The chromogranules disintegrate after the loss of granule contents in the following cases.

1) A part of chromogranules in hypodermal cells of larvae disintegrates in each instar, especially at the moulting stage. The granule membrane loses its normal function, the stored secretion products come out and then it disintegrates.

2) All of chromogranules in hypodermal cells of the larvae disintegrate at the full grown larval stage: They begin to issue forth their secretion products through their membrane subjecting it to some change. Losing the secretion products, the ghosts, *i.e.* the membranes, remain and then they also disappear. The density of chromogranules is remarkably reduced by loss of granule contents.

3) The fact that disintegration of chromogranules can be artificially

induced was reported (Tsujita and Sakurai, 1963). This disintegration process was electron-microscopically studied.

When mulberry leaves painted with 0.3 per cent melamine solution were continuously fed to normal or lemon yellow larvae from the beginning of their instar, disintegration of chromogranules started 2~3 days after the treatment, namely, the contents of granules were lost through their membranes and later the membranes disappeared.

All chromogranules disintegrate and pteridine compounds and uric acid are lost from hypodermal cells. Thus, larvae treated with melamine become highly transparent. Normal opaque hypodermis could develop in larvae with transparent hypodermis that were artificially produced, when they were fed for about two days with fresh mulberry leaves without melamine. In this recovery process a number of minute particles first appeared in the cytoplasm and they gradually increased in size. Although the skin of larvae in this stage exhibited semi-transparency, when those minute particles in hypodermal cells grew to usual size, the larvae showed white non-transparent skin.

4) It is difficult to fix the complete content of chromogranules because almost all of them are lost in the procedure of fixation and dehydration. For example, after fixation with osmic acid, dehydration was carried out through alcohol from low to high concentration. During this procedure the materials were examined by electron microscope and it was found that the granule contents were almost all lost through the granule membranes during dehydration.

Development of Chromogranules in the Larval Skin of the Silkworm

Mitsuo TSUJITA and Susumu SAKURAI

The developmental process of the chromogranules in larval skin cells of the silkworm was studied by electron microscopical observation of thin sections.

The larval skin cells of the silkworm larvae have generally the shape of cylinders and the elliptical nucleus occupies a central position. At first, numerous ribosomes, 150~200 Å in diameter, assemble here and there in the cytoplasm forming ribosomal aggregates. A small vesicle appears in the center of each aggregate. The vesicles gradually increase in size and spherical, oval or elliptical granules are formed. The ribosomes arrange themselves uniformly in a layer surrounding the granule membrane and some proteinous substance can be seen inside the vesicle. It may be said that the precursor of chromogranules is a type of endoplasmic reticulum.

It was substantiated (Tsujita and Sakurai, 1964) that soluble proteins combining either with pteridine pigments or with uric acid are present in the chromogranules. It may be safely said that those compounds are contained in the granule vesicles and squeeze out when the chromogranules are pressed upon.

The well delimited precursor of the chromogranules can be precipitated by a procedure leading to the separation of the mitochondrial fraction.

It was stated above that the process of chromogranule development from initial to mature stage can be followed up in the cytoplasm of hypodermal cells. The early steps of the same developmental process can be followed up on the sections through the mitochondrial fraction.

Gene dependent variations were electron-microscopically observed as to shape and size of chromogranules produced in larval skin cells of normal strains and mutants with transparent larval skin.

It may be inferred that a large amount of uric acid found in the larval hypodermis of normal strains is mostly contained in the chromogranules. On the contrary, size and shape of chromogranules produced in the hypodermal cells of larvae with transparent hypodermis are abnormal and the amount of mature granules is decreased. The larval skin becomes transparent to varying degree in accordance with the shape and the amount of chromogranules. For example, in hypodermal cells of larvae homozygous for w^{oz} or *w-a* no mature chromogranules are found. Consequently, uric acid or pteridine compounds can be scarcely detected in their hypodermis. As for other mutant strains, transparency of larval skin and the amount of uric acid and pteridine compounds varies from strain to strain according to the shape and amount of chromogranules, especially their amount.

In the course of granule formation, the development of vesicle (granule) membrane with attached to it numerous ribosomal particles, which play some important role in the secretion of polypeptides, is the most important process. When it proceeds normally the granules have complete or sufficient ability for secretion and storage, and abundant chromogranules packed compactly with secretion products are formed in larval skin cells.

So far as our experimental results are concerned it seems that uric acid production and pteridine metabolism are normally carried out in the employed mutant strains with transparent larval skin. Therefore, it is inferred that in hypodermal cells of those larvae, the function of the granules, *i.e.* secretion and storage, is defective owing to the abnormal development of the granules, especially of granule membrane.

It was found by us that the granule membrane protein consists of a homogenous single unit protein. It seems that the granule membrane protein is made up by polymerization of this unit protein which is the

most important constituent of granule membrane.

Some Properties of Nucleic Acid in Larval Skin Cells of the Silkworm

Kunihiro KOJIMA, Mitsuo TSUJITA and Susumu SAKURAI

It was reported in our previous paper (1966) that quite a considerable amount of RNA is contained in the chromogranules of the larval skin cells of the silkworm and that the RNA is found in the ribosomal particles surrounding the membrane of chromogranules.

The present experiments were undertaken to clarify some properties of the nucleic acid obtained from larval skin cells and from chromogranules.

Larvae at late moulting stage, on 3rd, 5th and 7th day of 5th instar, and early and middle mounting stages of C-124 strain were used as materials.

In order to gather chromogranules, the larvae were cut open at the dorsal side and all their inner organs were removed. Then, the cytoplasmic layer containing the chromogranules of hypodermis was scraped off and gathered by a pincette tip, and the chromogranules were separated according to the procedure previously reported (Tsujita and Sakurai, 1966).

The nucleic acid was extracted from purified chromogranules or from hypodermis by the following procedure.

First, the sample was homogenized gently with teflon homogenizer in a solution of 0.14 M NaCl—1 mM $MgCl_2$—0.05 M acetate buffer (pH 6.0) containing 17 mg bentonite per ml (volume ratio of sample to solution 1 mg: 9 ml). The homogenate was stirred gently with an equal volume of cold 90 per cent aqueous phenol for 30 minutes and centrifuged at 13,000×g for 30 minutes. The aqueous phase was mixed with two volumes

Table 1. C-124 Nucleic acid content in the hypodermis

Larval stage	Nucleic acid		
	Soluble RNA	DNA	Ribosomal RNA
Late moulting stage of 4th instar	0.522	0.255	2.90
3rd day of 5th instar	0.658	0.362	6.25
5th day of 5th instar	0.210	0.036	1.98
7th day of 5th instar	0.255	0.040	1.68
Early mounting stage	0.127	0.038	1.30
Middle mounting stage	0.135	0.060	1.90

The amount of nucleic acid is shown by mg per g hypodermis including cuticle.

of ethanol, and then CH_3COOK was added until the solution came to 2 per cent of this compound. In order to obtain purified RNA and DNA this precipitate was dissolved in 0.2 M NaCl—0.05 M phosphate buffer (pH 6.7) and dialysed against 2 per cent CH_3COOK for 12 hours. The dialyzate was mixed with two volumes of ethanol, and CH_3COOK was added until the solution came to 2 per cent of this compound. Then this mixture was centrifuged at 3,000 rpm for 15 minutes and the precipitate was dissolved in 0.2 M NaCl—0.05 M phosphate buffer (pH 6.7).

Nucleic acid fractions were chromatographed on a methylated albumin Kieselguhr column by a simplified procedure and the content of soluble RNA, DNA and ribosomal RNA was measured.

Nucleic acid contents are given in Table 1. This table shows that the largest amount of RNA was detected in the hypodermis in larvae on 3rd day of 5th instar.

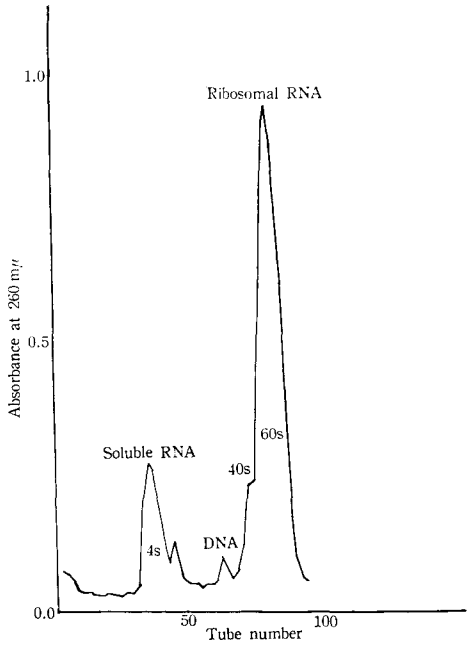

Fig. 1. Chromatographic profiles of the nucleic acid fraction from hypodermis on 3rd day of 5th instar.

Chromatographic profiles of RNA fraction obtained from hypodermis and chromogranules of larvae on 3rd day of 5th instar are shown in Figs. 1 and 2.

Those two chromatographic patterns indicate that both samples, of

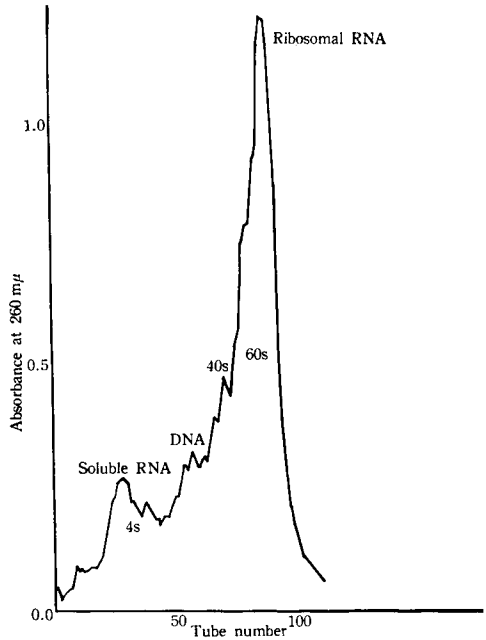

Fig. 2. Chromatographic profiles of the nucleic acid fractions from chromogranules on 3rd day of 5th instar.

hypodermis and chromogranules, contained 4 S soluble RNA, DNA and 40 S, 60 S ribosomal particles. Therefore, it may be said from these experimental results that the numerous minute particles surrounding the membrane of chromogranules are ribosomal particles.

Incorporation of ^{14}C-Glycine into Chromogranules of Larval Skin Cells of the Silkworm

Susumu SAKURAI and Mitsuo TSUJITA

In order to observe polypeptide synthesis in chromogranules, 0.3 μc of ^{14}C-glycine was injected into the body fluid of the silkworm at 4th moulting stage, on 3rd day and 5th day of 5th larval instar, and at full grown larval stage. The cytoplasm of the hypodermal layer was scraped off and gathered. The raw fresh cytoplasm was mixed with 0.25 M sucrose solution (volume ratio of sample to sucrose solution 1:10) and centrifuged at 900×g for 10 minutes.

The chromogranules were sedimented by centrifugation at 900×g for 10 minutes. Microsomes and mitochondria were isolated as shown in Fig. 1.

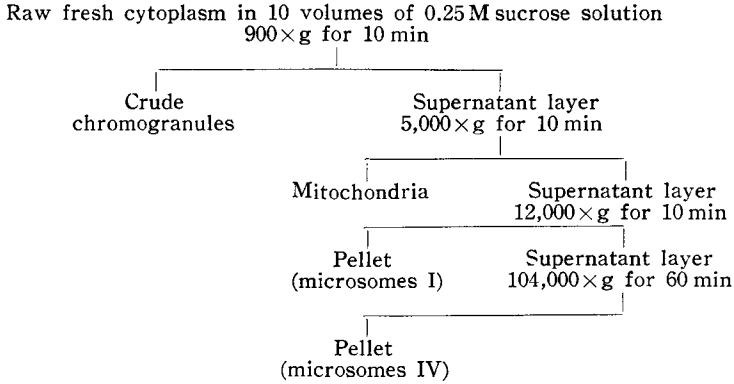

Fig 1. Isolation procedure of labeled cell fractions.

The supernatant layer was centrifuged at 5,000×g for 10 minutes. The resultant pellet was the fraction containing mitochondria and chromogranule precursors (Tsujita and Sakurai, 1966). The protein residues obtained from the labeled cell fractions according to the procedure of Schmidt-Thannhauser and Schneider (STS) were dissolved in 0.3 N-potassium hydroxide and the radioactivity was measured in a windowless gas flow counter.

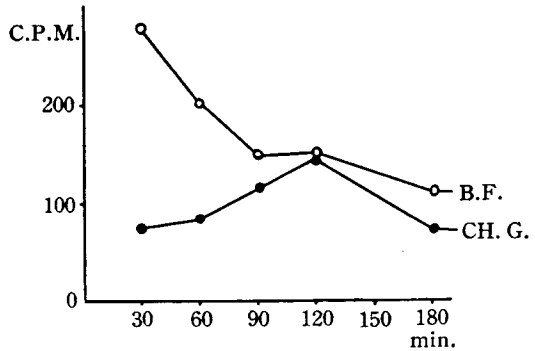

Fig. 2. Time course of incorporating reaction of ^{14}C-labeled amino acid into body fluid and soluble polypeptides of chromogranules.

C.P.M.: count per minute
B.F. : body fluid
CH. G.: chromogranules

As shown in Fig. 2, maximum incorporation of ^{14}C-glycine into chromogranules could be seen 2 hours after injection (Sakurai and Tsujita, 1966).

The chromogranules had higher radioactivity than the microsomes and mitochondria, and moreover ^{14}C was mostly distributed among polypeptide components of secretion products within the granules after incorporation of ^{14}C-labeled amino acid into the granules.

Chemical Characterization of Chromogranule Membrane in Larval Skin Cells of the Silkworm

Susumu SAKURAI and Mitsuo TSUJITA

Preparation and purification of insoluble protein from granule membrane—Purified chromogranules were dissolved in 2 per cent deoxycholate. The solution was dialysed against distilled water for overnight at 5°C. The precipitate formed was collected by centrifugation at 5,000×g for 10 minutes, suspended in 0.25 per cent sucrose solution and again centrifuged at 10,000×g for 10 minutes. The precipitate was dissolved in 8 M urea solution and 2-mercaptoethanol was added at pH 8.6 (volume ratio of sample to 2-mercaptoethanol 1 mg:1 ml). After 1 or 2 hours, the reaction mixture was eluted through Sephadex G 25 1.4×100 cm column which had been equillibrated with 0.001 M EDTA—0.5 per cent methylamine-HCl buffer at pH 8, to remove excess urea and 2-mercaptoethanol. The reduced membrane was then treated with *p*-chloromercuribenzoic acid(PCMB) at room temperature for 30 minutes and adjusted to pH 8 (Anfinsen 1961). The final mixture was dialysed against 0.5 per cent methylamine-HCl buffer at pH 8 and further dialysed against distilled water. The suspension of PCMB-membrane protein was lyophilized for storage.

Electrophoretic and ultracentrifugation analysis of PCMB-membrane protein—After removal of the PCMB by suspension in 0.001 M EDTA-0.5 per cent methylamine-HCl buffer at pH 8, 1 ml of mercaptoethanol per mg of the derivative was added and the fully reduced membrane protein was applied to the Sephadex column as described above. The reduced sample thus obtained was examined for electrophoresis. Polyacrylamide gels were prepared with 6 per cent cyanogum 41 in Tris-HCl buffer at pH 9. Electrophoresis was carried out in vertical position for 7 hours at 5 ma constant. Tris-glycine buffer at pH 8.7 was used as the electrolyte. The gels were stained with 0.1 per cent amido black 10 B in a solvent of methanol-water-acetic acid (5:4:1). Excess dye was removed by overnight washing the gel in a 10 per cent acetic acid.

As the electrophoretic pattern of Fig. 1a shows, purified granule membrane protein migrated as one clear-cut single band.

The mercaptoethanol reduced sample was dissolved in 5 per cent methylamine-HCl buffer at pH 8 and ultracentrifuged at 56,000 rpm.

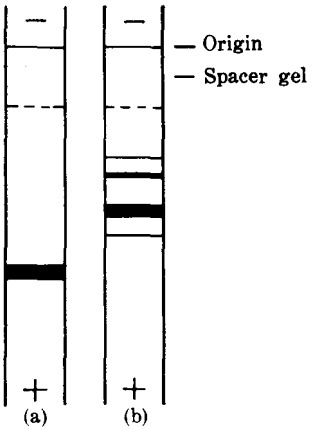

Fig. 1. Disk electrophoresis of protein from granule membrane of normal and oily mutant.
a. Protein from normal granule membrane.
b. Protein from granule membrane of oily mutant.

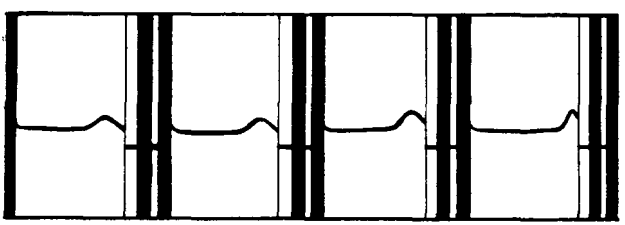

Fig. 2. Sedimentation diagrams of protein from normal granule membrane. Ultracentrifugation at 56,000 rpm at 4°C. The 0.25 per cent solution of the protein in methylamine-HCl buffer (pH 8). Photographs were taken at 8 minutes intervals.

As shown in Fig. 2, purified granule membrane protein of the normal strain exhibited a single symmetrical boundary in the analytical ultracentrifuge in 5 per cent methylamine-HCl buffer.

Thus, it may be inferred from the above-mentioned experimental results that the membrane protein of the chromogranules is composed of a homogeneous single unit protein.

According to our preliminary experimental results, the electrophoretic pattern of the membrane protein, obtained from chromogranules of hypodermal cells of ^{60}Co γ-ray induced *w-b* mutant with oily larval skin, exhibited two or three minor bands, not shown by normal granule membrane, and electrophoretic mobility of the major band differed from that of normal

granule membrane (Fig. 1b). This fact suggests that there is some defect in the formation of granule membrane protein and some important error occurs in the secretion and storage of polypeptides within the granules.

As to the constituent amino acids of the granule membrane protein, seventeen amino acids, lysine, arginine, histidine, aspartic acid, glutamic acid, threonine, serine, proline, glycine, alanine, cystine, valine, methionine, leucine, isoleucine, tyrosine and phenylalanine were detected using a Hitachi's amino acid analyzer.

Genetic and biochemical studies of the amino acid sequence in the unit protein of the granule membrane found in the present investigation and of the mode of polymerization of the unit protein in the formation of chromogranule membrane is now under way.

Genetic Effects of DNA in *Ephestia*

Saburo NAWA and Masaaki YAMADA

1. The experiment has been designed for inducing DNA-mediated genetic transformation in *Ephestia*. An imago of the wild type strain (NCR, a^+/a^+) is black eyed, while a homozygote (a/a) for the recessive gene a, which controls the enzyme tryptophan pyrrolase, is red eyed. Larvae of a/a were injected with DNA extracted from NCR adults. The injected larvae were permitted to develop into adults and were examined for eye-color. Some of them were crossed to untreated a/a to score the change in eye color in their progeny and in subsequent generation. Black-eyed animals have been obtained either among the treated animals or in subsequent generations. The results are summarized in Table 1. When

Table 1. Frequency of mutations among treated animals and in subsequent generations

Experiment	Treated generation		B_1		B_2	
	No. of adults examined	Black-eyed mutants	No. of adults examined	Black-eyed mutants	No. of adults examined	Black-eyed mutants
1	105	0	6,655	0	1,196	1
2	140	0	4,010	0	2,156	2
3	214	0	9,425	0	1,974	1
4	220	1	9,105	2	13,980	4
5	54	0	3,398	4	4,168	0
6	85	0	7,039	0	14,699	1
Total	818	1	39,632	6	38,173	9

transmission of the newly acquired black eye color has been followed for several generations by mating mutants with untreated a/a, it became evident that there were several types of mutants. 1) Most of the black-eyed mutants have been confirmed to be a^+/a heterozygotes. 2) One mutant male, which appeared in B_2 in Table 1, gave a^+/a heterozygotes and a/a homozygotes in the proportion of 6:1, when crossed with an a/a female, showing that the genotype of the mutant was not simply a^+/a. It may have been a mosaic. 3) A few mutants behaved as being a^+/a^+ homozygotes, since they gave only a^+/a progeny when crossed by a/a. These mutants appeared in the progeny (B_1 and B_2 in Table 1) derived from crosses of treated animals with untreated a/a. It is difficult to imagine the occurrence of a^+/a^+ homozygotes in the progeny from crosses with a/a. Special care has been taken to avoid contamination by migrating animals. 4) An unusual mutant, a black-eyed female, was obtained in the treated generation. When the female was crossed with an a/a male, all the B_1 animals were black, although only five were obtained, all females. All those females were crossed with a/a males. One pair gave no progeny. Three pairs gave in the total 356 imagos, all of which were black, but all were a^+/a heterozygotes. The fourth pair gave rise to 123 black-eyed offsprings, half of which were a^+/a heterozgotes, but the rest were not, and gave only black-eyed animals (a^+/a) in crosses with a/a. These observations could be interpreted on the assumption that a piece of DNA incorporated into the cell behaved as a particle which could reproduce itself, although its sudden disappearance cannot be plausibly explained. 5) One mutant female, which was apparently black-eyed, gave no black-eyed animals in subsequent generations when crossed with a/a. It seems likely that in this female the mutation occurred in somatic cells but not in germ cells.

Treatment of eggs of a/a with NCR-DNA has also been carried out, giving similar results.

In a reverse type of experiment, eggs of NCR (a^+/a^+) were treated with DNA extracted from a/a adults. When imagos developed from treated eggs were crossed with a/a, a red-eyed mutant has been obtained. Further experiments are now in progress.

2. Some properties of DNA used in the experiments have been described previously (Ann. Rep. No. 15). The molecular weight of the DNA was estimated from its viscosity and sedimentation. The intrinsic viscosity $[\eta]$ ranged from 70 to 110 for different preparations, using a rotating cylinder viscometer by Zimm and Crothers. The mean value of sedimentation coefficient ($S^0_{w,20}$) was 30. Using these values, we obtained 16 $\sim 23 \times 10^6$ as molecular weights for DNA prepared by the same method, which was used to extract DNA in the transformation experiments.

Peroxidase Isozymes in Leaves of *Pharbitis nil*

Toru ENDO

Although the biological function of plant peroxidases is yet poorly understood, it may be considered in relation to the role of indoleacetic acid oxidases. Peroxidases of intact cell sap were squeezed out from leaves of the Japanese mornning glory, *Pharbitis nil*, and were zymographically examined by starch gel electrophoresis, pH 8.5 borate buffer system. The enzymes were stained by slight modification of a method reported before (Endo, Rad. Bot. **7**:35). The final concentrations of the reactants were 0.03 per cent hydrogene peroxide, 0.1 per cent benzidine acetate and 0.01 M tris-acetic acid buffer, pH 4.0. Several isozymes were detected in six leaves of different age of a strain with normal leaves and blue flowers, TKS-046. The results showed that number and activity of isozymes generally increased with age.

On the zymogram of the youngest leaf, two or three blurred anodal bands were observed and one sharp as well as one weak cathodal band. In the oldest leaf, at least six anodal and two cathodal bands, sharp and heavy, were found. The sharp cathodal band showed similar activity in the course of development of the leaves examined. However, another similar strain, TKS-036 lacked the sharp band. In F_1 hybrids, TKS-046$\times$ TKS-036 and reciprocal, the isozyme band always appeared at the same location on the zymogram as that of TKS-046. No hybrid enzymes were detected.

Hormonal Enzyme Regulation in the Cultured Hypocotyl of *Pharbitis nil*

Toru ENDO

The *in vitro* effects of plant hormones and antihormones on enzyme systems were zymographically studied in cultured hypocotyls of *Pharbitis nil*. The hypocotyls were excised from 7 day-old seedlings, cut into pieces 15 to 25 mm long and cultured for three weeks on Linsmaier-Skoog medium (RM-1964) with or without hormones. The slant media, except for control, contained one of the following optional constituents; 10 ppm of kinetin (KT), gibberellic acid (GA), indole-3-acetic acid (IAA), naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) or 100 ppm of maleic hydrazide (MH), triiodebenzoic acid (TIBA), chlorocholine chloride (CCC), N-dimethylaminosuccinamide (B-9) and cyclohexaimide (CHI).

Morphologically, roots were formed on control, GA, CCC, B-9 and IAA

medium. A large callus developed, especially at the base of the cutting, on KT and IAA medium. A comparatively smaller callus was also found on NAA and 2,4-D, as well as, rarely, on control medium. No differentiation was observed on MH, TIBA and CHI media. Also, TIBA resulted in a browing of the cuttings except the parts near the ends.

The isozymes examined were those of peroxidase and esterase. Esterases were stained by a reacting mixture which contained 1 mM 1-naphthylacetate, 0.02 M phosphate buffer, pH 7.0, and 1 mg/ml of Fast Blue RR salt. Activities of both enzyme species were very weak in intact tissue, but in general increased considerably after culturing. The zymographic analyses showed, more or less, differences in enzyme pattern not only among the hypocotyl parts, callus and root, but also among the media containing different constituents. In the cultured hypocotyl separated from roots or callus, TIBA, NAA, 2,4-D, IAA and MH accentuated, in this order, the activity of several peroxidase isozymes, in comparison with the control. For instance, a few cathodal bands were newly detected in TIBA-cultured material. IAA was unique in that one sharp cathodal band was considerably activated. The other optional constituents were not very effective. Even CHI, inhibitor of protein systhesis, did not affect the formation and/or activation of peroxidases. At least four anodal and one cathodal esterase bands were observed in the hypocotyl after culturing. MH and 2,4-D appreciably activated those isozymes. A significant observation was made in TIBA-cultured material which did not show any esterase isozymes.

Variation in Peroxidase Isozymes of *Oryza perennis* and *O. sativa*

Yaw-En CHU

Peroxidase isozymes in various organs of a wild rice species, *Oryza perennis* Moench, were investigated using the starch-gel electrophoresis method. From leaf blade, leaf sheath, internode, basal node, and root, eight isozyme bands were found running toward the cathode and five bands running toward the anode. Each organ showed a specific pattern of band distribution, and gene dependent differences were found in the presence or absence of certain bands. In the leaf blade, leaf sheath and root, the activities of the bands were found to differ with the age of the organ.

In six *perennis* populations of different origin, within-populational variations in zymograms were examined. Populations of perennial habit, one from India and three from Africa, each showed several different zymo-

grams, but a population from the Amazon basin, supposedly of annual habit, was not polymorphic.

Further, the zymograms of leaf blade and leaf sheath were observed in 110 *perennis* strains collected from the natural habitats in different countries (each represented by one plant), 38 *sativa* strains from various Asian countries, and the intermediate *perennis-sativa* strains from Jeypore Tract, India. The zymograms of *perennis* strains were quite variable, and the frequencies of certain bands markedly differed among geographical strain-groups. Most African and American strains had bands 4A and 5C, which were not frequent in the Asian material. Of several zymogram types of leaf blade found among Asian *perennis* strains, two, peculiar to the Asian strain-group, were found to characterize *sativa* varieties, as shown in Table 1. Most Indica varieties had band 5C, which

Table 1. Comparison of leaf-blade zymograms between Asian *perennis* and *sativa* strains

Type no.	Zymogram	Asian *perennis*		Intermediate *perennis-sativa* from Jeypore			*sativa*	
		Perennial	Annual	Wild	Cultivated		Indica	Japonica
					Indica	Japonica		
1	2A	2	6	8	1	4	2	18
2	2A 5C	6	4	5	12	1	16	2
3	5C			1				
4	2A 4C 5C			1				
5	4A	7	5					
6	4A 5C	3	6					
7	4A 4C 5C							
8	2A 3A 4A 5C	1	1					
9	4C 5C		1					
No. of strains		19	23	15	13	5	18	20

most Japonica varieties did not show. The semi-wild strains from Jeypore Tract showed a similar variation pattern as found in *sativa* varieties.

These isozyme variations seem to suggest genic differences that may have some bearing on populational variability as well as on racial differentiation. It may be inferred that cultivated rice might have arisen from wild forms having certain peroxidase isozymes.

Characterization of Xanthine Dehydrogenase from *Drosophila*[1)]

Tomotaka SHINODA

Purification and general characterization of xanthine dehydrogenase of *Drosophila* were carried out employing wild types (ORNJ and PACN) and mutants (*lxd* and *Pm*; *Sb*/*Xa*). Some thousandfold purification of the enzyme was achieved by application of several methods and the purified enzyme was submitted to general characterization. The following results have so far been obtained (Table 1).

Table 1. General characteristics of xanthine dehydrogenase of *Drosophila*

	Strain				Complementation XDH
	ORNJ	PACN	$\frac{Pm:Sb}{Xa}$	*lxd*	
Km(AHP)$\times 10^{-6}$ M	7.4	8.1	7.0	3.2	—
Km(MB)$\times 10^{-5}$ M	4.6	6.8	5.8	—	—
Relative mobility	Fast	Intermediate	Slow	Intermediate	Fast
Specific activity	8,000	5,200	30,000	1,200	80
Heat stability	+	+	+	+	±
Molecular weight$\times 10^5$	2.5~2.6	2.5~2.6	2.5~2.6	2.5~2.6	2.5~2.6
% inhibition with 4 M urea	95.1	97.5	94.3	96.1	—

Many functional similarities were observed among the enzymes obtained from various strains. Both the electrophoretic mobility and the specific activity of the enzyme varied from strain to strain. These results may suggest that each enzyme of various strains has a different structure with common functions.

Multiple Molecular Forms of Xanthine Dehydrogenase in *Drosophila*[1)]

Tomotaka SHINODA

Xanthine dehydrogenase (XDH) of *Drosophila melanogaster* is affected by mutations of at least three loci, *ry*, *ma-l* and *lxd*. Of these, ry^+ is the structural gene for XDH, while *ma-l*$^+$ and *lxd*$^+$ play less direct roles in the regulation of XDH activity, probably by controlling a cofactor of XDH. In addition to genetic electrophoretic variants XDH exists in two forms (XDH-I, and XDH-II) which can be detected using DEAE column

[1)] This work was carried out at the University of North Carolina, Chapel Hill, North Carolina, U.S.A.

chromatography or polyacrylamide gel electrophoresis. In 4 per cent gel the XDH-I migrates approximately 8 per cent faster than the XDH-II. The kinetic properties of these forms do not differ greatly. Sucrose gradient centrifugation indicates that they sediment at slightly different rates. Conversion of XDH-I to XDH-II, but not *vice versa*, can be accomplished by incubation with *Drosophila* extracts that are deficient in XDH. Some properties of the enzyme are summarized in Table 1.

Table 1. Comparison of some properties of XDH-I and XDH-II

	XDH-I	XDH-II
Km(AHP)$\times 10^{-6}$ M	10.1	7.1
Km(MB)$\times 10^{-5}$ M	6.2	5.5
Sp. activity	4,700	10,200
Mobility	F	S
Mol. weight	260,000	250,000
% of remaining activity at 50°C, 1 h	68	61.2
Effect of *ma-l*$^+$(or *ry*$^+$) factor	+	−
% inhibition with 4 M urea	97.1	95.1

IV. EVOLUTIONARY GENETICS

An Intergeneric Hybrid between *Eremopyrum orientale* and *Henrardia persica*

Sadao SAKAMOTO

Henrardia is a monotypic genus in the tribe Triticeae represented by one species, *Hn. persica* (Boiss.) C.E. Hubbard. Morphologically this genus is different from other members of the tribe. In order to find phylogenetic relationships of this genus with other genera of the tribe, a strain of *Hn. persica* var. *glaberrima* (Hausskn.) C.E. Hubbard (2n=14, Iran) was crossed with various species of *Aegilops*, *Eremopyrum*, *Heteranthelium*, *Taeniatherum* and *Triticum* in 1965. From these crosses, 25 triploid F_1 hybrids were produced only when *Er. orientale* (Linn.) Jaub. et Spach. (2n=28, Iran) was used as the female parent.

Morphological characteristics of the F_1 hybrids were of *Eremopyrum* type, while the length of the spikes was intermediate. Disarticulation of the ripe spikelets was characterized by wedge type as that of the *Eremopyrum* parent. Average chromosome pairing per cell of the F_1 was $0.00_{III}+0.42_{II}+20.20_{I}$. Bivalents ranging from 0 to 4 were all terminally associated. Thus, no genomic homology was found between *Er. orientale* and *Hn. persica*. Complete sterility was observed. Colchicine solution (0.5 per cent) was applied to the tillering clones of the F_1 and 150 well-developed seeds were obtained in 1966.

Three Intergeneric Hybrids among *Heteranthelium piliferum*, *Eremopyrum buonapartis* and *Hordeum* sp.

Sadao SAKAMOTO

The genus *Heteranthelium* in the tribe Triticeae is monotypic with *Ht. piliferum* (Banks et Soland.) Hochst., having quite a specific spike characteristic, i.e. mixture of fertile and rudimental spikelets in one spike. In 1965 a strain of *Ht. piliferum* (2n=14, Afghanistan) was crossed with various species of *Aegilops*, *Eremopyrum*, *Henrardia* and *Hordeum*. The following two intergeneric hybrids were obtained: 1) two diploid hybrids, *Ht. piliferum*×*Er. buonapartis* (Spreng.) Nevski var. *buonapartis* (2n=14, Iran), and 2) a triploid hybrid, *Ht. piliferum*×*Hordeum* sp. (2n=28, material "No. 66w" was received from the All-Union Institute of Plant Industry, Leningrad, 1964). In the same year, 3) 19 triploid hybrids, *Er. buonapartis* var. *buonapartis*×*Hordeum* sp., were also produced.

Growth of hybrids under 1) was subnormal and the shape of the spikes

was of *Eremopyrum* type having no rudimental spikelets which are the character of *Heteranthelium* parent. Growth of the hybrid under 2) was vigorous and the spike morphology was intermediate between the parents. The spikelets had two empty glumes and two florets like the *Heteranthelium* parent but no rudimental spikelets were found. Growth of all hybrids under 3) was very vigorous and the shape of their spikes was of *Hordeum* type. However, three spikelets with two florets each and four empty glumes were observed at most spikelet nodes. Thus, the numerical composition at the spikelet node of the F_1 was intermediate between the parents. Sterility of all three hybrid combinations was complete.

Average chromosome pairing per cell of the F_1 hybrids was in 1) $0.04_{II}(0\text{-}2)+13.9_{I}(10\text{-}14)$, in 2) $0.00_{III}(0\text{-}1)+5.06_{II}(2\text{-}8)+10.88_{I}(5\text{-}17)$ and in 3) $0.00_{IV}(0\text{-}1)+0.00_{III}(0\text{-}1)+5.50_{II}(1\text{-}8)+9.97_{I}(5\text{-}19)$. The parentheses indicate the range of respective pairing. Judging from the chromosome pairing in 1), 2) and 3), 5.0_{II} and 5.5_{II} observed in 2) and 3) indicate autosyndesis of chromosomes derived from the *Hordeum* parent. The conclusion may be drawn that the *Hordeum* parent used in this experiment was a tetraploid species having two partially homologous genomes.

The tillering clones of 3) were treated with 0.5 per cent colchicine solution and 31 seeds were obtained in 1966.

Diallel Crosses among Sikkimese Rice Types. III

Tadao C. KATAYAMA

Diallel crosses were carried out using sixteen strains, namely, fourteen Sikkimese, one *indica* and one *japonica* strains. All crosses were successful and the F_1 seeds obtained were sown in April, 1965. This year, the characters of intact and husked F_1 seeds, such as length, width, thickness, ratio of length to width and grain weight, were compared with those of the parents. Investigation of many other characters of the hybrids, such as number of tillers, plant height, heading date, internode length, is underway.

In the previous paper (Katayama 1966), the Sikkimese strains could be divided into 10 *indica* type and 4 *japonica* type strains based on the pollen fertility of the F_1's produced by diallel crosses. However, from the results obtained at present, 3 strains, C7725, C7727 and C7734, formerly classified as *indica*, were found to be of intermediate type between *indica* and *japonica*.

Therefore, Sikkimese rice is composed of typical *japonica*, typical *indica* types and an intermediate type. It is assumed that Sikkim might be one of the differentiation centers of cultivated *Oryza sativa* into *japonica*

and *indica* types.

Further Studies on Embryo Transplantation in the Genus *Oryza*

Tadao C. KATAYAMA

In order to investigate the compatibility relation between embryo and endosperm in *Oryza* species, embryo transplantations were made between rice species. The method was described in a previous paper (Katayama 1965). In the present article, two experiments are reported.

Four strains of *O. sativa* (two *indica* and two *japonica* types) and one strain of each of the following nine species were used, namely, *O. officinalis*, *O. minuta*, *O. eichingeri*, *O. latifolia*, *O. australiensis*, *O. granulata*, *O. ridleyi*, *O. brachyantha* and *O. subulata*. Seedlings from intact seeds developed in addition to coleoptile, 1st, 2nd and 3rd leaves. Seedlings of *O. sativa* developed three to four roots and those of other species developed one to two roots. Embryos alone without endosperm produced only a coleoptile and no root appeared except in a *japonica* type of *O. sativa*.

In heterogeneous transplantation, embryos of *O. sativa* were grafted upon the endosperms of other species. In all cases, only a coleoptile has developed. In all reciprocal transplantations, also only a coleoptile developed. This means that the grafted embryos failed to absorb nutrients from alien endosperms which suggests a distant relation to *O. sativa*.

In the previous report, it was strongly indicated that nutrient absorption from endosperms of *indica* type strains by embryos of the other closely related species employed, was much better than from endosperms of *japonica* type strains. In order to study the details on this particular point, 10 strains of *O. sativa* (five *japonica* and five *indica* strains) as testers, and one strain of *O. sativa* var. *spontanea*, *O. perennis*, *O. barthii*, *O. glaberrima* and *O. breviligulata*, were used. When embryos of *japonica* strains were grafted upon the endosperms of other species, coleoptile and 1st leaf developed in most cases. In all reciprocal transplantations, i. e., when embryos of other species were grafted upon the endosperms of *japonica* strains, also coleoptile and 1st leaf developed. When embryos of *indica* strains were grafted upon the endosperms of other species, coleoptile and 1st leaf appeared in most cases. However, in the reciprocal transplantation, i. e., when embryos of the other species were grafted upon the endosperms of *indica* strains, coleoptile, 1st, 2nd and, in some cases, even 3rd leaf, developed.

From these results, the previous finding was confirmed which indicated that *indica* type is taxonomically nearer related to the other employed species than *japonica* type.

Geographical Distribution of Winter, Intermediate and Spring Types of Common Wheat

Yasuo NAKAI

The growing habit of about 1400 varieties of common wheat collected from various parts of the world was examined using growth chambers adjusted to a constant temperature, 20°C, with 16 hr illumination. After three months from sowing, when all spring standard varieties were heading, the growing habit of the tested varieties was determined; varieties that headed at that time were classified as spring, those with visible ears inside as intermediate and those showing no ear formation as winter types. The result is summarized in Table 1.

Table 1. Geographical distribution of three growth habit types in common wheat

Locality	No. var. tested	Spring (%)	Intermediate (%)	Winter (%)
Pakistan	38	58	18	24
Afghanistan	39	12	30	58
Iran	147	27	11	62
India	58	98	0	2
Tibet	19	100	0	0
China	180	27	12	61
Japan	169	37	11	52
Greece	9	44	12	44
Italy	54	52	2	46
Iberia	96	30	4	66
Russia	89	69	2	29
Sweden	34	100	0	0
Norway	13	100	0	0
Finland	15	100	0	0
U. S. A.	260	33	7	60
S. America	43	81	7	12
Australia	79	97	0	3
Total	1342			

As to the varieties from Pakistan, India, Tibet, Russia, South America and Australia, many were of spring or intermediate type. In China, Japan, Mediterranean countries and U.S.A., both winter and spring types were

equally frequent. Some strains of Afghanistan and most of eastern Iran were of spring type. With respect to the Scandinavia strains used in this examination they were of spring type. The distribution pattern of different growth habit types of *Aegilops squarrosa* in central Asia (Kihara and Tanaka, 1958) is identical to that found here for common wheat.

Embryosac Sterility of F_1 Hybrids between Strains of *Oryza perennis*

Yaw-En CHU and Hiko-Ichi OKA

Embryosacs were observed in F_1 hybrids between strains of *Oryza perennis* Moench, *O. sativa* L., *O. breviligulata* Chev. et Roehr, and *O. glaberrima* Steud., which are pollen sterile in varying degree (Ann. Rep. **14:** 74, **15:** 84 and **16:** 71). An embryosac with an egg nucleus, two polar nuclei and antipodes were regarded as normal; this estimation was verified by back-crossing. Most F_1 hybrids showing high pollen sterility had an appreciable percentage of normal embryosacs. For instance, the F_1's between American and Asian strains of *O. perennis* showed 45–70 per cent normal embryosacs, though their pollen fertility was almost zero.

In sterile F_1 hybrids, the deterioration of embryosacs seemed to take place in three different stages, namely 1) deterioration of the tetrad immediately after meiosis, 2) failure of mitosis producing two to four nuclei, and 3) failure of nuclear differentiation into egg and polar nuclei. The F_1 hybrids between the Oceanian strains and those of the other geographical groups of *O. perennis* gave relatively low embryosac fertilities, ranging between 25 per cent and 65 per cent. When the Oceanian *perennis* strains were crossed with *O. glaberrima* or *O. breviligulata*, the F_1 embryosacs showed deterioration immediately after meiosis which was normal, and had little or no normal embryosacs at the flowering time.

Pattern Analysis of Character Variations in *Oryza perennis*

Hiroko MORISHIMA and Hiko-Ichi OKA

Oryza perennis Moench is distributed throughout tropical countries of the world and comprises many varieties or forms. The Asian forms tend to differentiate into the perennial (*perennis* or *balunga*) and annual (*spontanea* or *fatua*) types. Our collections from New Guinea are also divided into perennial and annual types. From 1958 to 1965, a number of strains of this species, obtained from their natural habitats in different countries, were repeatedly grown in a greenhouse or in automatically controlled short-day plots, and records were taken regarding 18 metric

and 6 code-described characters. In each strain, representative values of metric characters were obtained by comparing the data with controls. In all the metric characters, variations among strains were found to be continuous.

To examine the pattern of character association among the strains, the technique of "quantification" was applied to the data for 65 strains, which had complete sets of records. This method, devised by Hayashi (1951) basing on a "multi-dimensional constellation model", is to conduct quantification of the data so as to maximize the correlation between, in our

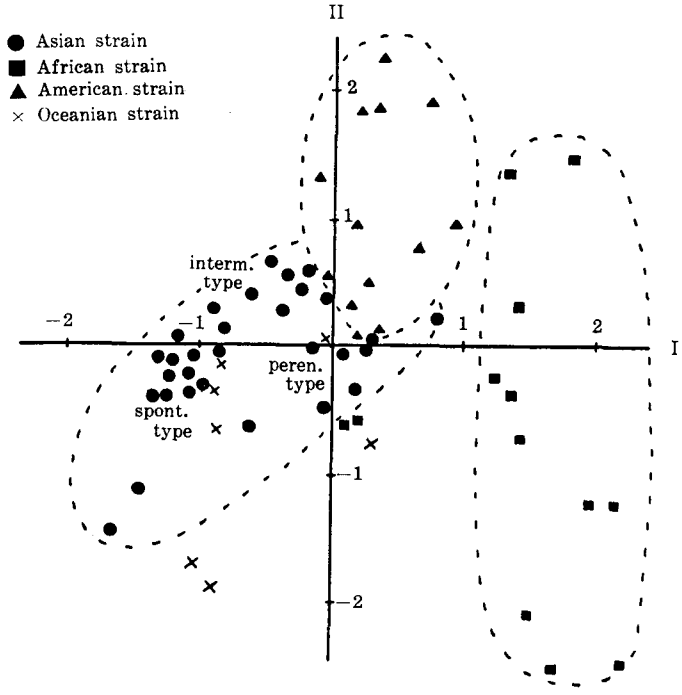

Fig. 1. Strains of *Oryza perennis* scattered on the basis of pattern analysis of variations in 24 characters.

case, strains and characters. The strains are then arranged in order in accordance with their overall similarity. They were scattered on the plane defined by two vectors given by two largest latent roots, as shown in Fig. 1. The strains of Asian, American origin formed different clusters, partly overlapping one another.

The differentiation of *perennis* strains into these geographical groups

could also be found from their F_1 sterility relationships (Ann. Rep. **14:** 74 and **15:** 84). The above results of computation indicate that the geographical groups can be distinguished by character-association patterns, though not by a single character. The figure also shows that in the Asian group, the perennial and annual types were separated, and in the American group, strains from Cuba, Surinam and the Amazon basin could be separated. It seems that the varieties of *O. perennis* distributed in different countries have different patterns of character correlation, possibly in relation to their different modes of adaptation to environment.

Population Survey of No. 1 Chromosome Polymorphism of Black Rats (*Rattus rattus*) Collected in Japan and Korea

Tosihide H. YOSIDA, Yukuo MORIGUCHI,
Yung Sun KANG[1] ond Kyojiro SHIMAKURA[2]

It was already reported that black rats (*Rattus rattus*) were characterized by a polymorphism of the largest No. 1 chromosome pair, present as a telocentric homomorphic pair (T/T), a telocentric and subtelocentric heteromorphic pair (T/S) and a subtelocentric homomorphic pair (S/S). The frequency of these three types in 124 rats collected in 7 localities of Japan and 9 animals from Seoul, Korea, was already reported in this annual report (No. **16,** 1966). Later we collected in total 236 rats in 9 lacalities of Japan and 26 rats in Seoul, Korea. The results of observation are given in Table 1. As the table shows, all animals collected in Sapporo, Niitsu (Niigata Pref.) and Obama (Fukui Pref.) had the T/T pair

Table 1. Frequency of black rats (*Rattus rattus*) with three types of No. 1 chromosomes, collected in Japan and Korea

Karyotype	Locality					
	Sapporo	Niitsu (Niigata)	Obama (Fukui)	Sugadaira (Nagano)	Hiratsuka (Kanagawa)	Misima (Sizuoka)
T T	100%	100	100	80	70	70
T S	0	0	0	20	30	30
S S	0	0	0		0	0
Total no. of animals	50	30	11	15	7	56

[1] Seoul University, Korea.
[2] Hokkaido University, Sapporo.

Karyotype	Locality			Total in Japan	Seoul (Korea)
	Hamamatsu (Sizuoka)	Ube (Yamaguchi)	Kusudomari and Nagasaki		
T T	57%	82	82	200 (84.8%)	9 (34.6%)
T S	29	18	18	35 (14.8%)	13 (50%)
S S	14	0	0	1 (0.4%)	4 (15.4%)
Total no. of animals	7	11	49	236 (100.0)	26 (100.0)

while in the other places in Japan, namely Sugadaira (Nagano Pref.), Hiratsuka (Kanagawa Pref.), Misima, Hamamatsu (Sizuoka Pref.), Ube (Yamaguchi Pref.) and Nagasaki (included Kusudomari), animals with T/S heteromorphic pair were observed at 18 to 33 per cent. Except for only one animal with S/S homomorphic pair which was found in Hamamatsu population, all the others showed the T/T homomorphic pair. Frequency of animals with T/T, T/S and S/S chromosomes in all 236 was 84.8, 14.8 and 0.4 per cent, respectively. On the other hand, among 26 animals collected in Seoul, Korea, about 50 per cent had the T/T pair, and about 15 per cent animals were of S/S type.

Based on the above investigations, it can be said that the frequency of animals with No. 1 T/T, T/S and S/S chromosomes was significantly different in different localities of Japan and in Korea.

Segregation of Three Chromosome Types in Black Rats Crossed in the Laboratory

Tosihide H. YOSIDA and Yukuo MORIGUCHI

Segregation of three types (T/T, T/S, and S/S) of No. 1 chromosomes in black rats, *Rattus rattus*, which were crossed in the laboratory was already reported for some animals (Ann. Rep. **16,** 1966). We got more data on the segregation of the three chromosome types from animals bred in our laboratory. The results of observations are shown in the following table (Table 1). Animals with S/S chromosomes were bred in the laboratory with success. From crossing a T/S female by an S/S male we obtained four litters. Average litter size of the hybrids was four. Chromosomes of two litters were observed. They segregated into 5 animals with a T/S chromosome type and 3 animals with an S/S type. On the other hand, by crossing an S/S female by an S/T male only one baby rat was obtained,

Table 1. Segregation of three chromosome types in black rats (*Rattus rattus*) bred in the laboratory

Karyotypes of parents	Segregation			Total no. of animals
	T/T	T/S	S/S	
T/S(♀)×T/S(♂)	18	43	12	73
T/T(♀)×T/S(♂)	37	39	0	76
T/S(♀)×T/T(♂)	59	61	0	120
T/T(♀)×T/T(♂)	48	0	0	48
T/S(♀)×S/S(♂)	0	3	2	5

but it died soon after birth. By mating an S/S female by a T/T male two baby rats were born, but they also died within one day after birth. Based on the above investigation, S/S females of the black rat may be unfit to bear and nurse their brood.

V. MATHEMATICAL AND STATISTICAL STUDIES ON POPULATION GENETICS

Simulation Studies on the Number of Neutral Alleles Maintained in a Finite Population by Mutation

Motoo KIMURA

The number of isoalleles that may be maintained in a finite population was studied theoretically for neutral and overdominant mutations by Kimura and Crow (1964), who derived, for neutral mutations, the following formula for the equilibrium distribution of allelic frequencies;

$$\Phi(x)=4M(1-x)^{4M-1}x^{-1}\,, \tag{1}$$

where $M=N_e u$ in which N_e stands for the effective population number and u stands for the mutation rate per generation. In deriving the above formula it was assumed that each mutant represents an allelic state not preexisting in the population.

The above distribution has the meaning that $\Phi(x)dx$ gives an approximation to the expected number of alleles whose frequencies in the population lie within the range x to $x+dx(0<x<1)$.

Using the above formula, the average and the effective number of alleles in a population of size N may be given as follows:

$$n_a=\int_{1/(2N)}^{1}\Phi(x)dx=4M\int_{1/(2N)}^{1}(1-x)^{4M-1}x^{-1}dx\,, \tag{2}$$

$$n_e=1\Big/\int_0^1 x^2\Phi(x)dx=4M+1\,, \tag{3}$$

The average number of alleles is defined as the reciprocal of the mean frequency of alleles contained in a population, while the effective number of alleles is defined as the reciprocal of the sum of squares of allelic frequencies.

In order to test the validity of the above formulae, simulation studies were carried out by using computer IBM 7090. Fig. 1 illustrates a result of Monte Carlo experiment, assuming a population consisting of 50 males and 50 females, of which only 25 males and 25 females actually participate in breeding ($N=100$, $N_e=50$). In the experiment, the process of mutation and random sampling of gametes was simulated by generating pseudo-random numbers in the computer. In each generation, 100 male and 100 female gametes are randomly chosen respectively from 25 breeding males and 25 breeding females to form the next generation. Mutation to a new (not pre-existing) allele is induced in each gamete with probability 0.005

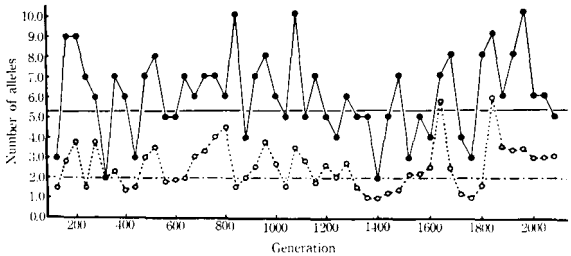

Fig. 1. Neutral alleles, Random mutation, $N=100$, $N_e=50$, $u=0.005$.
●—— Average (actual) number ○--- Effective number

prior to the formation of zygotes ($u=0.005$). The initial population (at the 0th generation) was set up such that it contained 200 different alleles; outputs both of average and effective numbers of alleles were given at the interval of 20 generations and the experiment was carried out until generation 2,100 (actually illustrated in the figure at the interval of 40 generations). The balance between mutation and random extinction of alleles was found to be attained well before generation 100. From the outputs over generations 120–2,100, the average and the effective number of alleles were computed as follows:

$$n_a=6.05\,, \qquad n_e=2.07$$

The corresponding values derived from equations (2) and (3) are

$$n_a=5.30\,, \qquad n_e=2.00$$

Thus, a fairly good agreement was observed between the actual values from the simulation experiment and the theoretical predictions based on the diffusion approximation.

Two Loci Polymorphism as a Stationary Point

Motoo KIMURA

In a previous note (cf. Ann. Rep. No. **16**: 86) a remarkable property inherent in the linked gene system was reported under the term *quasi linkage equilibrium*. The purpose of the present note is to show the relevance of this concept in the study of two loci polymorphism.

Let X_1, X_2, X_3 and X_4 ($\sum_1^4 X_i=1$) be respectively the frequencies of four chromosome types A_1B_1, A_2B_1, A_1B_2 and A_2B_2 in a population, in which A_1 and A_2 are a pair of alleles in the first locus and B_1 and B_2 are a pair in the second locus. It is convenient here to give these chromosomes the

numbers, 1, 2, 3 and 4, so that the frequency of chromosome i is X_i ($i=1$, 2, 3, 4) and the fitness (in selective values) of the genotype formed by the union of chromosomes i and j may be denoted by w_{ij}. In a large random mating population, if c is the recombination fraction between the two loci, the amount of change in one generation of the chromosome frequencies may be given as follows:

$$\left.\begin{aligned} \Delta X_1 &= \{X_1(w_{1.}-\bar{w})-cD_w\}/\bar{w} \\ \Delta X_2 &= \{X_2(w_{2.}-\bar{w})+cD_w\}/\bar{w} \\ \Delta X_3 &= \{X_3(w_{3.}-\bar{w})+cD_w\}/\bar{w} \\ \Delta X_4 &= \{X_4(w_{4.}-\bar{w})-cD_w\}/\bar{w} \end{aligned}\right\} \qquad (1)$$

where

$$w_{i.}=\sum_{j=1}^{4} w_{ij}X_j\ , \qquad \bar{w}=\sum_{ij} w_{ij}X_iX_j\ ,$$

and

$$D_w=w_{14}X_1X_4-w_{23}X_2X_3\ .$$

Thus, at the genetic equilibrium in which $\Delta X_i=0$ ($i=1, 2, 3, 4$), we have

$$\begin{aligned} X_1(w_{1.}-\bar{w}) &= -X_2(w_{2.}-\bar{w})=-X_3(w_{3.}-\bar{w}) \\ &= X_4(w_{4.}-\bar{w})=cD_w\ . \end{aligned} \qquad (2)$$

The equilibrium chromosome frequencies $\hat{X}_i$ ($i=1, 2, 3, 4$) may be obtained by solving the above set of equations.

I will now show that the equilibrium point ($\hat{X}_1$, $\hat{X}_2$, $\hat{X}_3$, $\hat{X}_4$) is a stationary point of $\bar{w}$ with the two side conditions,

$$\sum_{i=1}^{4} X_i=1\ ,$$

and

$$(X_1X_4)/(X_2X_3)=R_0\ ,$$

where R_0 is a constant giving the ratio between the frequencies of coupling and repulsion phases at equilibrium, i.e. $R_0=(\hat{X}_1\hat{X}_4)/(\hat{X}_2\hat{X}_3)$. The stationary point of $\bar{w}$ with these two restrictions is equivalent to the stationary point of

$$\phi=\bar{w}-2\mu\,(\sum_i X_i-1)-2\lambda\left(\log\frac{X_1X_4}{X_2X_3}-\log R_0\right)\ ,$$

in which no restrictions are imposed among X_i's (-2μ and -2λ are Lagrange multipliers). Thus

$$\partial\phi/\partial X_1 = 2w_{1.} - 2\mu - (2\lambda/X_1) = 0 ,$$
$$\partial\phi/\partial X_2 = 2w_{2.} - 2\mu + (2\lambda/X_2) = 0 ,$$

etc., giving

$$X_1(w_{1.} - \mu) = -X_2(w_{2.} - \mu) = -X_3(w_{3.} - \mu) = X_4(w_{4.} - \mu) = \lambda , \quad (3)$$

in which, it is easy to show that

$$\mu = \bar{w} ,$$

and

$$\lambda = (w_{1.} - w_{2.} - w_{3.} + w_{4.}) / \sum_{i=1}^{4} (1/X_i) .$$

Thus, the relation (3) together with

$$(X_1X_4)/(X_2X_3) = R_0 ,$$

should be equivalent to (2).

The Mutational Load with Epistatic Gene Interactions in Fitness

Motoo KIMURA and Takeo MARUYAMA

The mutational load in a large population was first calculated by Haldane in 1937 without assuming an epistatic component in fitness. Later, a similar but more detailed calculation was carried out by Kimura (1961). Also, the mutational load in a small population was studied by Kimura, Maruyama and Crow (1963).

In the present work, the effect of epistasis on the mutational load was studied using a model which assumes that fitness is a function of the number of mutant genes in an individual. The results are summarized as follows:

1) If the deleterious effect of the mutant genes is proportional to the square of their number, the load under random mating becomes roughly half as large as in the case of no epistasis, provided that the average number of such genes per individual is fairly large. Under asexual reproduction, however, the epistasis has no effect in reducing the load. The situation is intermediate for a random mating population of a hypothetical organism having only one pair of chromosomes between which no crossing over takes place.

2) The mutational load may also be reduced under random mating if fitness is a threshold character.

3) Epistatic interaction in fitness among deleterious mutant genes may be classified into two types, namely, the reinforcing type and the diminishing type. In the former, the deleterious effect becomes disproportionately large as their number in an individual increases. On the other hand, in the latter, the deleterious effect per mutant gene becomes smaller as their number increases. Consideration of mutational load as well as developmental homeostasis involved suggests that the reinforcing type of epistasis among deleterious mutant genes must be more common than the diminishing type in nature.

For the details, see Kimura and Maruyama (1966), Genetics **54**: 1337–1351.

Eigenvalues in a Genetics Problem

Takeo MARUYAMA

The smallest and the second smallest eigenvalues in a genetics problem are important, for they determine respectively the asymptotic rate of fixation in a case with absorbing boundaries and the asymptotic rate of approach to a steady state if it exists. Kimura has given a power series expansion of the smallest eigenvalue for the general case with absorbing boundaries. Miller also gave an analytic method and numerical examples in cases of heterosis. The purpose of the present work was to show that an analogous method to the perturbation method in quantum mechanics can be used to obtain all eigenvalues in a genetics problem, including of course the first and second eigenvalues, when we know the characteristic pairs of unperturbed equation.

The mathematical model considered is

Genotype	A_1A_1	A_1A_2	A_2A_2
Fitness	$1-s_1$	1	$1-s_2$

We assume no mutation and no migration, and that the effective size of a population is equal to N. Then the diffusion equation is

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

Letting $\phi(x;t)=T\cdot X$ be the solution of the above equation, we have

$$4N\frac{T'}{T}=\frac{1}{X}\left[\frac{d^2}{dx^2}\{x(1-x)X\}+4N\hat{s}\frac{d}{dx}\{(2x-1-z)x(1-x)X\}\right]=-\lambda\ , \qquad (1)$$

where

$$\hat{s}=(s_1+s_2)/2\ ,\quad \hat{x}=s_1/(s_1+s_2)\ ,\quad 2x-1=z\ ,\quad \text{and}\quad 2\hat{x}-1=\hat{z}\ .$$

Define

$$\left.\begin{aligned} H &= \frac{d^2}{dz^2}(1-z^2) \\ h &= \frac{d}{dz}\{2(z-\hat{z})(1-z^2)\} \end{aligned}\right\} \quad \text{linear operators}$$

Then the right hand side of (1) is equivalent to

$$(H+\alpha h)\phi_m(z)=E_m\phi_m(z)\ , \tag{2}$$

where

$$\alpha=N\hat{s}\ .$$

Kimura has solved all the characteristic pairs of $H\phi_m=\xi_m\phi_m$. They are

$$\xi_m=-(m+1)(m+2)$$
$$\phi_m=T_m^{(1)}(z) \qquad \text{(Gegenbauer polynomials).}$$

We now expand E_m and $\phi_m(z)$ in powers of α, that is

$$E_m=\sum_{n=0}^{\infty}\alpha^n E_m^{(n)} \quad \text{and} \quad \phi_m(z)=\sum_{n=0}^{\infty}\alpha^n \Phi_m^{(n)}\ . \tag{3}$$

Substituting the above E_m and ϕ_m in (2), we obtain

$$H\sum_{n=0}^{\infty}\alpha^n \Phi_m^{(n)}+h\sum_{n=0}^{\infty}\alpha^{n+1}\Phi_m^{(n)}=\sum_{n=0}^{\infty}\alpha^n\sum_{i=0}^{n}E_m^{(i)}\Phi_m^{(n-i)}\ . \tag{4}$$

Equating the coefficients of like powers in (4), we have

$$\left.\begin{aligned} &\Phi_m^{(0)}=\phi_m=T_m^{(1)} \\ &E_m^{(0)}=\xi_m=-(m+1)(m+2) \\ &(H-\xi_m)\Phi_m^{(1)}+h\phi_m=E_m^{(1)}\phi_m \end{aligned}\right\} \tag{5}$$

Letting $\Phi_m^{(1)}=\sum_{j=0}^{\infty}a_{mj}\phi_j$ (this is permissible, because the Gegenbauer polynomials are a complete set), substituting this $\Phi_m^{(1)}$ in the last equation of (5) and using the fact that $H\phi_j=\xi_j\phi_j$, we have

$$H\sum_j a_{mj}\phi_j-\xi_m\sum_j a_{mj}\phi_j=E_m^{(1)}\phi_m-h\phi_m\ . \tag{6}$$

Multiplying both sides of (6) by $(1-z^2)\phi_m$, integrating over the interval $(-1, 1)$, and using the orthogonality of ϕ_m, we have

$$E_m^{(1)}=-\frac{(\phi_m, h\phi_m)}{\|\phi_m\|^2}\ ,$$

where

$$\|\phi_m\|^2=\int_{-1}^{1}(1-z^2)\phi_m\phi_m dz \quad \text{and} \quad (\phi_m, h\phi_m)=\int_{-1}^{1}(1-z^2)\phi_m h\phi_m dz\ .$$

In a similar, but more complicated way, 2nd, 3rd, 4th terms in (3) can be obtained. They are

$$E_m^{(2)}=\frac{1}{\|\psi_m\|^2}\left(-\sum_{\substack{j=0\\ j\neq m}}^{\infty}\frac{(\psi_j, h\psi_m)^2}{\|\psi_j\|^2(\xi_j-\xi_m)}\right),$$

$$E_m^{(3)}=\sum_{i\neq m}\sum_{j\neq m}\frac{(\psi_m, h\psi_i)(\psi_i, \bar{h}\psi_j)(\psi_j, h\psi_m)}{(\xi_i-\xi_m)(\xi_j-\xi_m)},$$

where $\bar{h}=h-(\psi_m, h\psi_m)$,

and

$$E_m^{(4)}=-\sum_{i\neq m}\sum_{j\neq m}\sum_{k\neq m}\frac{(\psi_m, h\psi_i)(\psi_i, \bar{h}\psi_j)(\psi_j, h\psi_k)(\psi_k, \bar{h}\psi_m)}{(\xi_i-\xi_m)(\xi_j-\xi_m)(\xi_k-\xi_m)}$$
$$-E_m^{(2)}\sum_{i\neq m}\sum_{j\neq m}\frac{(\psi_m, h\psi_j)(\psi_j, h\psi_m)}{(\xi_i-\xi_m)(\xi_j-\xi_m)}.$$

A Diffusion Process with Heterosis

Takeo MARUYAMA

As Wright has emphasized, finite populations may be important in the course of evolution because random drift in gene frequency can move the population from one adaptive peak to another on a fitness surface diagram. Although it is true that random drift can eventually shift a finite population from one peak to any other peak, we would like to know the time required for it. As a first attempt to solve the problem, a simple model was constructed of a fitness surface with only one peak, representing a polymorphism due to heterosis, and an approximate solution of the gene frequency distribution was obtained.

The diffusion equation corresponding to heterotic selection in a finite population is

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

where $V_{\delta x}=x(1-x)/2N$ and $M_{\delta x}=x(1-x)(s_1-(s_1+s_2)x)$. A solution to equation (1) with initial condition $\lim_{t\to 0}\phi(p, x; t)=\delta(p-x)$ has not yet been solved. However if we restrict the behavior of the process at the local neighborhood of $x_0=s_1/(s_1+s_2)$, the point where the process starts, we have roughly $V_{\delta x}=x_0(1-x_0)/2N$ and $M_{\delta x}=x_0(1-x_0)\{s_1-(s_1+s_2)x\}$. Then (1) becomes

$$\frac{\partial \phi}{\partial t}=\alpha\frac{\partial^2\phi}{\partial y^2}+\beta y\frac{\partial \phi}{\partial y}+\beta\phi\,, \tag{2}$$

where $y=s_1-(s_1+s_2)x$, $\beta=s_1s_2/(s_1+s_2)$ and $\alpha=s_1s_2/4N$. If we assume the solution of (2) to be $\phi=T(t)Y(y)$, then we have

$$\frac{1}{T(t)}\frac{dT(t)}{dt}=\frac{1}{Y(y)}\left\{\alpha\frac{d^2Y}{dy^2}+\beta y\frac{dY}{dy}+\beta Y\right\}=\lambda\,. \tag{3}$$

It is easy to see that $T(t)=e^{\lambda t}$. The right hand side of (3) becomes

$$\alpha\frac{d^2Y}{dy^2}+\beta y\frac{dY}{dy}+(\beta-\lambda)Y=0\,.$$

If we let $Z=\sqrt{\frac{\beta}{\alpha}}y$ and $Z(z)=Y(z)$, we have

$$\frac{d^2Z}{dz^2}+z\frac{dZ}{dz}+\left(1-\frac{\lambda}{\beta}\right)Z=0\,, \tag{4}$$

equation (4) is a Hermite differential equation and its integrable solutions are $Z_n=e^{-z^2/4}D_n(z)$ $n=0,1,2,\cdots$ where $-\frac{\lambda}{\beta}=0,1,2,\cdots$ and

$$D_n(z)=(-1)^ne^{z^2/4}\frac{d^n}{dz^n}e^{-z^2/2}\,.$$

Hence the solution of (2) is

$$\phi(t;x)=\sum_{n=0}^{\infty}a_ne^{\lambda_nt}e^{-z^2/4}D_n(z)\,.$$

Now we want to determine the coefficients a_n such that $\lim_{t\to 0}\phi(t;x)=\delta(x_0-x)$. The principle in determining a_n is $\int\delta(x-x_0)f(x)dx=f(x_0)$ for all function $f(x)$ and the orthogonatity of $D_n(z)$. The final form of the solution is

$$\phi(x;t)=\left[\frac{N(s_1+s_2)}{s_1s_2t\pi}\right]\exp\left\{-\left(\frac{s_1}{s_1+s_2}-x\right)^2\Big/\frac{s_1s_2t}{2N(s_1+s_2)^2}\right\}\,.$$

Although this solution is valid for only a short time, it has a pleasing form; $\phi(x;t)$ is a normal distribution (in particular it is the Wiener transition function) with variance proportional to time and the inverse of the population size.

An Application of Kimura's Formulae to Define the Evolutionary Load in a Small Population

Takeo MARUYAMA

Gene frequencies or the distribution of gene frequencies are determined by selection coefficients, population size, mutation rate etc. If the environment changes the relative fitness of genotypes, a new equilibrium will be attained by natural selection. If we assume for this discussion that the change is such that the previously advantageous gene becomes disadvantageous, the shift of gene frequencies will be large. This case is called a gene substitution. If we further assume that there is neither mutation nor migration during the process, the substitution will be complete. This change in the relative fitnesses causes a genetic load, called the evolutionary load. Symbolically if we let $l(t)$ be the genetic load at time t, then the evolutionary load for the whole process is given by

$$\int_{t=0}^{\infty} l(t)dt \ .$$

It has been shown by Kimura that the load in an infinitely large population is

$$-\frac{1}{h}\left\{\log p_0+(1-h)\log\frac{1-h}{h+(1-2h)p_0}\right\} \ ,$$

where h is the degree of dominance and p_0 is the initial gene frequency. The main trouble with finite populations is that, even if we assume p_0 to be positive, some populations will loose the advantageous gene and will stay in the lost class until mutation occurs. Therefore it is not possible to define the evolutionary load in analogy to the case of an infinite population. However we may measure the load as follows; for each sample path we calculate the difference in fitnesses between the best genotype and the average fitness at a given time, add them from time zero to infinity and take the expectation with respect to the collection of all sample paths. Because this is the expected total genetic death spent in the substitution of the gene, we divide it by the fraction which went to fixation. We call it the evolutionary load. Applying Kimura's solution $\phi(p, x;t)$ to the diffusion equation in genic selection and his solution $u(p)$ to the probability of ultimate fixation we can write the load in a finite population, in a case of genic selection, as

$$\frac{1}{u(p)}\int_{t=0}^{\infty} dt \int_{x=0}^{1} sx\phi(p, x;t)dx \ . \qquad (1)$$

The explicit formulae for $\phi(p, x;t)$ and $u(p)$ due to Kimura are

$$\phi(p, x;t)=\sum_{k=0}^{\infty} C_k e^{-\lambda_k t+2cx} V_{lk}^{(1)}(z) ,$$

where $z=1-2x$, $c=Ns$, and C_k and λ_k are constants, $V_{lk}^{(1)}=\sum_{n=0.1}' f_n{}^k T_k^{(1)}(z)$ where $f_n{}^k$ are constants and $T_k^{(1)}(z)$ are Gegenbauer polynomials, and

$$u(p)=\frac{1-e^{-4Nsp}}{1-e^{-4Ns}} .$$

Therefore using the relation

$$I_{nm}\equiv\int_{-1}^{1} T_n^{(1)}(z)\, T_m^{(1)}(z)=\begin{cases}0 \text{ if } m-n=\text{odd}\\ (n+1)(n+2) \text{ if } m\geqq n \text{ and } m-n=\text{even}\end{cases}$$

(1) can be expressed as

$$\frac{e^c s}{4}\sum_k \frac{C^k}{\lambda_k}\sum_{n=0.1}' f_m{}^k\left(\sum_n d_n I_{mn}\right) ,$$

where

$$d_n=\frac{\int_{-1}^{1}(1-z^2)(1-z)e^{cz}T_n^{(1)}(z)\,dz}{\|T_n^{(1)}\|^2} ,$$

$$\|T_n^{(1)}\|^2=\int_{-1}^{1}(1-z^2)\{T_n^{(1)}(z)\}^2\,dz=\frac{2\cdot 3\cdot\cdot(3+n-1)}{\left(n+\frac{3}{2}\right)n!} .$$

Dimensionality of Human Migration

Norikazu YASUDA

A pattern of human migration has been considered as two-dimensional, that is, man moves in all directions from where he was born. However, this is not true in a population with barriers.

Suppose that an individual moves from his birthplace to a place to where he is attracted by job or mate. This exploration range will be proportional to the inverse of dimensional power of marital distance (x), defined as the distance between the birthplaces of consorts. An acceptable frequency distribution is $m(x)=ab/(1+ax)^{b+1}$, where $b+1$ represents dimension and a is a scale parameter or "attraction index".

Examples of estimated parameters are given as follows:

Population	Dimension $(b+1)$	Attraction index (a)
Brazil (grandparent)	1.90±0.06	0.09±0.01
Brazil (parent)	1.90±0.06	0.06±0.01
Japan (Ohdate)	1.49±0.02	1.62±0.15
Italy (M area)	1.60±0.03	2.86±0.32
Italy (P area)	1.99±0.04	0.56±0.03

The estimated dimensionality in Italian samples agrees impressively with the results of Cavalli whose approach was completely different. Whether one or two in dimensionality is one of the important parameters in a discussion of the relationship between the inbreeding coefficient and distance. The above results then suggest that dimensionality in man is two and deviation from it may be understood to be due to physical or sociological barriers.

In addition, the attraction index may be used for a characterization of a population: the smaller is the value of a, the more migration takes place.

Dimensionality and Distance in Human Migration

Norikazu YASUDA

Direction of human migration may depend upon the geography of the place where people live. By introducing the *isotropic index*, a method was devised to ascertain the effect of distance on dimensionality in man.

Suppose that an individual was born at a place $P(x_1, y_1)$ and moved into a place $Q(x_2, y_2)$ (both points might be same). The isotropic index θ is defined as:

$$\theta = \tan^{-1}\left|\frac{y_1-y_2}{x_1-x_2}\right| .$$

The expected variance of θ is zero if a person migrates in one direction while it will be $\pi^2/48$ (measured in radians) if isotropic migration takes place. It may not be zero variance even if one dimensional migration is observed, since the direction is often curvilinear. This will be serious when the line makes a right angle. The method, however, is still applicable in such a case by dividing the population into two parts.

Dimension (d) is thus evaluated from $d=1+s^2/\sigma^2$, where $s^2=[\sum\theta^2-(\sum\theta)^2/n]/(n-1)$, n is the number of pairs and $\sigma^2=\pi^2/48$.

Applying the method in two studies on distribution of ABO blood groups

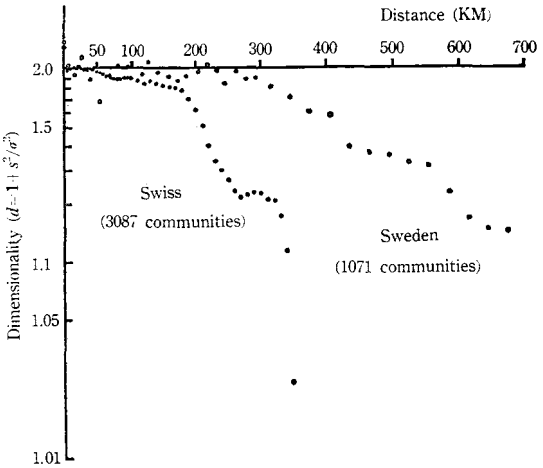

Fig. 1. Dimensionality and distance in man.

in Switzerland (Rosin, 1956) and in Sweden (Beckman, 1959) where individuals are grouped by their birthplace so that the distribution of communities may be considered approximately as an expected distribution of human migration, and making all possible pairwise combinations of 3087 communities in Switzerland and of 1071 in Sweden, we found that the isotropic distances are about 100 km. in Switzerland and about 200 km. in Sweden, reflecting the geography of each country. Dimension becomes one for further remote distances (see figure).

A Statistical Singularity at the ABO Blood Group System

Norikazu YASUDA

Suppose that the observed and the expected numbers of individuals in the standard ABO blood group classification are given by:

Phenotype	*Genotype*	*Observed*	*Expected*
O	*OO*	n_0	$N[r^2+(r-r^2)\alpha]$
A	*AA, AO*	n_A	$N[p^2+2pr+\{p-(p^2+2pr)\}\alpha]$
B	*BB, BO*	n_B	$N[q^2+2qr+\{q-(q^2+2qr)\}\alpha]$
AB	*AB*	n_{AB}	$N[2pq-2pq\alpha]$

where p, q and r are gene frequencies of A, B and O, respectively, α is the inbreeding coefficient, and $N=n_A+n_B+n_{AB}+n_0$. Then the maximum likelihood scores under the null hypothesis that $\alpha=0$ are

$$U_p=n_0\left[-\frac{2}{r}\right]+n_A\left[\frac{2r}{p^2+2pr}\right]+n_B\left[\frac{-2}{q+2r}\right]+n_{AB}\left[\frac{1}{p}\right],$$

$$U_q=n_0\left[-\frac{2}{r}\right]+n_A\left[\frac{-2}{p+2r}\right]+n_B\left[\frac{2r}{q^2+2qr}\right]+n_{AB}\left[\frac{1}{q}\right],$$

and

$$U_\alpha=n_0\left[\frac{1-r}{r}\right]+n_A\left[\frac{q-r}{p+2r}\right]+n_B\left[\frac{p-r}{q+2r}\right]+n_{AB}\left[-1\right].$$

It is easily verified that

$$U_\alpha=-(pU_p+qU_q)/2\,,$$

indicating that no reliable estimate of α can be obtained from this material alone although one degree of freedom is left for it. The situation is not improved either by subtyping A into A_1 and A_2 and AB into A_1B and A_2B or B, A_1B and A_2B in the same manner as A and AB. Furthermore, if there exist m alleles which are codominant to each other, but are dominant to the silent allele O, we found that

$$U_\alpha=-\sum_{i=1}^{m}p_iU_{p_i}/2\,.$$

An example of $m=3$ was observed in the esterase-2 system in *Drosophila virilis* (Ohba, personal communication).

No such difficulty, however, was observed from mating type frequencies in the ABO system.

VI. EXPERIMENTAL STUDIES ON POPULATION GENETICS

Deleterious Genes in the Second Chromosome Concealed in Natural Populations of *Drosophila melanogaster*[1)]

Chozo OSHIMA and Takao K. WATANABE

Among 905 second chromosomes isolated from natural populations at Kofu and Katsunuma territory in late October 1965, the frequency of lethal chromosomes was 15.47 per cent. The frequencies of lethal chromosomes did not fluctuate to a remarkable extent during the past several years, but those of semilethal and subvital chromosomes in the extracted chromosomes in 1965 were decreased as compared with their earlier frequencies, as shown in Table 1.

Table 1. Frequencies of the second chromosomes carrying deleterious genes in natural populations of the Kofu and Katsunuma territory

Year	No. of the second chromosomes isolated	Frequency of			
		Lethals	Semilethals	Subvitals	Normals
1959	144	13.19±2.82	19.44±2.30	16.67±3.11	50.69±4.17
1963	668	20.21±1.55	18.11±1.49	25.15±1.68	36.53±1.86
1964	740	15.54±1.33	19.59±1.46	7.57±0.98	57.30±1.82
1965	905	15.47±1.20	6.85±0.84	3.76±0.63	73.92±1.46

A tester strain, having the dominant marker genes *Cy* and *Pm* on each second chromosome, was used in the analysis, but its genetic background (X and third chromosomes) was previously substituted with normal chromosomes originated from the same natural population. Consequently, the genetic background was assumed to be highly heterozygous. On the contrary, the genetic background of the tester strain, which had been used in the former experiments, was assumed to be homozygous, as it was substituted with homozygous chromosomes of an isogenic Samarkand strain.

The frequencies of chromosomes carrying deleterious genes except for complete lethals, could have been influenced by the state of genetic background of the tester strain. Therefore, the frequencies of semilethals and subvitals, found in the past years (1959, 1963, 1964), would be nearer

1) The following six investigations were supported by a grant from the Japan Society for the Promotion of Science as part of the Japan—U.S. Cooperative Science Program.

to the real frequencies in the natural populations than those obtained in 1965.

Distribution of Persistent Lethal Genes in Natural Populations

Chozo OSHIMA and Takao K. WATANABE

From six different populations, distributed over the Kofu and Katsunuma territory ($15 \times 3\,km^2$), 132 lethal chromosomes were isolated, and allelism tests between lethal genes were performed. The results are represented in Table 2.

Table 2. Allelism tests between lethal genes of 132 lethal chromosomes

	Whole populations	Within Kofu populations	Within Katsunuma populations	Between Kofu & Katsunuma populations
No. of lethals	132	63	69	132
No. of crosses	8646	1953	2346	4347
No. of allelic crosses	169	54	38	77
Allelic rate (%)	1.95	2.76	1.62	1.77

Seventy-nine lethal chromosomes, isolated from the same populations in 1964, have been maintained individually by the *Cy*-balanced condition. Allelism tests between newly isolated 132 lethal chromosomes and the old ones were completely performed by the diallel cross. Among the total of 10428 crosses, 248 were allelic and allelic rate was determined to be 2.38 per cent, being similar to that (2.35 per cent) between lethals isolated in 1963 and 1964.

Table 3. Frequency of persistent lethals among all isolated lethals and their persistence periods

Period		No. of lethals tested	No. of persistent lethals	Frequency (%)
1 Year	1963-1964	100	35	35.0
	1964-1965	140	52	37.1
2 Years	1963-1965	140	29	20.7
4 Years	1959-1963	114	10	8.8
5 Years	1959-1964	100	9	9.0
6 Years	1959-1965	140	10	7.1

From the results of such successive allelism tests, some lethal genes were confirmed to have persisted for a long time in the Kofu and Katsunuma populations, as shown in Table 3.

Eighty lethal genes among 132 lethals were assumed to be newly arisen lethals and the allelic rates between them were very low, as shown in Table 4.

Table 4. Allelic rates between newly arisen lethal genes

	Whole population	Within Kofu population	Within Katsunuma population	Between Kofu & Katsunuma population
No. of lethals	80	38	42	80
No. of crosses	3160	703	861	1596
No. of allelic crosses	15	3	7	5
Allelic rate (%)	0.47	0.43	0.81	0.31

Recessive Visible Mutant Genes on the Second Chromosome Concealed in Natural Populations

Chozo OSHIMA and Takao K. WATANABE

Among 905 chromosomes, 89 (9.8 per cent) had a recessive visible mutant gene. A mutant gene, making bristles slender and short, was named tentatively 'reduced' (*rd*) and similar mutant genes were found in 63 chromosomes. Another mutant gene, manifesting brown eye, was identified to be the *bw* gene (2-104.5); it was found in 12 chromosomes in addition to five kinds of mutant genes (tentatively named spread wing, excised wing, upturned wing, reduced wing and small body) observed in a few chromosomes.

For the past two years, many chromosomes, having the *rd* gene, were isolated from the Kofu and Katsunuma populations. By the results of allelism tests between them, these *rd* genes could be divided into five groups and the loci of *rd*-1 and *rd*-4 were determined to be 66.9± and 51.8± respectively. The manifestation of *rd* gene is very similar to that of Minute genes, of which about 20 loci have been recorded on the second chromosome, most of them clustering near the centromere. It may be assumed that by some mechanisms a fairly number of *rd* genes have been maintained in the natural populations in spite of lower viability of heterozygous flies (*rd*/+).

Segregation Distorter (SD) Genes and Their Linked Lethal Genes in *Drosophila melanogaster*

Takao K. WATANABE and Chozo OSHIMA

Among 420 second chromosomes, which were extracted from natural populations at Kofu and Katsunuma in late October of 1965, 15 chromosomes (3.6 per cent) were found to have segregation distorter genes, as a result of checking segregation ratios in heterozygous males for each second chromosome and the *cinnabar brown* chromosome. Seven chromosomes among them had each a lethal gene. The lethal gene on five of them was clarified to be a persistent one (*l* 401, locus: 47.9), which had been maintained in high frequency in the Kofu and Katsunuma populations for at least two years.

Most SD chromosomes (86.7 per cent) had *In*(2R)C on the right arm, whose equilibrium frequency in the natural populations was about 25 per cent. The SD gene was assumed to be located near the proximal end of the right arm. Then, the linkage between SD and the inversion could be strong, but that between SD and *l* 401 was weaker, because the lethal gene was located on the left arm.

An SD chromosome was marked with *Lobe* (locus: 72.0) gene and was used to analyze the varying segregation ratio in heterozygous male flies in about 390 chromosomes extracted from the natural population. The mean (k) segregation ratio was estimated to be 0.77, but it varied widely from 0.4 to 1.0. At 0.5 as k value, the effect of the segregation distorter was suppressed and at 1.0 as k value, complete expressibility of the gene was assumed.

The segregation of homozygous male flies for SD genes was found normal, but in the offspring the number was reduced. Several hypotheses illustrating the mechanism of segregation distortion have been proposed, but the problem still remains unsolved.

A Mechanism of Persistence of Some Lethal Genes in Natural Populations of *Drosophila melanogaster*

Takao K. WATANABE

Two lethal genes (*l* 404, *l* 407) on the second chromosome have been maintained at high frequency for at least 6 years in the same natural populations. One hundred twenty female flies, heterozygous for *Cy* and the persistent lethal chromosomes (*Cy*/*l* 404 or *Cy*/*l* 407) and the same number of female flies heterozygous for *Cy* and normal chromosomes (*Cy*/n_i) were mated with male flies having two normal chromosomes in heterozygous condition.

Each female was mated with three males in a vial and transferred every day to a new vial containing the culture medium. In the course of experiments, the longevity of the two kinds of flies was examined, and the number of their progeny was compared as to productivity. The ratio of non-*Cy* flies was estimated. Then, the viability of lethal heterozygotes (l/n_i) could be compared with that of normal heterozygotes (n_i/n_j).

The mean longevity of *Cy*-lethal heterozygous females was about 6 days shorter than that of *Cy*-normal heterozygous females, but the former produced 509 flies on an average during 10 days after emergence and the latter produced only 460 flies. The viability of lethal heterozygotes and normal heterozygotes is given in Table 5.

Table 5. Comparison of viabilities (pooled basis) of lethal and normal heterozygous flies

	Period					
	1–5 days		6–10 days		11–41 days	
	No. of flies	Viability*	No. of flies	Viability	No. of flies	Viability*
l/n_i	28351	1.0035±0.01192	22861	1.0174±0.01346	20772	1.0377±0.01440
n_i/n_j	27244	0.9519±0.01154	25383	1.0021±0.01258	30370	0.9867±0.01132

* 1% level significance.

The data were divided into three parts according to the period of egg laying. From the results, significantly more lethal heterozygous flies than normal heterozygous flies were not only produced by younger parents but also by older parents. This could be an effective mechanism of the persistence for those lethal genes.

Further Study on Chromosomal Polymorphism in the Kofu and Katsunuma Natural Populations

Taishu WATANABE[1] and Chozo OSHIMA

About 800 male flies were captured from two natural populations in the Kofu and Katsunuma territory in late October of 1965. These male flies were individually mated with several virgin females of an isogenic Samarkand strain. The salivary chromosomes of 8 or 10 F_1 larvae of each culture were observed. The chromosomal types of 573 male flies could be determined and the frequency of each kind of chromosomes was recorded. The expected numbers of all possible homozygous and heterozygous com-

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binations of the standard second and third chromosomes and various inversion chromosomes were calculated on the basis of Hardy-Weinberg's law.

The observed numbers and expected numbers of three groups; homozygotic, single inversion heterozygotic and double inversions heterozygotic, were compared with each other as shown in Table 6.

Table 6. Frequencies of nine combinations of homozygotic, single inversion heterozygotic and double inversions heterozygotic second and third chromosomes

Combination		No. of flies		
Chromosome 2	Chromosome 3	Obs.	Exp.	Deviation
Homozygotic	Homozygotic	66	64.27	+ 1.73
Single heterozygotic	Homozygotic	85	92.20	− 7.20
Homozygotic	Single heterozygotic	100	109.52	− 9.52
Single heterozygotic	Single heterozygotic	155	156.98	− 1.98
Total of single heterozygotic		340	358.70	−18.70
Double heterozygotic	Homozygotic	34	30.86	+ 3.14
Double heterozygotic	Single heterozygotic	55	52.55	+ 2.45
Homozygotic	Double heterozygotic	32	22.87	+ 9.13
Single heterozygotic	Double heterozygotic	31	32.79	− 1.79
Double heterozygotic	Double heterozygotic	15	10.95	+ 4.05
Total of double heterozygotic		167	150.02	+16.98
Grand total		573	572.99	+ 0.01

The heterotic effect of inversions, which have been maintained in fairly high frequencies (6~42 per cent) in the natural populations, could be recognized at least in the presence of two or three inversions on one arm of one chromosome.

Lack of Chromosomal Interaction with Respect to Overdominance in *Drosophila melanogaster*[1)]

Terumi MUKAI

As previously reported (Genetics **50**: 1-19, 1964), spontaneous mutant polygenes controlling viability have been accumulated independently in 104 second chromosome lines which originated from a single normal chromosome. Based upon the test for the heterozygous effects of these mutant polygenes located only in one of the homologous chromosomes in Generations 32, 60, and 78, we have proposed that an optimum level of heterozygosity exists for manifestation of overdominance (Genetics **52**: 493-501, 1965; Ann. Rep. **16**). Furthermore, it was reported that the optimum level of heterozygosity in the second chromosomes was independent of the heterozygosity of the third chromosomes (*Sb*/+ vs. +/+ third chromosome genetic background) (Ann. Rep. **16**). In order to confirm the second finding, an experiment was conducted in Generation 85. Homozygous and heterozygous viabilities of 50 chromosome lines selected from the above 104 lines were tested both in homozygous and heterozygous genetic backgrounds (*Sb*/*Ubx* third chromosome). The outline of the result is presented in Table 1. Forty-six lines out of 50 showed overdominance

Table 1. Means of the standardized viabilities of homozygotes and heterozygotes and their correlation coefficients

	Homozygous viability	Heterozygous viability	Correlation coeff. between homo- and heterozygotes
Homozygous genetic background	0.3329***±0.0455	1.0611***±0.0045	−0.1180
Heterozygous genetic background	0.3113***±0.0468	1.1007***±0.0074	−0.2443

Notes 1. Number of chromosome lines=50.
2. Control viability=1.0000.
*** Significantly different from 1.0000 ($P<0.001$).

in both genetic backgrounds, two showed it only in homozygous genetic background, and the remaining two only in heterozygous genetic background. Thus, it is confirmed that the manifestation of overdominance in the second chromosome is independent of the heterozygosity in the

1) This work was conducted in the Department of Medical Genetics, University of Wisconsin, Madison, Wisconsin, U.S.A. and was supported by a PHS Grant GM-07666.

third chromosome, contrary to the interaction among genes located within chromosomes. If the second and the third chromosomes are not independent, overdominance is not to be expected in the second chromosomes under the optimum heterozygosity hypothesis, because the level of heterozygosity of the third chromosomes (*Sb/Ubx*) is probably much higher than the optimum level for the second chromosomes.

The Detrimental Load to the Lethal Load Ratio (D:L Ratio) of Newly Arising Mutations in *Drosophila melanogaster*[1)]

Terumi MUKAI and James F. CROW

Greenberg and Crow (1960) have proposed a method for testing the degree of dominance of detrimental genes by comparing the D:L ratio in equilibrium population and that for newly arising mutations. The former ratio has been estimated to be 0.5~1.0 by Greenberg and Crow (1960), Band (1964), and Temin (1966).

In order to estimate the D:L ratio for newly arising mutations, spontaneous mutations controlling viability have been accumulated at a minimum pressure of natural selection in 150 second chromosomes which originated

Table 1. Summary of experimental results in Generations 10 and 20

	(CH)**			(PQ)**			(RT)**		
	Gen.0	Gen.10	Gen.20	Gen.0	Gen.10	Gen.20	Gen.0	Gen.10	Gen.20
Number of lethal lines	0	1	3	0	1	2	0	2	6
Mean viability including lethals	(34.10)	32.08	29.48	(33.51)	31.64	29.82	(28.11)	25.02	21.20
Mean viability excluding lethals	(34.10)	32.73	31.36	(33.51)	32.28	31.05	(28.11)	26.06	24.01
D:L ratio			1.35			1.89			1.27

The number of chromosome lines is 50 in each group; each originated from a single chromosome extracted from a natural population.

** Stands for the original Madison chromosomes.

1) This work was conducted in the Department of Medical Genetics, University of Wisconsin, Madison, Wisconsin, U.S.A. and was supported by a PHS Grant GM-07666.

from 3 Madison chromosomes (50 from each Madison chromosome). Homozygous viability of each chromosome was tested in Generations 10 and 20. The result is presented in Table 1. The D:L ratio was calculated for each chromosome group which originated from a single Madison chromosome. The results are 1.27~1.89 which are larger than those in equilibrium populations. This indicates that the degree of dominance of detrimental genes is larger than that of lethal genes which was estimated to be $\bar{h}$ =0.02~0.04 by several workers (e.g. Crow and Temin 1964; Hiraizumi and Crow 1960; Stern, *et al.* 1952).

Studies on the Competition between Races 1A and 21B of Wheat Leaf Rust

Keizo KATSUYA

Physiologic races of various plant pathogenic fungi continuously vary in prevalence in nature. The change in prevalence of races 1A and 21B of wheat leaf rust, *Puccinia rubigo-vera* Winter *f. sp. tritici* Mains, was investigated by comparing in growth cabinets aggressiveness of the two races and the factors that might influence them.

When a mixture of urediospores of the two races was used to inoculate two susceptible wheat varieties, Norin No. 61 and Shinchunaka, for five uredial generations race 21B quickly became predominant at 15° and 20°C, and after four uredial generations race 1A was nearly eliminated from the mixtures. Relative competitive abilities were not found to be influenced by the density of pustules on infected leaves. The rate of growth of pustules on the two wheat varieties grown at 15° and 20°C was determined by measurement with a microscope. Pustules of race 21B grew faster than those of race 1A at 15°C, and at twelve days after inoculation pustule size of race 21B was about 1.6 times larger than that of race 1A. At 20°C, however, pustules of race 21B grew almost the same as those of race 1A. Experimentation showed that the urediospores of race 21B were more infective than those of race 1A on the two wheat varieties and that the incubation period of race 21B was remarkably shorter than that of race 1A at 15°C. The predominance of race group 21 of wheat leaf rust in north Japan may have resulted from the greater aggressiveness of that race.

Interaction among Genotypes for Migration in *Drosophila melanogaster*

Takashi NARISE

In the previous migration experiment with wild and *vestigial* flies of *Drosophila melanogaster* it was found that the migratory activity of the *vestigial* flies increased due to mixing them with wild flies (see this Annual Report No. 12). It then become the immediate task to find out if the gene *vestigial* itself was responsible for making sensitive to the stimulation, or was an interaction between the gene and its genetic background responsible. The present experiment was conducted to resolve this problem.

Employed were five wild strains, MS-1, Niihama, Oshoro, Gifu, and Tosu, and their substitution *vg* strains. They were mixed one with another in a manner shown in Table 1. One hundred flies were introduced into the migration-tube in the either mixing or control experiment. The mixture was composed of 50 flies of a wild strain and 50 flies of the *vg*-substituted strain. The experimental results are presented in Table 1 and Table 2.

Table 1. Effect of *vestigial* genotype on migratory activity of five wild strains

	Migratory activity of wild type strains				
	MS-1	Tosu	Gifu	Niihama	Oshoro
MS-1 (*vg*)	13.2	22.0	25.6	20.8	31.2
Tosu (*vg*)	16.0	10.0	35.4	15.2	20.8
Gifu (*vg*)	19.8	12.4	22.0	16.0	22.4
Niihama (*vg*)	21.2	16.0	37.2	22.0	21.6
Oshoro (*vg*)	20.8	13.2	27.2	19.2	19.6
Single	26.8	28.4	21.6	28.8	24.6

Table 2. Effect of wild type on migratory activity of five *vestigial* strains

	Migratory activity of *vestigial* substituted strains				
	MS-1 (*vg*)	Tosu (*vg*)	Gifu (*vg*)	Niihama (*vg*)	Oshoro (*vg*)
MS-1	17.2	16.4	16.4	19.6	24.4
Tosu	23.6	11.6	16.4	12.0	20.0
Gifu	21.6	20.8	19.2	17.6	22.8
Niihama	18.8	27.6	16.8	8.0	18.8
Oshoro	24.8	14.4	18.8	15.6	13.6
Single	11.0	11.0	18.8	5.8	13.5

In these tables, MS-1 (*vg*) means the MS-1 strain substituted with the *vestigial* gene into the genetic background of MS-1 and so on.

It is found from Table 1 and Table 2 that the high migratory activity of wild type flies generally decreases by being mixed with a *vestigial* strain except for the Gifu strain. The activity of *vestigial* strains, however, increases due to being mixed with a wild strain except for Gifu (*vg*) strain. It is noteworthy that the migratory activity of a strain increased when the strain was mixed with strain having different genetic background in comparison with that having the same genetic background except for Niihama and Gifu (*vg*) strains.

The Relation between Migratory Activity and Competitive Ability in *Drosophila melanogaster*

Takashi NARISE

In order to find if migratory activity is in some way or other related to competitive ability in *Drosophila melanogaster*, an experimental study was conducted with a wild strain, MS-1. A selection experiment started with two hundred flies of the strain for high migratory activity. The selection was conducted using the migration-tube set, and the description of the apparatus and method of experiment have been repeatedly reported in the preceding issues of this Annual Report (see Annual Reports No. 7 and No. 8).

The selection was continued through eight generations, when the migratory activity of the selected strain attained 34.8 per cent in comparison with the original population with 20.5 per cent migration.

Competitive ability of the selected strain was tested in the 8th selection generation. Five pairs of flies of the selected strain were mixed with five pairs of the tester, a wild type of *D. simulans*, and that were kept five days in a half-pint milk bottle. Only emerged male flies of both species were counted because of the difficulty in distinguishing female flies between the two species. The number of all emerged male flies per bottle in a single culture of selected and original *melanogaster* strains was 193.53 and 193.68, respectively, suggesting that the two strains were not different with regard to propagating capacity. The capacity of *D. simulans* in the single culture was as low as 68.20. Table 1 presents the number of emerged male flies under mixed condition.

The findings from this experiment are : 1) the competitive ability of the selected strain was far lower than that of the original one, and 2) the number of emerged flies in the next generation was not different between the original and the selected strain suggesting that the fitness of these two

Table 1. Number of all emerged male flies per bottle under mixed condition

	melanogaster	*simulans*
Original	72.40	0.73
Selected	49.24	5.96

strains had little changed.

From this experiment, the conclusion can be drawn that migratory activity and competitive ability are negatively associated at least in the present fly population of *D. melanogaster*. For generalization of this fact, however, more study is without doubt required.

Experimental Induction of Bilateral Asymmetry in Wings of *Drosophila melanogaster*

Takashi NARISE and Kan-Ichi SAKAI

This study deals with an experimental induction of bilateral asymmetrty in the wings of *Drosophila melanogaster*. It has been observed that the wings of wild flies are almost completely symmetrical. Accordingly, there seemed to be little hope to obtain flies having asymmetrical wings by mere selection in wild populations. Finding, however, that the *vestigial* flies are almost more or less asymmetrical in their wings, selection for high as well as for low asymmetry was attempted in the population of *vestigial* stock. It was found that the selection was successful only for higher, but unsuccessful for lower asymmetry. The selected *vestigial* strain with high asymmetry in wings or, for simplicity, the *vg*-As strain, was crossed with wild type flies whose wings are completely symmetrical. The crossing was made between 25 wild type males and 125 *vg*-As females or 25 sets were set up each of 1 wild male and 5 *vg*-As females, yielding 125 families in total. The experiment was conducted at 25°±1°C.

Of the 25 sets of the hybridization experiment, 15 showed no sign of asymmetry. In ten, however, one or more than two families gave rise to bilateral asymmetry in wings of some flies. Those ten sets are shown in Table 1.

It is found from Table 1 that the complete symmetry in wings of normal wild flies can be upset by the effect of genetic system favoring asymmetry, integrated by selection in the *vestigial* flies.

Table 1. The sets of one wild ♂×5 *vg*-As ♀♀, in which flies with wing asymmetry arose in at least one family. 0 means that no F_1 had asymmetrical wings. The two figures without and with brackets stand for number in per cent of flies with asymmetrical wings and average value in 0.1 mm of asymmetry, respectively

Set No.	Female No.				
	1	2	3	4	5
1	30.00 (0.058)	0	—	0	0
3	0	0	0	0	7.7 (0.150)
6	0	0	38.40 (0.090)	19.00 (0.088)	—
9	0	0	24.30 (0.083)	0	—
10	0	0	0	0	92.90 (0.069)
12	2.7 (0.100)	0	0	0	0
14	0	20.00 (0.088)	0	0	0
15	0	0	0	0	14.70 (0.080)
21	23.50 (0.075)	13.60 (0.067)	0	0	0
24	11.80 (0.050)	0	0	0	0

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN ANIMALS

Post-Irradiation Modification and Mechanism of Reverse Dose-Rate Effect on Mutation Induction in Silkworm Gonia

Yataro TAZIMA and Toshihiko SADO

An enhancing effect on radiation-induced mutation was observed in the silkworm after fractionated exposure of gonial cells. As a possible interpretation of this phenomenon, some kind of modification in cellular metabolism was assumed. The modified state, which becomes detectable by increased mutation frequency, persists at least for 48 hours and is characterized by a peak appearing 18-24 hours after the initial exposure. The non-decay type curve suggested that this was not due to a biochemical disturbance but presumably to cell synchronization.

The results of an experiment, where two split doses were given to two experimental series in reciprocal order (250 R+750 R and 750 R+250 R) 6 hours up to 48 hours apart, showed that the peak appears earlier for the initial 250 R than for the initial 750 R series. By labelling S phase cells with H^3-thymidine, Sado observed that irradiated cells are blocked in their progression at G_2 and their proportion increases remarkablly in the cell population. This observation favours the hypothesis of cell synchronization. If this were the case, a decrease in mutation frequency should also be detected when second irradiation were given at an appropriate interval after the initial exposure. This, however, was not observed up to 72 hour interval, suggesting that synchronization, if any, is destroyed immediately in the subsequent cell cycle. Presumably the cells are released from the blockade at a reduced rate.

Based on those results, we examined the validity of the same mechanism for the interpretation of the reverse dose-rate effect. Cytological observations revealed that the mitotic process of gonia at a late stage is inhibited, being blocked at G_1 and G_2, during chronic exposure. Furthermore, it was confirmed by a new series of chronic exposure experiments lasting three days which were started every 24 hours that the chronic treatment becomes more effective for mutation induction than the acute one as early as one day after hatching, coinciding with the initiation of cellular activity. This observation explains why the dose-rate effect is reversed within a very short period.

(Abstract of a paper presented at the third International Congress of Radiation Research, Cortina D'Ampezzo)

Repair of Radiation Induced Premutational Damages Revealed by Fractionated Irradiation of Silkworm Spermatids

Yataro TAZIMA and Kimiharu ONIMARU

The results of our study, for past six years, on dose-rate effect of radiation, strongly suggested the existence of a mechanism of repair of premutational damages. Furthermore, the assumption of a repair phenomenon allowed a consistent interpretation of several kinds of experimental data obtained so far. Our knowledge, however, was rather scanty as to the mechanism of repair of premutational damages in higher organisms.

A new project has, therefore, been undertaken in our laboratory for the investigation of such a mechanism. As a first step of this project efforts have been made to find out good evidences for the repair of premutational damages which could be utilized as an experimental system for further studies.

For the elucidation of repair, two methods can be applied. One is a split dose irradiation with an appropriate time interval. The other is a post-irradiation treatment with various agents. In both cases, repair of premutational damages, if any had occurred, would be detected by a decrease in observed mutation frequency.

As reported previously, a remarkable enhancing effect has been observed with regard to mutation frequency when we irradiated silkworm spermatogonia with two split doses of 500 R+500 R. The mechanism was soon revealed in a cytological study by Sado (1966). Namely, after initial exposure the proportion of G_2 cells increased among the germ cell population and if the second exposure was given to those cells an enhancement in mutation response was the result. Accordingly, those cases can not be regarded as due to true repair.

In order to obtain conclusive evidences for the repair, fractionation experiments have been carried out with spermatids. Silkworm spermatids appear most abundantly in full-grown larvae. Furthermore, at this stage the cells are fairly homogeneous with respect to their developmental stage. Total dose of 1000 R was given to full-grown larvae in two fractions: 500 R +500 R. Intervals between the two exposures were 0, 6 and 12 hours. The results are given in Fig. 1.

It can be seen from the figure that split dose irradiation clearly reduces the induced mutation frequency in comparison with the same dose given singly.

Similar experiments were carried out at V-4.5 (fifth instar day 4.5) and mid-pupal stage, but the results were less conclusive for both stages.

Since irradiation of full-grown larvae almost completely excludes the

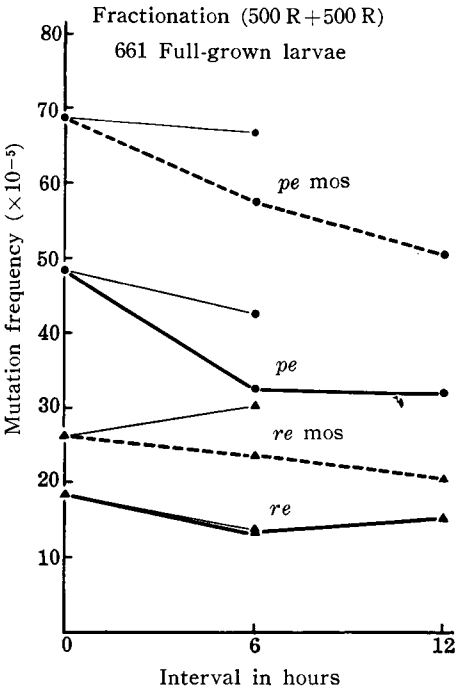


Fig. 1. Results of split dose irradiation of full-grown larvae: 500 R + 500 R with 0, 6 and 12 hour intervals. Heavy lines, solid and broken, represent the split dose irradiation and light lines denote single dose irradiation.

risk of contamination with cells irradiated at other stages than spermatids, the observed reduction in mutation frequency can be regarded as the reflection of a repair of premutational damages produced by radiation.

Modification of γ-Ray-Induced Mutation Frequencies in the Silkworm by Post-Treatment of Spermatids and Spermatozoa with Nitrogen Gas

Yataro TAZIMA and Kimiharu ONIMARU

As reported in the foregoing article (this Annual Report), a direct evidence of a repair of premutational damages was obtained by fractionated irradiation of spermatids. Another evidence has been obtained from the comparison of the effects of post-irradiation treatment between nitrogen and oxygen.

Both spermatids and spermatozoa were chosen as the object of this study. Irradiation of spermatids was performed when the larvae attained maturity and irradiation of spermatozoa was carried out after emergence. The material used was strain C108. For the detection of mutation, specific loci method was used by employing *pe* and *re* genes as markers. The effect of N_2 post-treatment was examined both for N_2 pre-treated and non-pretreated cells. For N_2 pretreatment, the wild type males were placed in a nitrogen gas chamber 25 minutes before irradiation and there they were exposed to γ-rays. In the non-pretreated group, irradiation of wild type males was carried out in the air. Irrespective of the pretreatment, the irradiated insects were divided into two groups: one was subjected to N_2 post-treatment for 25 or 50 minutes and the other to O_2 post-treatment for the same duration.

The results of experiments obtained for spermatids are shown in Fig. 1

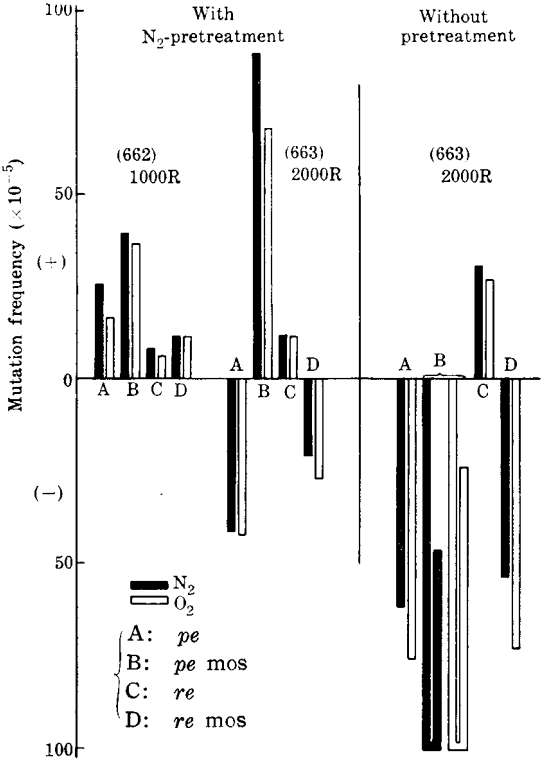


Fig. 1. Effect of post-treatment with N_2 as compared to that with O_2. γ-irradiation of spermatids. Details in the text.

In this figure cases in which post-treatment with N_2 was mutagenically more effective than with O_2 are shown in the upward histogram and those where N_2 was less effective are represented in the downward histogram.

Our experiments show that post-treatment with N_2 is in most cases mutagenically more effective than that with O_2 for spermatids irradiated under anaerobic condition. However, the effect becomes reverse when the spermatids are irradiated under aerobic condition.

In contrast, N_2 post-treatment of spermatozoa resulted in a lower mutagenic effectiveness than O_2 post-treatment when the insects had been pretreated with N_2 and irradiated under N_2.

Those results almost agree with those obtained by Sobels *et al.* for *Drosophila* and may be mentioned as another evidence of a repair of premutational damages.

Mechanisms of Mutation Induction by Mitomycin-C in the Silkworm

Yataro TAZIMA and Kimiharu ONIMARU

During the course of our experiments of post-irradiation treatment of silkworm spermatogonia with various metabolic inhibitors, we observed that mitomycin-C enhanced markedly the mutagenic effect of γ-rays. The enhancing effect was more pronounced when the chemical had been administered 12 hours after irradiation.

Concerning the effect of mitomycin-C upon biological systems, numerous papers have been published by many workers and at least three different mechanisms have been assumed: *i.e.*, 1) inhibition of DNA synthesis, 2) degradation of DNA and 3) cross-linking of DNA.

Since a strong mutagenic effect upon silkworm spermatozoa has been elucidated for some alkylating agents which cross-link DNA (Nakao, 1961), experiments have been carried out to examine if mitomycin-C is mutagenically effective when given to mature spermatozoa.

The chemical was injected as a saline solution into wild type male pupae of C108 strain two or three days before emergence. The administered dose of the chemical were 0.02～0.05 mg per capita. Mutation frequencies were estimated by specific loci method as usual. The results are shown in Table 1.

As may be clearly seen from the table, injection of mitomycin-C resulted in a high incidence of mosaic mutations as well as whole type recessive mutations. The most striking was an extraordinarily high incidence of mosaics. Their frequency was from 10 to 20 times as high as that of the whole type.

Assuming that the incidence of mosaics was due to a single helix (of

Table 1. Results of injection of mitomycin-C into male pupae (654) of the silkworm

pe re ♀ × treated + + ♂

Injected dose per capita	Observed number of eggs*				Mutants detected**			
	Unfertilized	Early dying	Surviving	Total	*pe*	*pe*, + mos	*re*	*re*, + mos
0.05 mg	3,174 (5.8)	2,225 (4.1)	49,005 (90.1)	54,404	46 (94)	471 (961)	30 (61)	661 (1,349)
0.02 mg	3,517 (4.9)	2,401 (3.4)	65,788 (91.7)	71,706	135 (205)	823 (1,251)	71 (108)	959 (1,458)

Numerals in parentheses are (*) percentage of eggs observed and (**) mutation frequency per surviving eggs represented in the order of 10^{-5}.

DNA) type mutation and that of whole type mutations to a simultaneous occurrence of two single helix type mutations at the same locus, the probability of the incidence of whole type mutations could have been expected to be the square of the probability of the mosaics. The observed frequency for the whole type was about one order higher than such expectation. This suggests that whole type mutants were also produced by a single mutational event provoked on a double helix of DNA.

Anyhow, the above results indicate that mitomycin-C can act on one of the double helices of spermatozoa DNA, where DNA synthesis is no more in progress.

Mutagenicity of a Nitrofuran Derivative Applied to Silkworm Germ Cells

Yataro TAZIMA and Yosoji FUKASE

It has been reported by Endo *et al.* (1963, 1966) that a nitrofuran derivative, 3-amino-6[2(5-nitro-2 furyl) vinyl]-1, 2, 4,-triazin hydrochloride, NFT, exhibits in several respects a similar action to that of mitomycin-C. According to their report, the chemical is a potent phage inducer in *Escherichia coli* K_{12} (λ) cells and inhibits DNA synthesis without affecting RNA and protein synthesis in non-lysogenic bacteria.

Since mitomycin-C has been proved to have a strong mutagenicity in the silkworm, we thought that it would be interesting to examine the mutagenic effect of NFT in comparison with that of mitomycin-C.

NFT used in this experiment was kindly supplied by Prof. H. Endo, Cancer Institute, Kyushu University. Two series of experiments have been carried out. Materials and methods used were almost the same as those reported in the foregoing article if not otherwise specified.

1) Feeding experiment with third instar larvae

NFT was administered to the third instar larvae by painting an aqueous solution of the chemical on mulberry leaves. The administered doses are given in the foot notes of Table 1.

Table 1. Results of feeding NFT to the third instar larvae (664)

pe re ♀ × treated + + ♂

Treatment	No. of eggs observed	No. of mutants detected				Mutation frequency ($\times 10^{-5}$)			
		pe	*pe*, + mos	*re*	*re*, + mos	*pe*	*pe*, + mos	*re*	*re*, + mos
None	134,116	31	6	0	2	23	5	0	2
*NFT (L)	123,483	28	10	1	4	23	8	1	3
**NFT (H)	126,031	51	14	3	3	41	11	2	2

*NFT (L): 10 mg of NFT was given to 1,000 larvae at one time.
**NFT (H): 50 mg of NFT was given to 1,000 larvae in five times.

It may be clearly seen from Table 1 that an increased dose of the chemical increases the mutation frequency both for whole and mosaic mutants.

2) Injection experiment at post pupal stage

The chemical was injected as a saline solution to wild type male pupae of C108 strain two or three days before emergence. Injections with known mutagenic agents, nitromine (NM) and ethyl methane sulphonate (EMS), were performed in parallel. The results are given in Table 2. In this experiment the mutagenic effect of NFT could be again observed. The

Table 2. Results of injection of NFT into pupae (663)

pe re ♀ × treated + + ♂

Treatment	No. of eggs observed	No. of mutants detected				Mutation frequency ($\times 10^{-5}$)			
		pe	*pe*, + mos	*re*	*re*, + mos	*pe*	*pe*, + mos	*re*	*re*, + mos
Control	149,908	42	12	2	2	28	8	1	1
NFT 0.01%	98,540	55	15	1	5	56	15	1	5
〃 0.1%	121,026	80	11	4	5	66	9	3	4
NM 0.025%	114,869	59	25	4	9	51	22	4	8
EMS 0.01%	135,386	50	55	4	43	40	44	3	34
〃 0.1%	103,516	59	1,065	3	602	57	1,029	3	582

* Injected amount was 0.05 m*l* of physiological salt solution of the chemical at the concentration specified.

induced mutation frequencies were clearly enhanced for whole mutations, while those for mosaics were not significantly increased.

Those results are in sharp contrast to those obtained for NM, EMS as well as mitomycin-C, which produced a high incidence of mosaics. NFT seems to have a specific action by attacking both DNA helices at one event.

Changes in the Mutation Response of Post-Meiotic Silkworm Germ Cells to γ-Rays with Progressing Spermiogenesis

Yataro TAZIMA

The double helix model of DNA suggests that there could be two types of mutations concerning the site of occurrence, *i.e.* those occurring in one of the double helices and those occurring in both helices. Identification of those two types may be possible if post-meiotic germ cells are irradiated. When mutants are obtained as mosaics, they may be attributed to the single helix type. Whereas, mutants in whose whole body a recessive character is expressed may correspond to the double helix type mutation.

It has often been observed in the silkworm that irradiation of spermatozoa resulted in the production of a higher proportion of mosaics than when early spermatids were irradiated.

In order to know if mutation response varies according to progressing spermiogenesis, data obtained from different experiments have been arranged in accordance with the development of the insects. Although the extent of mutation response varies depending on the irradiated stage, those data can be compared so far as the ratio of mosaics to whole mutants is concerned.

In Fig. 1 calculated ratios of mosaics to whole mutants at each locus are plotted against the progression of the developmental stages of the insect. In the male, meiosis takes place most frequently around V-2 (instar V day 2), and thereafter the germ cells enter into the spermatid stage. When the larva attains maturity (Full G.) fully formed spermatozoa appear in the testis and increase in number progressively thereafter until the mid-pupal stage (M.P.).

As can be clearly seen from the figure, the incidence of mosaics in relation to whole mutation is very low before meiosis, reaches to the unity around V-4.5 and then increases rapidly with the progress of spermiogenesis.

The relation can be more intuitively understood from the curves marked with rightward arrows. Those curves represent changes in induced fre-

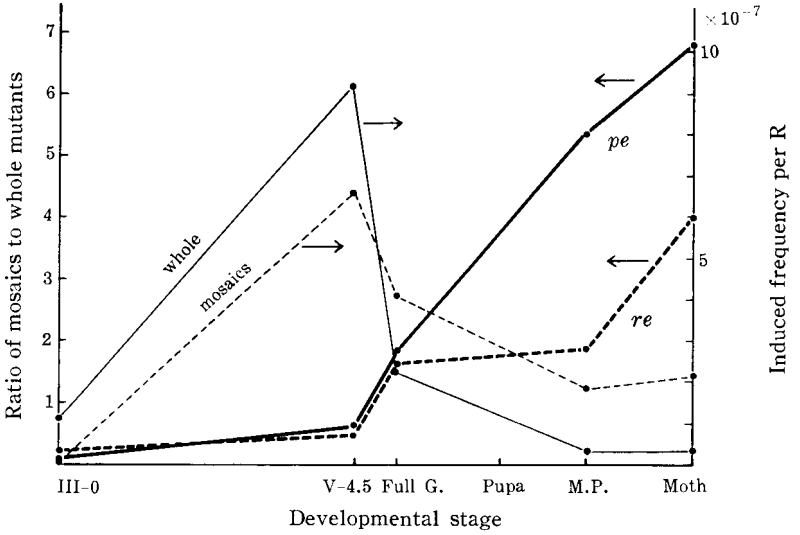


Fig. 1. Changes in the mutation response of silkworm spermatogenic cells with the progress of development. Curves with rightward arrows are to be read against the right ordinate and *vice versa*.

quencies per R for whole and mosaic mutants, in an average for two loci.

It can be inferred from those results that the site of mutation response becomes reduced in size with the progression of spermiogenesis. Presumably this change might be correlated with a decreasing facility to repair premutational damages.

Studies on Strain Differences in Radiosensitivity in the Silkworm
I. Screening of Sensitive and Resistant Strains to Embryonic Radiation Killing

Akio MURAKAMI and Yataro TAZIMA

A comparison of radiation effects between strains of different radiosensitivity may help to understand better the mechanism of radiation effects on living organisms. From this view point, experiments have been carried out in order to select sensitive and resistant strains to embryonic radiation killing. A worldwide collection of silkworm strains has been maintained for nearly 50 years at the Sericultural Experiment Station, Tokyo. Among 250 strains, 30 representative strains were chosen and their radiosensitivity to embryonic killing was examined. X-irradiation

was applied to over-wintered eggs on the 30th of March, 1966. Irradiation conditions were: 173 kVp, 25 mA and 1.0 mm Al filter. Four different doses, 800, 1,200, 1,600 and 2,000 R, were given at dose-rate of 250 R/min. After hatching of the surviving embryos, hatchability was calculated and LD_{50} was estimated from dose-response curves. The results are shown in Table 1. The average LD_{50} for 30 strains was 910 R. It may be clearly

Table 1. Differences in radiosensitivity among 30 strains

Race	Strain	LD_{50} (R)	Race	Strain	LD_{50} (R)
Japanese	Aojuku	1,460	Chinese	Dai-ankyô	860
	Akajuku	1,320		Dai-kôjyô	1,050
	Kojiki	230		Ben-yô	800
	Tadamisan	940		Santô-sanmin	800
	Matamukasi	1,320		Sisen-sanmin	980
	Chûsu	890	China-southern Asian	Daizô	930
Korean	Kôrai-sanmin	650		Ringetu	670
Ryukyuan	Ryûkyû-tasanken	1,110	European	Toruko-ôken	1,100
Chinese	Kansen	1,580		Ascoli	140
	Kin-ô	800		Biohne	520
	Seikyo	1,160		Maruke-ôken	610
	Sekko	800		Maiera-zebra	760
	Daientô	800		Rosa	1,020
	Hikô	1,340		Var	1,280
	Syôkô	1,080		Kokuga	1,500

seen from the table that there were striking differences among strains with regard to radiosensitivity. The LD_{50} values for the most radio-resistant three strains were approximately 1,500 R. In contrast, two strains were extremely sensitive, *i.e.*, LD_{50} was 140 R for Ascoli and 240 R for Kojiki. These differences in radiosensitivity might have been, in part, due to different dosages of the relevant genes accumulated in those strains.

Table 2. LD_{50}'s obtained for 16 combinations of diallelic crosses

Male		Female			
		Kansen	Aojuku	Kojiki	Ascoli
Kansen	(1,580)	1,580	1,700	1,480	1,750
Aojuku	(1,460)	1,750	1,540	1,060	1,660
Kojiki	(230)	1,750	1,460	230	1,600
Ascoli	(140)	1,750	1,625	1,600	140

In order to investigate the mechanism, the radiosensitivity of F_1 eggs was examined in all combinations of diallelic crosses between some resistant and sensitive strains. Those eggs were irradiated one day after HCl treatment for artificial hatching. The results obtained are given in Table 2. LD_{50} values thus obtained for self-bred batches were almost the same as that observed for over-wintered eggs. In cross-bred batches, however, LD_{50}'s were roughly the same as those observed in inbred batches of the resistant strains. Those results suggest that the radiosensitivity of silkworm embryos is controlled by two major genes at least, and that the effect of maternal or cytoplasmic factors is relatively small.

Studies on Strain Differences in Radiosensitivity in the Silkworm
II. Relation between Sensitivity to Embryonic Killing and Mutability

Akio MURAKAMI and Yataro TAZIMA

As reported in the foregoing article, some strains sensitive or resistant to radiation were discovered with regard to embyronic killing. Using those strains, an investigation has been carried out to ascertain if sensitivity to mutation runs parallel to sensitivity to killing. The strains used were: (resistant) Kansen, Aojuku and Hiko, (moderate) Sekko and (sensitive) Ascoli and Kojiki. The mutability was measured by the specific locus method, using egg colour markers. Irradiation conditions of X-rays were: 173 kVp, 25 mA and 1.0 mm Al filter throughout this study. Doses delivered were 250 R per minute.

Irradiation of gonial cells: Both spermatogonia and oögonia were irradiated in the larvae after hatching. The dose applied was 1,000 R only. The results are given in Table 1 a and b, separately for spermatogonia and oögonia, respectively.

Table 1. Mutation rates for the gonial cells in five representative strains

a. Spermatogonia

Strain	Mutation-rate			
	pe locus		*re* locus	
	Whole	Mosaics	Whole	Mosaics
	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-8}$
Kansen	37.4	1.6	6.2	1.3
Aojuku	58.5	5.8	13.0	0.0
Sekko	471.3	3.1	144.9	4.1
Ascoli	232.1	2.2	49.1	0.0
Kojiki	135.4	15.6	41.6	10.4

b. Oögonia

Strain	Mutation-rate			
	pe locus		*re* locus	
	Whole	Mosaics	Whole	Mosaics
	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-8}$
Kansen	—	—	2.4	0.0
Aojuku	19.0	9.0	1.4	0.0
Sekko	173.5	12.7	122.5	7.8
Ascoli	—	—	109.8	3.9
Kojiki	35.0	7.0	19.6	1.4

It can be seen from the table that the mutation rates are higher for spermatogonia than for oögonia in all strains studied. It is of interest to note that the mutation rate of a Chinese strain, Sekko, was extraordinarily high, although as to embryonic killing it belonged to the moderate group. Except for Sekko, it may be roughly inferred that sensitive strains to embryonic killing are also sensitive to mutation in both sexes.

Irradiation of oöcytes: The sensitivity of oöcytes and mature spermatozoa was tested after irradiation of newly emerged adults. The results for mature oöcytes are given in Table 2. In this experiment X-ray doses

Table 2. Mutation rates for the mature oöcytes in five representative strains

Strain	Mutation-rate			
	pe locus		*re* locus	
	Whole	Mosaics	Whole	Mosaics
	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-8}$
Kansen	470.6	28.4	500.6	27.0
Aojuku	386.0	30.2	372.3	14.9
Hiko	634.8	35.5	766.1	23.1
Ascoli	1,012.4	130.3	447.9	77.7
Kojiki	1,111.7	72.3	732.0	55.6

given were 1,000 R and 2,000 R. The mutation-rates are shown in an average for the two doses. It appears that sensitive strains for embryonic killing, Ascoli and Kojiki, were also sensitive with respect to mutation.

Irradiation of mature spermatozoa: The results of the experiment with mature spermatozoa are presented in Table 3. In this experiment X-ray doses given were 3,000 R and 6,000 R. The mutation rate is expressed as an average of the two doses. No distinct correlation was found be-

Table 3. Mutation rates for the mature sperms in five representative strains

Strain	Mutation-rate			
	pe locus		*re* locus	
	Whole	Mosaics	Whole	Mosaics
	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-8}$
Kansen	16.7	37.6	8.9	12.4
Aojuku	16.1	26.3	8.7	14.2
Hiko	22.8	34.3	4.7	18.7
Ascoli	26.9	24.2	8.9	22.8
Kojiki	19.9	38.8	5.8	18.2

tween radiosensitivity to embryonic killing and mutability. This finding is in sharp contrast to those described above for the three types of germ cells. The discrepancy might be due to either to the lack of recovery of radiation damage or to a different repair system in mature spermatozoa.

Relation between Sensitivity to Killing and Mutation Observed during a Mitotic Cycle of Silkworm Cleavage Nuclei

Akio MURAKAMI

Cyclic change in radiosensitivity to killing has been revealed for early cleavage nuclei of the silkworm (Murakami, 1966). The most sensitive phase within a cell cycle was found to be the metaphase/early telophase, whereas the least sensitive was the interphase. This finding suggested that dividing chromosomes are sensitive.

The purpose of this study was to investigate the change in mutation response in relation to the progress of the mitotic cycle during early cleavage. Materials used were eggs laid by ++/++ females mated with *pe re*/*pe re* males. These eggs were collected every 10 minutes and allowed to develop for a definite duration until they were irradiated. From 170 minute to 230 minute old eggs after oviposition were irradiated at 10 minute intervals. Radiation dose of 1,000 R was delivered in one minute. Mutation frequencies were estimated by specific locus method using the *pe* and *re* genes. The lethality was also examined at the same time. The mitotic phase of the irradiated cells was determined cytologically. The results are shown in Fig. 1. Mutations most frequently found in this experiment were fractionals. The highest peak for embryonic killing was observed at 190 minute age. In contrast, the highest mutation frequency was observed at 200 minute age. Cytological observation indicated that the

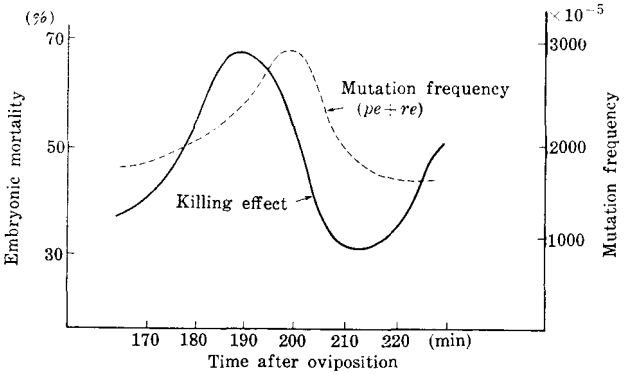


Fig. 1. Changes in sensitivity to killing and mutation during a mitotic cycle as expressed after oviposition as time function.

phase of maximum embryonic killing corresponded to metaphase/early telophase (or M+G_1) and the highest mutation response appeared at late telophase/early interphase (or G_1+early S). The discrepancy between the peak stages for cell killing and for mutation response suggests that the mechanisms involved are different. Presumably we may assume that the target or targets responsible for cell killing are chromosomes and those for mutation response are DNA strands.

The Effect of 5-Bromodeoxyuridine (BUDR) on the Frequency of 14 MeV Fast Neutron Induced Mutations in the Gonial Cells of the Silkworm

Akio MURAKAMI

It was reported in our previous paper (Murakami and Tazima, 1964) that a post-irradiation treatment of silkworm spermatogonia with BUDR or 5-Bromouracil (BU) increases the frequency of specific locus mutations by about two or three times at the maximum (Murakami and Tazima, 1964). This enhancing effect was assumed to be caused by an inhibition with such chemicals of the recovery process of pre-mutational lesions.

It was thought, therefore, to be of interest to study whether the radiation lesions produced by higher LET (Linear Energy Transfer) radiations could be modified by a similar treatment with those chemicals. Hence, the effect of BUDR treatment of 14 MeV fast neutron induced mutations was studied in gonial cells of the silkworm.

Materials and method used were the same as described in the previous paper (Murakami and Tazima, 1964). BUDR was administered to newly

hatched larvae of a wild type by feeding them on leaves supplemented with the chemical (20 mg per 1,500 heads) immediately after 14 MeV neutron irradiation.

The results are given in Table 1 a and b, for males and females, separately.

Table 1. Effect of BUDR on 14 MeV fast neutron-induced mutation frequency in silkworm gonia

a. Males

Treatment	Total No. observed	*pe* locus				*re* locus			
		Whole		Mosaics		Whole		Mosaics	
		Mut. dltd.	Mut. freq.	Mut. dltd.	Mut. freq.	Mut. dltd.	Mut. freq.	Mut. dltd.	Mut. freq.
			$\times 10^{-5}$		$\times 10^{-5}$		$\times 10^{-5}$		$\times 10^{-5}$
Control	176,245	58	32.9	3	1.7	4	2.3	0	0.0
BUDR*	145,184	48	33.1	5	3.4	4	2.8	1	0.7
14 MeV neutrons 1,000 rad	219,521	172	78.4	6	2.7	33	15.0	2	0.9
14 MeV neutrons 1,000 rad+BUDR*	147,976	133	89.9	8	5.4	40	27.0	2	1.4

b. Females

			$\times 10^{-5}$		$\times 10^{-5}$		$\times 10^{-5}$		$\times 10^{-5}$
Control	212,420	21	9.9	8	3.8	14	6.6	0	0.0
BUDR*	177,346	8	4.5	8	4.5	3	1.7	1	0.6
14 MeV neutrons 1,000 rad	244,530	68	27.8	9	3.7	28	11.5	2	0.8
14 MeV neutrons 1,000 rad+BUDR*	147,212	17	11.6	14	9.5	22	14.9	2	1.7

* BUDR: 20 mg per 1,500 heads.

The mutagenicity of BUDR treatment alone is not clear. However, when combined with 14 MeV neutrons, mutation frequencies were slightly enhanced in the experiment with spermatogonia. Whereas, no enhancing effect was observed in the experiment with oögonia. In the former the enhancing effect was higher at the *re* locus than at the *pe* locus. As to the enhancing effect of BUDR, neutron induced mutations responded to a lesser extent than X-ray induced mutations. This might have been due to the fact that neutron induced pre-mutational damages are less responsive to repair than those produced by low LET radiation.

Relative Biological Effectiveness of 14.1 MeV Neutrons in the Induction of Dominant Lethal Mutations in the Mouse

Kiyosi TUTIKAWA

The effectiveness of fast neutrons was compared with that of acute X-rays in the induction of dominant lethal mutations following irradiation of spermatozoa.

The irradiation of 14.1 MeV neutrons from T(d, n)He reaction was carried out at the Research Institute of Nuclear Medicine and Biology, Hiroshima University, Hiroshima. Two to three months old male mice of CBA strain were irradiated with doses of 242.5 and 485 rad neutrons, respectively. Other males of the same strain were given whether doses of 222 or 445 rad X-rays (dose rate 47.5 r/min.), for comparison of dominant lethality with that for neutrons.

One day after the irradiation each male was outcrossed to two adult females of the ICR stock. Matings were detected by daily examination of all females for vaginal plugs. Those females becoming pregnant were autopsied on or about 14th day of gestation, when number of corpora lutea, and of live and dead implants were recorded.

The dominant lethality was estimated from the live embryo/corpus luteum ratio, the induced dominant lethality being given by 1—(ratio in irradiated series/ratio in control series). The comparisons of fast neutrons and X-rays results to be considered here was based on the early matings during first one week after irradiation.

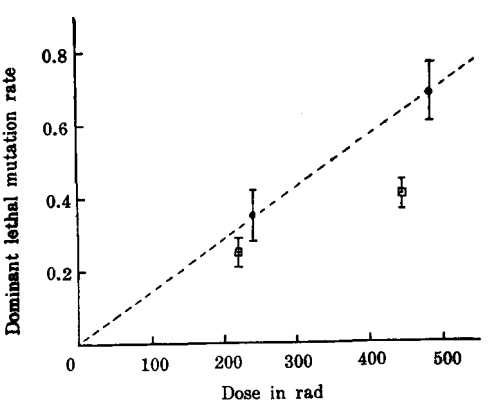


Fig. 1. Estimated dominant lethal mutation rates per gamete induced by 14.1 MeV neutrons and X-rays in mouse spermatozoa.

●: Neutrons ⊡: X-rays

Using $-\log_e(1-P)$ as an estimation of the dominant lethal mutation rates per gamete induced by two kinds of radiation, it was shown that the mutation frequency from neutrons is higher than that from X-rays, and the dose-frequency curves are in linear relationship (Fig. 1). If we equate the mutation rate of 445 rad X-rays with that of 242.5 rad neutrons around the 50 per cent level of dominant lethality, results suggest that 14.1 MeV neutrons are about 1.8 times as effective as acute X-rays for the induction of dominant lethal mutations in mouse spermatozoa.

VIII. RADIATION GENETICS IN PLANTS

RBE of Radiations in E-1 Hole of Kyoto University Reactor (KUR)

Seiji MATSUMURA, Etsuo AMANO and Masaru HAYASHI

To obtain basic data for future use of Kyoto University Reactor (KUR), dry seeds of rice plant and Einkorn wheat were irradiated in E-1 exposure hole of KUR under nitrogen gas or air atmosphere. As preliminary experiments, in June, 1966, hulled seeds of *Oriza sativa* were sealed in polyethylene vials after being flushed with nitrogen gas so that the desired atmospheres were obtained, and, three days later, they were irradiated in E-1 hole for 180 minutes at 1,000 kW. Radiation dosage of each sample was adjusted by varying the distance from the core side end, and thermal neutron doses were calculated from activation of gold foils and atomic composition of the material. γ-ray from 6,000 Ci ^{137}Cs source of National Institute of Genetics was used as standard radiation.

Results of seedling height measurement of the reactor radiations irradiated seeds showed that 50 per cent seedling height reducing doses were obtained at 21 cm for nitrogen gas and 22 cm for air atmosphere, from core side end of the E-1 sample holding boat, revealing distinct protective effect of nitrogen gas. Existence of such oxigen effect strongly support the heavy contamination of γ-rays measured by physical dosimetry (cf. KURRI-TR-16).

In October, 1966, seeds of *Triticum monococcum* were prepared as samples sealed in specially designed two celled air tight acryl resin containers. Nitrogen atmospheres were achieved by several times flushings of nitrogen gas after reducing gas pressure by a vacuum pump each time, and sealed in nitrogen gas stream. Exposure to reactor radiation was done three days later in E-1 hole of KUR. As *T. monococcum* seeds are more radiation sensitive than *O. sativa* seeds, 90 minutes of exposure at the same 1,000 kW gave 50 per cent seedling height reducing dose at about 24 cm for air preparation and 22 cm for nitrogen atmosphere, from the core end of the boat. Seedling height measurement on seventh day after sowing again revealed oxigen effect as shown in Figure 1 in which E-1 radiation dose was shown by thermal neutron component. Calculations of RBE of the reactor thermal neutrons free of contaminating γ-rays were tried at the 50 per cent seedling height reducing dose, assuming that the contaminating γ-rays in E-1 radiations might have the same RBE as that of ^{137}Cs γ-ray, and that the difference of dosages of the two γ-rays might be attributed to thermal neutrons.

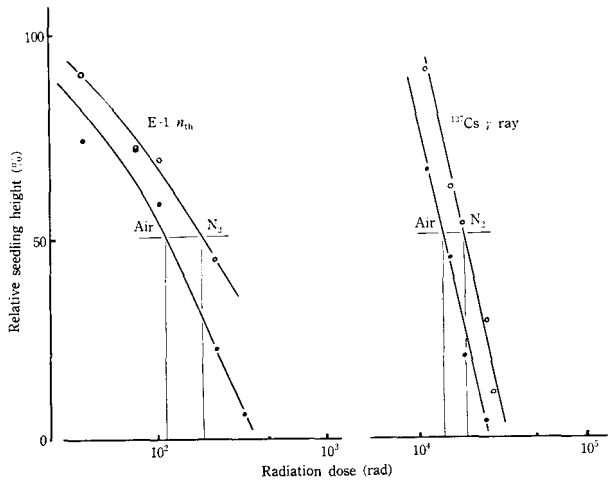


Fig. 1. Effect on seedling height of *T. monococcum* of KUR E-1 thermal neutrons and ^{137}Cs γ ray.

Under air atmosphere, the difference in γ-rays was 2,000 rads, effect of which was comparable to 131 rads of thermal neutrons, giving RBE value 15.3. Since efficiency of the γ-rays might be reduced under nitrogen gas atmosphere the difference in γ-rays was 5,000 rads and equivalent effect might be brought about by 181 rads of thermal neutrons, giving RBE value 28.3. Although fluctuation of γ-ray contamination and difficulty of its dosimetry are the problems still to be worked out about the reactor radiations, following calculations were tried further. With the same assumption about efficiency of γ-rays, it might be considered that 131 rads of thermal neutrons reduced final 13 per cent of the seedling height to reach 50 per cent height of control in case of air, and 181 rads reduced final 17 per cent in case of nitrogen atmosphere, to get to 50 per cent height, suggesting the same efficiency of thermal neutrons in seedling height reduction regardless of the atmosphere.

As for RBE values of overall E-1 radiations to ^{137}Cs γ-ray, 1.12 for exposure in air and 1.35 in nitrogen atmosphere were obtained.

Since contribution of thermal neutrons to the effect on the botanical materials used was only 13.6 per cent (air) or 26.7 per cent (nitrogen), RBE being considered, the values estimated may subject to be corrected when advanced dosimetry for contaminating γ-rays can supply further information.

Comparison of Mutagenic Efficiency between EMS and γ-Rays

Seiji MATSUMURA and Tarô FUJII

Seeds of a normal strain and heterozygotic F_1 seeds from a cross between normal and *chlorina* mutant of *Triticum monococcum* were soaked in water for two hours and then treated with 0.1, 0.3 and 0.5% EMS sulutions for 22 hours. For comparison, the seeds soaked for 24 hours were subjected to 0.5, 1.0 and 1.5 kR of γ-rays. Moreover, EMS and γ-ray treatments were combined to examine the synergestic effect of both treatments.

In the normal strain, several types of stripes were observed in many plants during the growth period of EMS treated lots, while they appeared very seldom in γ-ray lots. About 20 per cent survived plants had *chlorina*-like yellowish green stripes in 0.3% lot, and a similar frequency was also observed in EMS 0.3%+γ-ray 1.0 kR lot, while such type of stripes was never seen in the three γ-ray lots, as shown in Table 1.

Table 1. Appearance of *chlorina*-like streaks in the treated generation

Strain	Treatment	No. of seeds sown	Total No. of spikes	*Chlorina* or *chlorina*-like streaks		
				No. of plants	No. of spikes	(%)
Normal	Control	300		0		
	EMS 0.1%	200	7183	2	3	(0.042)
	〃 0.3%	200	4185	23	42	(1.004)
	γ-ray 0.5 kR	200	7990	0		
	〃 1.0 kR	200	5887	0		
	〃 1.5 kR	200	5226	0		
	EMS 0.3%+γ-ray 1 kR	200	4270	18	42	(0.984)
F_1	Control	300				
	EMS 0.1%	400	7447	0		
	〃 0.3%	400	4703	20	46	(0.978)
	γ-ray 0.5 kR	348	6965	1	3	(0.043)
	〃 1.0 kR	400	7138	2	4	(0.056)
	〃 1.5 kR	400	5987	2	6	(0.100)
	EMS 0.3%+γ-ray 1 kR	400	1607	9	24	(1.493)

Seeds from some tillers with stripes and from normal green tillers of the same individual were sown and segregation of *chlorina* seedlings was examined. *Albina* or other kinds of chlorophyll mutants were segregated among these X_2 seedlings, but no *chlorina*-like segregants was observed.

From this fact, we may say that no somatic mutations from dominant green to recessive *chlorina* occurred in F_1 by EMS treatment.

In the F_1 strain, number of *chlorina* or *chlorina*-like stripes in EMS 0.3% lot amounted to 0.98 per cent and the frequency was almost the same as in the normal strain. Rates of striping in γ-ray lots increased with increase of dosage but they were much lower than those of EMS 0.3% lot. The rate in the EMS 0.3%+γ-ray 1.0 kR lot was 1.49 per cent which is higher than the sum from EMS 0.3% and γ-ray 1.0 kR lots.

Segregation of several kind of chlorophyll mutants were observed in the X_2 generation of the normal strain. Chlorophyll mutation rate was similar in EMS 0.1% and γ-ray 1.0 kR lot, but the in EMS 0.3% lot was very high. These results show that the mutagenicity of EMS is very high in high dosages when the mutability is compared on the basis of their survival rates.

Comparison of the Killing Effect of γ-Rays and Thermal Neutrons

Tarô FUJII

Dry and 48 hour steeped seeds were used and exposed to thermal neutrons were done by the pneumatic tube which was provided in the reactor of Kyoto University; the length of irradiation time were 7 sec in minimum and 180 sec in maximum at the out put of 1 MW. The 7 sec irradiation of dry seeds showed merely 19 per cent of survival rate, and no survival plants were observed in other lots.

From the above experience, out put of reactor was diminished to 50 kW in the second experiment and similar observation was repeated; the details of irradiation and survival rates were given in Table 1 with the results of γ-ray irradiations. γ-ray irradiations for dry seeds had almost no effect, but slight decrease of survival rate was observed in 100 kR lot. Decrease of survival rate was marked in wet seeds lots. In the neutron lots, killing effects were severe and no survival plant was observed by 180 sec irradiation of dry seeds while contaminated γ-ray was 45 kR. Higher killing effects in the wet seeds were also seen in the neutron lots, but it might be the results of contaminated γ-rays because the environmental modification of radiation effect is very small in high LET radiations, while it is rather large with sparsely ionizing radiations. These results show that the killing effect of thermal neutrons was very high. RBE of thermal neutrons in somatic mutations will be examined in the next step of experiment and RBE versus LET relations should be determined.

Table 1. Killing effects of thermal neutrons and γ-rays for dry and wet seeds

Dose (sec)	Total neutron flux* (N_{th}/cm^2)	Contaminated γ-ray (kR)**	Survival rate (%)	
			Dry	Wet
0	—	—	93.9	—
5	1.39×10^{12}	1.25	—	80.6
10	2.44 〃	2.5	97.1	68.0
20	4.58 〃	5.0	69.5	63.5
30	7.48 〃	7.5	88.9	37.8
40	9.48 〃	10.0	71.9	43.3
60	14.5 〃	15.0	70.6	0.0
90	22.4 〃	22.5	59.0	0.6
120	26.6 〃	30.0	17.2	0.0
180	51.5 〃	45.0	0.0	0.0
300	71.9 〃	75.0	0.0	0.0
Dose of γ-rays from Cs^{137} (kR)				
	10		91.6	92.7
	30		90.8	75.6
	50		92.8	21.7
	70		93.3	0.0
	100		82.4	0.0

* Estimated from the activation of Au-foil.

** Calculated from the measurement of γ-rays at 1 MW.

On the Determination of Absorbed Dose in Heavy Ionizing Particles

Tarô FUJII

Dry F_2 seeds obtained from F_1 hybrids between a hairless mutant and the wild strain of *Arabidopsis* were subjected to γ-rays from a Cs^{137} source and to He^4, C^{12} and Ar^{40} ions from the Hilac. From the experiments we could recognize a severe killing effect of heavy ionizing particles, and also dramatically high mutation rates and marked growth inhibition. RBE for somatic mutation at 0.5 per cent frequency was roughly estimated as 10, 35 and 5 for He-, C- and Ar-ions, respectively[1]. The range of heavy ionizing particles is very short. The average seed volume was calculated roughly from the weight and measurements of length and width of the

[1] Fujii, T., M. Ikenaga, and J. T. Lyman, 1966. Rad. Bot. **6**: 297-306.

seeds, to be 20.5×10^{-3} mm^3. From this calculation, it was concluded that Ar-ions must be stopped within the seeds but He- and C-ions certainly could penetrate and pass through seed tissue. Moreover the energy transfer varies according to the position of the range or tissue which may be seen from the Bragg Curve[2].

Further experiment about killing efficiency of heavy ionizing particles was done according to above questions. Dry seeds were irradiated with C-ions in five different lots. Each lot received the same number of par-

Table 1. Relation between the survival rates and the thickness of absorbers

	Absorber (mg/cm^2)	Range (micron)	Survival rate (%)
Control	—	—	71.6
Lot 1	0	525	66.0
2	10.2	400	64.8
3	19.7	315	57.6
4	29.9	215	46.7
5	39.3	125	46.8

ticles per cm^2, but the energies of the ions were varied for the different lots by different thickness of absorbers and lot 1 received a surface dose of 400 rads (Table 1). Survival rates decreased with the increase of absorber's thickness. The results suggest that the irradiation of C-ions with maximum stopping power will show more severe killing effect, because the maximum of the stopping power is 6×10^3 Mev/g/cm^2 at the range of 45 mg/cm^2. Namely, RBE value of C-ions should be more larger than that obtained in the previous experiment when the embryo was attacked by the ions with maximum stopping power. Further studies are now under examination.

Photoreactivation of an UV-Induced Mutation in Maize

Seiji MATSUMURA and Tomoo MABUCHI

A normal maize strain (*Su*) concerning the sugary endosperm gene of a local variety and a recessive line (*su*) of Golden Cross Bantam were used in this study. Table 1 summarizes the data obtained from the experiments with UV- or γ-ray induced mutations at *Su* locus after visible

[2] Brustad, T., P. Ariotti and J. T. Lyman, 1960. Lawrence Rad. Lab. Rep. UCRL-9454.

Table 1. Frequencies of mutations from *Su* to *su* in maize pollen grains induced by UV and γ-rays treated with VL and dark after irradiation

Irradiation	Dose	Post-irradiation treatment	No. of seeds set	No. of seeds set per ear	No. of endosperm mutants		
					Whole Obs. (%)	Chimeras Obs. (%)	% of the whole mutations
Control	0	VL	4164	134	1(0.024)	3(0.072)	25.0
	0	Dark	4023	108	1(0.025)	1(0.025)	50.0
UV	2.7×10^3 ergs	VL	3420	148	6(0.17)	15(0.44)	28.5
	5.4×10^3 ergs	VL	4556	85	18(0.39)	35(0.76)	33.9
	〃	Dark	2333	41	22(0.94)	23(0.98)	48.8
	8.1×10^3 ergs	VL	913	43	5(0.55)	11(1.20)	31.2
	〃	Dark	1557	45	25(1.60)	19(1.22)	56.8
γ-rays	550 R	VL	4122	132	25(0.60)	11(0.27)	69.4
	〃	Dark	5579	143	35(0.62)	13(0.23)	72.9
	1100 R	VL	2134	152	44(2.06)	18(0.84)	70.9
	〃	Dark	4154	122	122(2.40)	22(0.52)	81.9

light (VL) and dark post treatments. The percentage of seed set was high after the two doses of γ-rays, while it decreased with the increase of UV-dose to about 50 per cent of the control lot at the highest dose. Induced whole (endosperm wholly deficient for the dominant character) and chimeral mutants (endosperm consisting partially of *su* and *Su* phenotypes) were scored separately for VL and dark posttreatment. No marked differences in the frequency of whole and chimeral mutants between VL and dark posttreatments were observed. As to the frequency of the two types, whole mutation was more frequent than the chimeral one. The frequency of chimeras fits roughly a linear relation with increasing doses, while it is rather exponential than linear for whole mutation.

It is noted that the frequency of mutations induced by γ-irradiation was not affected by subsequent VL treatment either for whole or chimeral mutants. On the other hand, the frequency of whole and chimeral mutations from UV-irradiation showed almost linear relation. The frequency of whole mutation was considerably decreased to about one third by exposure to visible light, while there was no marked difference in the frequency of chimeras between VL and dark posttreatment.

Photoreactivation of UV-Induced Damage in Maize Pollen

Etsuo AMANO and Tomoo MABUCHI

As the positive proofs for existence of photoreactivation of ultraviolet (UV) induced mutation in maize pollens had been obtained (cf. Ann. Rep. No. **16**: 112–114), further experiments to study the type of repairable damages were tried using linked endosperm marker genes on chromosome 9, *C*, *sh*, *bz* and *wx* which located on short arm of chromosome 9 in this order being *C* the most distal.

The experimental procedures were basically the same as reported before (Ann. Rep. ibid.). Pollens of a multiple dominant stock, C^I *Sh Bz Wx*, were irradiated in a single layer in Petri dish with UV light from a Toshiba germicidal lamp at a dose rate of 136 ergs/mm²/sec for 30, 60 or 90 seconds. Immediately after the UV treatments, half of the pollens were exposed to visible light from two 400 W high pressure mercury fluorescent lamps (6,400 lux) for 30 minutes, and the other half were kept in dark. They were pollinated in dark room under a natrium lamp illumination to a corresponding multiple recessive tester stock. Potted tester plants were transferred to field after wrapping the pollinated silk and ear with aluminum foil to shield from outside light. The multiple dominant

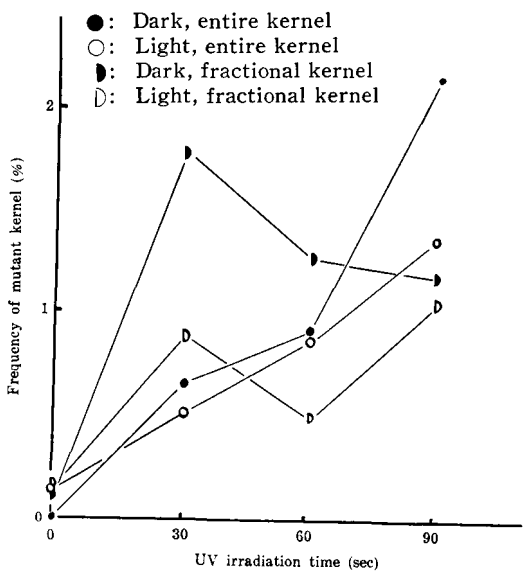


Fig. 1. Effect of post-irradiation exposure to visible light on UV induced endosperm mutation in maize.

stock used as male parent carried also a dominant yellow endosperm gene *Y* on chromosome 6 besides the aforementioned genes, and this *Y* was useful to confirm fertilization by the treated pollen to *y* (white endosperm) carrying tester, in case of loss of all the four chromosome 9 marker phenotypes.

Results of experiment are shown in Figure 1, in which B-F-B type mosaic kernels were included in either whole or fractional (more than 1/8 of surface area of the kernel) kernel mutation, according to the area of mosaics. In this experiment, photoreactivations of UV induced damages were observed as lowered mutation frequencies in the groups which were treated with visible light after UV irradiations compared to the groups without the post exposure to visible light.

Contrary to the previously reported results on *su* locus, in which whole kernel type showed more photoreactivation, present results showed more photoreactivation in fractional or sectorial loss of the dominant phenotypes than in whole kernel type. This discrepancy might be due to the difference of the stocks used, commercial variety vs. genetical pure strain, or the systems used, single specific locus or multiple loci, but, for a conclusive explanation, this should be studied further.

As for the type of damages which could be repaired by the post exposure to visible light, simultaneous loss of a few neighboring marker genes and B-F-B type mosaics were reduced by the post exposure. This strongly suggests that some of the UV induced damages which would result in chromosome breakage might be repaired by post exposure to visible light.

Since the number of mutated kernels at a single locus were not many enough, conclusion about repairability of the damages which would result in so called point mutations should be reserved until further data will be accumulated.

Distribution of initiating points of the B-F-B cycles seemed to be proportional to map distances.

On the Somatic Variations in Corn and Chrysanthemum under Chronic γ-Irradiation

Seiji MATSUMURA and Tarô FUJII

Heterozygotic F_1 seeds from the cross between normal green (Yg_2) and yellow green mutant (yg_2) of corn, and white flowering variety of chrysanthemum were planted in the γ-greenhouse with four different lots on July 18, and relation between the frequency of somatic mutation or variagation and dose rate effect were examined. Radiation intensity was 0.29–3.06 R/hr, total irradiation time being 1741 hours; therefore, total dosage

in each lot was 430, 910, 2370 and 4500 R.

In corn, $Yg_2 \rightarrow yg_2$ mutation was detected by yellow green stripes on leaves. Lengths of mutated stripes were different according to the developmental stage and occurrence of mutation; the number of the stripes longer than 1 cm was counted in the present experiment. Dose versus mutation frequency shows almost linear relations when the results shown in Table 1 are plotted on the figure.

Table 1. Somatic mutation frequency in corn under chronic γ-irradiations

Dose rate (R/h)	Total dosage (R)	No. of examined leaves	No. of stripes (frequency)
—	0	109	0
0.29	430	294	5 (0.050)
0.62	910	223	9 (0.132)
1.38	2470	318	30 (0.266)
3.06	4500	199	28 (0.429)

Somatic variegation in chrysanthemum from white to yellow was observed in many flowers. When the variegation occurred in early stage, yellow sectors appeared on several ray flowers and when it occurred in late stage streak appeared on single ray flower. Dose versus variegation curve also showed almost linear relation like that in corn. From these results, no dose rate effect was observed in the present experiment, namely 0.29–3.06 R/hr, because when the recovery of radiation damage occurred at low dose rate range, effect at higher dose rate range must be much higher than the present results.

Table 2. Somatic variegations in chrysanthemum under chronic γ-irradiations

Dose rate (R/h)	Total dosage (R)	No. of examined flowers	No. of streaks (frequency)	No. of sectors (frequency)
—	0	46	5 (0.11)	0
0.29	430	50	18 (0.36)	4 (0.08)
0.62	910	50	48 (0.96)	3 (0.06)
1.38	2470	50	50 (1.00)	4 (0.08)
3.06	4500	50	153 (3.06)	29 (0.58)

Endosperm Mutations Induced by UV in Corn

Tarô FUJII

A wild strain (*Bz*) with respect to the bronz color of endosperm and a recessive homozygotic strain (*bz*) was used. Pollen grains of the dominant line were irradiated by UV-rays, emitting primary 2537 Å, at a dose rate of about 140 ergs/mm²/sec. Just after the irradiation, a half of the irradiated pollen grains was exposed to visible light of 6500 lux for 30 minutes, the pollen was crossed with recessive strain under the sun light (PR treatment). The other half of the irradiated pollen was held for 30 minutes in darkness and pollinations with recessive strain were done in dark room with natrium lamp (Dark treatment). Plants of the dark treatment lot were kept under the dark condition for 24 hours and removed to the field. For comparison, γ-ray irradiated pollen grains were pollinated after PR or dark treatments with the same manners as those

Table 1. Frequencies of *Bz*→*bz* mutations induced by UV and γ-rays with PR and dark treatment

Treatment	Dose	Post-irradiation treatment	No. of seeds	No. of mutation (frequency)	
				Whole	Fractional
Control	—	PR	1246	0	0
		Dark	5052	0	0
UV	15 sec	PR	6673	1 (0.02)	8 (0.12)
		Dark	2733	4 (0.15)	17 (0.62)
	30 sec	PR	—		
		Dark	371	2 (0.54)	4 (1.08)
	60 sec	PR	4289	2 (0.05)	5 (0.12)
		Dark	389	5 (1.29)	5 (1.29)
	90 sec	PR	4007	2 (0.05)	17 (0.42)
		Dark	3035	20 (0.66)	64 (2.11)
γ-ray	300 R	PR	3211	5 (0.16)	2 (0.06)
		Dark	4200	6 (0.14)	8 (0.19)
	600 R	PR	4823	30 (0.62)	16 (0.33)
		Dark	4680	21 (0.45)	7 (0.15)
	900 R	PR	5017	47 (0.94)	23 (0.46)
		Dark	3841	25 (0.65)	28 (0.72)

of UV-irradiations. Results are summarized in the Table 1; the whole mutation means that the endosperm is wholly deficient for the dominant

character, and fractional mutation means that the endosperm has partially *Bz* and *bz* characters. In the γ-ray treated lots, mutation rate at higher dosage lot was rather higher than the linear relation. Frequency of fractional and whole mutations were almost same in each lot and these frequencies were not much different between PR and dark treatment. On the other hand, dose versus mutation frequency was almost linear in UV treated lots, and frequency of fractional mutation was higher than that of the whole one in each lot in dark treatment. Frequency was markedly decreased according to the PR treatment but rate of fractional mutation is still high. From the experiments, photorecovery of UV-damage could be observed in *Bz* gene like that of *Su* gene by Matsumura and Mabuchi, and further detailed experiments in relation to the sector size of fractional mutation are now underway.

IX. MICROBIAL GENETICS

Genetic Map of *H1* Gene in *Salmonella*

Shigeru YAMAGUCHI and Tetsuo IINO

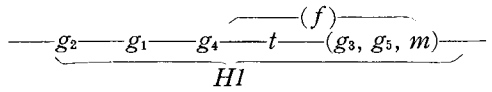
Flagellar protein, flagellin, of *Salmonella* has complex antigenicities; that is, there are several serologically different antigenic determinants on one flagellin molecule. The set of the specificities of this complex antigen is determined as a whole by one of the allelic genes at the two loci, *H1* and *H2*, which are the structure genes for flagellin. Based on the assumption that the antigenic specificity-determing regions corresponding to the respective antigenic determinants distribute linearly within *H* gene, it was attempted to assign the positions of these regions on the genetic map of *H* gene, *H1* in the present experiment, by establishing the system of intra-*H1* recombination. Three derivatives of SJ241, a stable phase-1 strain of *Salmonella abortus-equi*, given different alleles of *H1-g*.. by transduction were used. Their designations and their compositions of the antigenic specificities are as follows; Tr6 g_1, g_2, g_3, t, Tr16 g_3, g_4, f, and Tr17 g_1, g_2, g_4, g_5, m.

The establishment of the system of intra-*H1* recombination was achieved by use of *H1*-linked *fla*$^-$ mutants. The principle of the system is that occurrence of a *fla*$^+$ transductant in transduction between a pair of *fla*$^-$ mutants, whose mutation sites are closely linked to *H1* and are on the opposite sides of it, should require a crossover at the region between the two *fla*$^-$ sites which includes *H1* locus; therefore the closer the *fla*$^-$ sites are to *H1*, the larger is the relative frequency of the occurrence of intra-*H1* recombinants among the *fla*$^+$ transductants.

About fifty *fla*$^-$ mutants from each of Tr6, Tr16, and Tr17 were isolated by selection in which phage M8, a host-range mutant of bacteriophage chi, was used as selecting agent. In several mutants showing high frequency of co-transduction of *fla*$^-$ with *H1*, the relative positions of their *fla*$^-$ sites with respect to *H1* locus were examined by carrying out the reciprocal transductions with a standard *H1*-linked *fla*$^-$ strain, a *fla*$^-$ mutant of SJ241, and then comparing the ratios of the number of *fla*$^+$ transductants having the donor-type flagellar antigen to that of *fla*$^+$ transductants having the recipient-type between each pair of reciprocal crosses. Two classes of *fla*$^-$ mutants which have their *fla*$^-$ sites on the opposite sides of *H1* locus were found among the mutants originated from each of Tr6, Tr16, and Tr17.

Transductions were then carried out in all pair-wise combinations among these mutants which have different alleles of *H1-g*.. each, and the *fla*$^-$ sites are on the opposite sides of *H1*. Antigenic compositions of *fla*$^+$

transductant clones were examined by use of anti-factor(s) serums. Among 1266 *fla*$^+$ transductant clones examined, 26 clones showed the antigenic compositions of recombinant-types. From the consideration of the antigenic compositions of these intra-*H1* recombinants it was shown that each of antigenic specificity-determining sites corresponding to respective antigenic determinants locates as a unit and as a whole they are in a linear array within *H1*. The arrangement established so far is as follows.



f is somewhere right of g_4. Relative order of the regions in parentheses has not yet been determined.

A Straight Flagellar Mutant in *Salmonella*

Tetsuo IINO and Michiko MITANI

A mutant of *Salmonella typhimurium* was found to produce straight flagella in phase-2 (antigen-*1.2*) and normal flagella in phase-1 (antigen-*i*). The straight flagella were observed by both light and electron microscopy either with or without formalin fixation. Flagellar bundles of the mutant bacteria prepared in 0.25 per cent methylcellulose (w/v) and examined by dark field microscopy were also found to be straight. It was shown by electron microscopy that the component flagella of the straight flagellar bundles were in most instances irregularly twisted around each other. Heteromorphous cells which had both straight flagella and either normal or mini-small-amplitude flagella were seen at the frequency of about 10 per cent among the bacteria in phase-2.

The bacteria with straight flagella were non-motile but they were sensitive to bacteriophage-chi, which has been known to infect motile bacteria of salmonella species.

In transduction with P22 phage from a normal flagellar strain to the phase-2 straight strain, transductional clones with normal flagella in both phase-1 and phase-2 were obtained. The transductional clones showed the antigen of the recipient in phase-1 and that of the donor in phase-2. This indicates that the straight mutant originated by a mutation of the structural gene of phase-2 flagellin.

In absorption-agglutination experiments with antisera prepared against flagella of either normal-*1.2* or straight-*1.2*, no antigenic difference between normal and straight flagella could be detected.

Flagellin Biosynthesis in *Salmonella* Spheroplasts

Hideho SUZUKI and Tetsuo IINO

Salmonella typhimurium grown in a modified 3XD medium (Na_2HPO_4 14.3 mM, KH_2PO_4 6 mM, NH_4Cl 20 mM, $MgSO_4$ 2 mM, $CaCl_2$ 0.2 mM, glycerol 300 mM, Casamino acid 1.5 per cent, Gelatin 0.002 per cent) was washed with 5 mM tris-buffer (adjusted to pH 8 at 30° with HCl) and suspended in 50 mM tris-buffer containing 10 per cent sucrose at a concentration of $2\sim4\times10^9$ cells/ml. The cell suspension was treated with lysozyme (100 μg/ml) for ten minutes and then EDTA (5 mM) was added. After another ten minutes, not less than 99 per cent of cells were converted to spheroplasts as determined by microscopic observation and viable counts. Then BSA (2 mg/ml) was added and the spheroplasts were centrifuged, suspended in a small amount of saline containing 10 per cent sucrose and dispersed into minimal medium supplemented with 10 per cent sucrose and 2 mM amino acids to give a concentration of approximately 5×10^8 cells/ml. At starting incubation, ^{14}C-alanine (0.2 μc/ml) or ^{3}H-leucine was added. Flagellin fraction was prepared from cells which incorporated radio active amino acid for thirty to sixty minutes, purified through reconstitution and analyzed with starch-gel electrophoresis. The results indicated that the spheroplasts do synthesize flagellin to an extent comparable to the control cells. A microscopic observation of stained preparation, however, revealed that the spheroplasts still retained flagella. As the intracellular pool is considered to be extremely small, if any, in *Salmonella*, active flagellin synthesis of the spheroplasts could be interpreted as a reflex of intactness of flagella forming apparatus on the persistent cell wall rather than an accumulation of flagellin in the cytoplasm of the spheroplasts.

Genetic Fine Structure of the *mot* Loci in *Salmonella typhimurium*

Masatoshi ENOMOTO

Flagella of *mot* (motility) mutants which though flagellated are nonmotile do not differ from those of the wild-type motile cell in antigenicity, number per bacterium, shape under electron microscope and configuration observed by X-ray diffraction. Both the wild type and the *mot* mutants show flagellar antigenic phase variation. These facts suggest that the paralysis is not due to any defect of flagella themselves, but to a defect of the flagellum-activating mechanism within the bacteria. As a part of investigations to clarify this mechanism, genetic analysis of *mot* loci was carried out.

Ninety-seven *mot* mutants derived from *S. typhimurium* strain TM2 were divided into three complementation groups by abortive transduction tests: *motA* with 64; *motB* with 30; and *motC* with 3. *motA* and *motB* were found to constitute adjoining functional units because of the occurrence of joint transduction of *motA* and *motB*, and of a deletion mutation covering the whole *motB* and a part of *motA*. *motC* was transduced jointly with *H1*, a phase-1 flagellin gene, at a fairly high frequencies and the order of the three mutational sites within *motC* was determined: *H1-C244-C272-C279*. The order of the representative mutational sites in *motA* and *motB* was also determined as shown in Fig. 1 by deletion mapping and two-factor transduction tests.

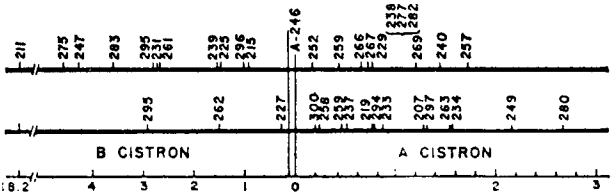


Fig. 1. Genetic map of *motA* and *motB*. The results of three series of transduction tests are arranged as follows: crosses between *A-246* and *motA* mutants (top, right); between *A-246* and *motB* mutants (top, left); and between *A-246* and weak-complementing mutants (bottom). The scale indicates recombination frequencies (precent). The upper and lower maps are comparable with each other except in respect of close mutational sites. *A-238*, *-277*, and *-282* of the upper map, and *A-294* and *-297* of the lower map are deletions.

Mapping of Three *mot* Loci in *Salmonella* by Linkage Analysis

Masatoshi ENOMOTO

The chromosomal location of *motA* and *motB* and the position of *motC* relative to *H1* were investigated. In order to analyse the linkage relationship between *mot* loci and other genes, systems of sexual recombination were adopted. Hfr mutant SW1391 (*met⁻ H1b H2enx*) derived from *Salmonella abony* strain SW803 and nonmotile F⁻ mutants, SJ694 (*leu⁻ his⁻ ser⁻ motB⁻ H1b H2enx*), SJ703 (*leu⁻ his⁻ ser⁻ motB⁻ H1i H2enx*), SJ700 (*leu⁻ motA⁻ H1i H2enx*), and SJ608 (*motC⁻ H1i H21.2*), were used. Selection of recombinants was made for the donor *mot⁺* and for the recipient *met⁺*. In some crosses *H1* and *H2* were added to the selection markers. The motile recombinants thus obtained were examined for their unselected auxotrophic markers. The order of the *mot* and other loci

involved in this work was inferred to be *met-ser-H2-his-motC-H1-motB-motA-leu*.

Difference in Frequencies of Cotransduction of *motC* with *H1* Gene in *Salmonella*

Masatoshi ENOMOTO and Shigeru YAMAGUCHI

In P22 mediated transduction from a motile wild-type strain to nonmotile mutants of *Salmonella*, *motC* (motility) and some *fla* (flagellation) genes are transduced jointly with *H1* (phase-1 flagellin) gene. When motile donor strains having *H1* which was introduced from a certain *Salmonella* species are used in transduction to a nonmotile mutant serotypically different from the donor, frequencies of cotransduction (percent fraction of motile transductant with donor *H1* allele) are different from each other among the combinations, though the donors are phenotypically indistinguishable. In order to elucidate this phenomenon, the following experiment was carried out. *Salmonella dublin* strain SJ11 (*H1gp motC*$^+$ *flaK*$^+$), mutants *motC-244* (*H1i*) and *flaK-48* (latent *H1i*) derived from *S. typhimurium* strain TM2 were used. The order of the genes in this chromosomal region is *flaK-H1-motC*. By means of transduction from SJ11 to *motC-244* and *flaK-48*, motile transductants *motC*$^+$ *H1gp* and *flaK*$^+$ *H1gp* were isolated. The former transductants received at least the chromosomal region between *H1* and *motC-244* from SJ11 and the latter received the region between *flaK* and *H1*. Using these transductants as donors and *motC-244* as the recipient, transduction tests were carried out and the frequencies of cotransduction were examined. With donors obtained from *motC-244* the frequencies of cotransduction were 32 to 44 per cent and with those obtained from *flaK-48* they were 4.5 to 16 per cent, whereas the frequency with SJ11 was 19 per cent. In the cotransduction of *motC* with *H1*, a crossover between *flaK* and *H1* is required as well as a crossover at the right region of *motC* (This Ann. Rept. **16:** 91). It is inferred that the decreased frequency of cotransduction with the donors obtained from *flaK-48* is the result of reduced crossover between *flaK* and *H1gp*, due to the heterogeneity of the chromosomal region between *S. typhimurium* and *S. dublin*, and that the increased frequency with the donors obtained from *motC-244* is the result of relative increase in the crossover between *flaK* and *H1gp*, due to the reduced crossover between *H1* and *motC*.

Infection of Bacteriophage-Chi to *Serratia marcescens*

Tetsuo IINO

It was found that bacteriophage-chi, which has been known to attack motile cells of both *Salmonella* and *Escherichia* species, is also able to infect motile strains of *Serratia marcescens*. The *Serratia* strains used were B-181-2 through B-181-10, B-182-1 through B-182-9, B-183-2 and B-183-3. One of these 20 strains, B-181-6, was non-flagellate and consequently non-motile. The remaining 19 strains were motile having peritrichous flagella with normal curvature.

Sensitivities of those *Serratia* strains to chi and its host range mutant M8, which is able to attack *Salmonella* possessing the g-complex antigen, were examined by the spotting method of E.W. Meynell. The bacteriophage used was propagated on *Salmonella abortus-equi* strain SJ241 which is sensitive to both chi and M8. The non-motile strain, B-181-6, was completely resistant to both chi and M8, whereas the remaining 19 motile strains were lysed by both chi and M8. The plaques formed with the latter strains were clear and indistinguishable in size and shape from those formed with *Salmonella* strain SJ241.

A spontaneous non-flagellate mutant was isolated from motile strain B-181-8 by screening on semi-solid nutrient agar medium. The mutant was found to be resistant to both chi and M8. Chi-phage can be used as a selective agent for the isolation of non-motile mutants from actively motile strains of *Serratia* by the same procedure which has been applied to *Salmonella* and *Escherichia coli*.

Chi-phages were propagated in *S. marcescens* B-181-7, *S. abortus-equi* SJ241 and *E. coli* W3110, and the stocks were designated chi (Ser), chi (Sal) and chi (E.c) respectively. The combinations of chi (Sal) and *Serratia*, and chi (Ser) and *Salmonella*, gave efficiencies of plating (EOP) not significantly different from those of the homospecific combinations, i.e. the combinations in which the indicator strain used was the same as the propagating one. However, when chi (Sal) or chi (Ser) were tested with *Escherichia*, the EOP decreased markedly. The infection by chi (E.c) of either *Salmonella* or *Serratia* also resulted in decreased EOP as compared with the homospecific combinations. When chi (E.c) was grown on *S. abortus-equi* SJ241 or *S. marcescens* B-181-7, the progeny phages then exhibited high EOP on these bacteria while the EOP on *E. coli* W3110 decreased markedly. This suggests the presence of host induced modification in the system.

Anti-chi serum, prepared by immunization of a rabbit with a phage stock of chi (Sal), was absorbed with the propagating strain, diluted 1000 fold and neutralization velocity constants with chi (Sal), chi (Ser) and

chi (E.c) were measured at 37°C. The constants found were 495±69, 497±61 and 521±73 respectively (95 per cent confidence interval). Thus the efficiency of inactivation of chi (E.c) by anti-chi (Sal) serum was not significantly different from those of both chi (Sal) and chi (Ser).

Normal Repressed Level of Ornithine Transcarbamylase Activity in Crude Extracts of an Arginine Sensitive Mutant of *Salmonella typhimurium*

Jun-ichi ISHIDSU

Among various enzymes having a relation to arginine and uracil biosyntheses, the activity of ornithine transcarbamylase which couples ornithine and carbamyl phosphate to citrulline was measured using crude extracts prepared from *arg-s-1* cells grown under various culture conditions and compared with the activity of wild type cells.

Arg-s-1 was first grown in a synthetic minimal medium (Ishidsu, Ann. Rep. No. **14,** 1963) supplemented with 10^{-3} M arginine and 10^{-3} M uracil to middle log phase. Cells were collected, washed and transferred without changing original titer to the following four media: 1) minimal with no supplementation (full derepression), 2) minimal with 10^{-3} M uracil (derepression for the arginine synthesizing system), 3) minimal with 10^{-3} M arginine (derepression for the uracil synthesizing system) and 4) minimal with 10^{-3} M uracil and 10^{-3} M arginine (continuous repression for both systems). After 2 hours' incubation, cells of each culture were collected, washed, resuspended in a small amount of potassium phosphate buffer (0.05 M, pH 7.0, containing 10^{-4} M EDTA) and sonicated. After the removal of cell debris by centrifugation, the supernatants were subjected to enzyme assay as crude extracts.

Results are summarized in the following table.

Culture condition	Specific activity	% Repression
Minimal	0.807	0
10^{-3} M uracil	0.713	11.6
10^{-3} M arginine	0.00173	99.8
10^{-3} M uracil + 10^{-3} M arginine	0.00161	99.8

These results are completely comparable with those obtained with wild type cells.

X. HUMAN GENETICS

Evaluation of the Family Planning Programme in Japan

Ei MATSUNAGA

An official programme on family planning in Japan was launched in 1952, in order to replace the increasing practice of artificial abortion by contraceptive methods. Placing the emphasis on the guidance of low income families, local health centers have been playing a leading and supervising role in the programme, with the aid of some ten thousand public health nurses and private work midwives. Besides, a number of voluntary organizations have assisted the government's programme, and mass communication by women's magazines has contributed greatly towards the popularization of the knowledge about contraception. As a result, present users of some kind of contraceptive measures have been rapidly increasing among every social stratum during a short period.

On the other hand, the decline in births has still been accompanied by a tremendous number of notified abortions. Taking into account the likewise tremendous number of unnotified ones—presumably some 60 to 100 per cent of the notified cases—, a question has occasionally been raised as to the effectiveness of the family planning programme as described above. However, when measured per 1,000 women aged 15-49, the number of notified abortions has steadily been decreasing from the maximum of 50 in 1955 to 30 in 1965, while the number of births dropped to 65 in 1957—just half the number of 1948—and remained thereafter almost constant, implying this being the number of "wanted births" of the present Japanese.

Under these demographic trends, a method was devised to evaluate the respective roles in the lowering of birth rate of 1) induced abortions, 2) contraceptive practice, 3) legalized sterilization, and 4) postponement of marriage. The result showed that, while the family planning programme had scarcelly been successful during the first few years, the abortions have been, even allowing for as many unnotified as for the notified ones, increasingly replaced by contraception since 1957. It should be noted, however, that even in 1965 about two thirds of the reduction in births was presumably based on induced abortions.

Details of this work will be published elsewhere.

Association of Ear-Wax Types with Susceptibility to Arteriosclerosis—A Preliminary Report[1]

Mitsuo MIYAHARA[2] and Ei MATSUNAGA

It is now well known that the ear-wax types in man are determined by a simple genetic mechanism, the wet type being inherited dominantly over the dry type. There is a marked variation in the incidence of the respective types among various ethnic groups; more than 80 per cent of Japanese belong to dry type, whereas this type of cerumen is exceptional among both Caucasians and Negroes. Since there is an apparent association between wet cerumen and development of apocrine sweat glands or axillary odour, it may be assumed that the effect of the allele pair responsible for ear-wax types is concerned with certain metabolic patterns presumably of lipid material. It has been hoped that investigations will be carried out to seek for an association between ear-wax types and some internal diseases, such as arteriosclerosis and other related diseases (Matsunaga,[3] 1962).

Data on ear-wax types have been collected from in- and outpatients in the Department of Internal Medicine of the Sapporo Medical College Hospital, Sapporo, during the last two years from January 1964 to December 1966. The diagnosis of "arteriosclerosis" of our patients was made, in most instances, if they were affected with coronary arterial sclerosis or ischemic heart disease, and in the remaining cases, if they had a history of either apoplectic stroke or encephalomalacia, or they showed calcification of the arc of the aorta as revealed by roentgenography. The conditions were then divided into two groups, *i.e.*, arteriosclerosis with and without hypertension. Patients with other diseases were also included in the study. In the classification of the disease entries, those with uncertain diagnosis were all included in the "miscellaneous" group.

Of 2,448 patients so far examined, there were 39 or 1.6 per cent whose ear-wax type could not be determined with certainty. Although our experience suggests that ambiguous cases are most likely to be of wet type, they were excluded from the subsequent analysis. The incidence of wet cerumen among the remaining 2,409 was 16.9 per cent, which was not significantly different from the 19.0 per cent ($\chi^2=2.92$, $d.f.=1$, $P>0.05$) previously obtained by a survey (Matsunaga *et al.*[4], 1954) of 1,639 pupils

[1] This work was supported by a grant from the Toyo Rayon Foundation for the Promotion of Science and Technology.

[2] Department of Internal Medicine, Sapporo Medical College, Sapporo.

[3] Matsunaga, E., 1962. The dimorphism in human normal cerumen. Ann. Human Genet. **25**: 273-286.

of a middle school in Sapporo.

The distribution of the patients differed between sexes according to the disease, but for each disease entry there was no significant difference in the distribution of ear-wax types according to sex, so that the data were pooled for male and female patients, and the results are summarized in Table 1.

Table 1. Association of wet cerumen with a variety of diseases

Groups of patients affected with	No. examined	Wet type		Relative risk for wet type	χ^2
		No.	%		
1. Arteriosclerosis	96	29	30.2	1.85	7.05*
2. Arteriosclerosis with hypertension	138	19	13.8	0.68	2.26
3. Essential hypertension	290	50	17.2	0.89	0.49
4. Diabetes mellitus	47	4	8.5	0.40	3.08
5. Endocrine disorders	80	16	20.0	1.07	0.05
6. Heart diseases	122	17	13.9	0.69	1.89
7. Kidney diseases	70	13	18.6	0.97	0.01
8. Liver diseases	90	15	16.7	0.85	1.89
9. Blood diseases	30	3	10.0	0.47	1.49
10. Gastrointestinal diseases	429	77	17.9	0.93	0.24
11. Lung diseases	45	6	13.3	0.66	0.90
12. Miscellaneous	972	158	16.3	0.83	3.08
Total	2,409	407	16.9		
Control	1,638	311	19.0		

* Significant at the 1 per cent level.

From the table it can be seen that the incidence of wet cerumen among the patients with arteriosclerosis, not accompanied by hypertension, was strikingly high—as high as 30.2 per cent—, whereas it was only 13.8 per cent among the arteriosclerotic patients with hypertension, the difference being significant at the 1 per cent level ($\chi^2=9.39, d.f.=1, P<0.01$). In other groups of patients the incidences of wet cerumen were all below 20 per cent. Using the figure of 19.0 per cent as control, the risks of the wet type relative to the dry in the susceptibility to various diseases have been calculated by means of Woolf's method[5] (1955). The item for which

[4] Matsunaga, E., S. Itoh, T. Suzuki, and S. Sugimoto, 1954. Incidence and inheritance of the ear-wax types. Sapporo Med. J. **6**: 1-4.

[5] Woolf, B., 1955. On estimating the relation between blood group and disease. Ann. Human Genet. **19**: 251-253.

significance was shown was only arteriosclerosis without hypertension; for this condition the risk of individuals with wet cerumen was apparently increased as much as 85 per cent as compared with individuals with dry cerumen.

It has been widely recognized that there is a variation in the incidence of arteriosclerosis among various ethnic groups; for example, coronary arterial sclerosis is much more common in the United States than in Japan. Since the Japanese immigrants in the United States show higher incidence of coronary arterial sclerosis than the Japanese in Japan, environmental factors such as dietary habits seem to predominate in the causation of the disease, but genetic factors underlying racial differences cannot be excluded. The present study indicates that, while there is no association between ear-wax types and arteriosclerosis accompanied by hypertension, individuals with wet cerumen are more susceptible to arteriosclerosis without hypertension. This association may account, to some extent, for the observed difference in the incidence of arteriosclerosis among various ethnic groups.

This work is still in progress and the data will be analyzed with reference to cholesterol level of the blood in the patients.

Maternal Age of Mosaics with Down's Syndrome

Ei MATSUNAGA, Akira TONOMURA[1] Hidetsune OISHI and Yasumoto KIKUCHI

The overwhelming majority of cases with Down's syndrome is now known to be primary G-trisomics, and the etiological importance of maternal age is well established. For the cases with translocations, maternal age is generally accepted to be of no significance, while less attention has been paid to maternal age of the mosaics.

In order to test for a possible effect of maternal age upon *mitotic* non-disjunction, data have been collected from the literature, including our own cases, concerning maternal age of 31 cases of mosaicism with varying degree of clinical signs of Down's syndrome. When they were divided into two classes, class A with predominantly normal cells and class B with predominantly G-trisomic or tetrasomic cells as revealed by either blood or skin culture, the mean maternal age was 35.1 ± 1.6 for class B mosaics, while it was 29.4 ± 2.0 for class A mosaics, the difference being significant at the 5 per cent level ($t=2.24$, $d.f.=29$, $P<0.05$). Since class A mosaics are most likely to have developed from fertilized eggs with normal karyotype, it appears that mitotic non-disjunction may not

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be affected by maternal age. However, more data are needed to draw a definite conclusion.

Chromosome Replication in Down's Syndrome

Yasumoto KIKUCHI and Hidetsune OISHI

Autoradiographic techniques have been used mainly for the study of DNA replication pattern of human chromosomes. This technique may be utilized also for chromosome identification. We report here a preliminary study of autoradiographic analysis of the G group chromosomes in patients with Down's syndrome and in normal subjects.

Four female patients with the standard type of G-trisomy, two normal females, and two normal males were studied. Leucocytes obtained from venous blood of each subject were cultured according to standard technique. Chromosome replication at the end of the DNA synthesis period (S period) was analysed by means of autoradiography, tritiated thymidine and continuous labeling technique.

Metaphase plates selected for analysis were scored according to the number of small acrocentrics labeled per cell. The results are shown in Table 1. In normal subjects, cells with 4 labeled G chromosomes occurred

Table 1. Number of cells showing the distribution of labeling among acrocentrics of the G group

Subjects	No. of G chromosomes labeled						Total No. of cells examined
	0	1	2	3	4	5	
Normal males	12	11	24	22	31	—	100
Normal females	5	14	27	18	37	—	100
Female patients with Down's syndrome	10	8	18	32	19	18	100

most frequently (31 per cent in males and 37 in females); cells with 2 labeled G chromosomes were found less frequently, while the frequencies of cells with 1 or 3 labeled G chromosomes were still lower. This indicates that most of the homologous pairs of G group replicate synchronously. In female patients with Down's syndrome, 32 out of 100 cells were observed to have 3 labeled and 2 unlabeled G chromosomes, while there were only 18 cells with 2 labeled and 3 unlabeled G chromosomes. The results may suggest that the sequence of replication of the extra chromosome is similar to the original homologous pair.

For detail analysis of grain distribution, 20 metaphase plates were

observed from a normal female and the same number from a female patient with Down's syndrome. In 12 cells from the normal female, 2 G chromosomes were generally labeled over the long arm (late replicating pair), but chromosomes of another pair were completely unlabeled or lightly labeled only over the centromere region or the short arm (early replicating pair). The remaining cells did not show a consistent pattern. In the patient with Down's syndrome, 12 cells had 3 late replicating G chromosomes and 2 early replicating ones. On the other hand, 5 cells had 2 late and 3 early replicating G chromosomes. In the remaining cells, different patterns were observed.

In the autoradiographs of chromosomes from each patient other than the G group, the characteristic pattern of late replication was observed in 1 X chromosome, in 2 of the D group (Nos. 13-15) and in Nos. 16 and 18.

The above results suggest that the extra chromosome associated with Down's syndrome belongs to the late replicating pair of G group, and that its presence does not affect DNA synthesis of chromosomes other than G group.

Phenotypes and Sex Chromosomes in Five Patients with Turner's Syndrome

Hidetsune OISHI, Yasumoto KIKUCHI and Ei MATSUDA

It is now widely accepted that monosomy of X or X deletion is responsible for clinical conditions called Turner's syndrome. However, individuals with this chromosome aberration do not always show all of the classical signs of Turner's syndrome (shortness of stature with sexual infantilism, cubitus valgus, and webbing of the neck). During our current survey, in collaboration with some University Hospitals in Tokyo, of human chromosomes in various congenital disorders and sex anomalies, we examined the chromosomes in four cases of XO patients and in one with XO/XXX mosaicism. The results of clinical and laboratory examinations are summarized in Table 1. In all cases, short stature, shield chest, cubitus valgus without webbing of the neck, lacking or scanty pubic hair, and a small, scarcely palpable uterus were noticed.

Analyses of dermal patterns of fingers and hands were performed on three patients, and the formulas are given in Table 2. Since the total ridge counts of the finger tips in Japanese females are on the average 137.70 ± 41.09 (Matsunaga), our patients seem to have higher ridge counts. The characteristic t''s were observed in the left palms of cases Nos. 4 and 5, while the axial triradius was absent in both hands of case No. 3.

Table 1. Some clinical data in five patients under study

Case No.	1	2	3	4	5
Sex chromosome constitution	XO	XO	XO	XO	XO/XXX
Drumstick	−	−	−	−	+(14/500)*
Maternal age at the birth of the patient	37	27	30	24	24
Paternal age at the birth of the patient	45	35	35	28	28
Birth weight (kg)		3.10	3.18	2.15	2.45
Age examined (years)	17	16	15	10	10
Height (cm)	143	134	130	106	121
Weight (kg)	41.5	34.7	40.0	23.5	25.5
Arm span (cm)	141	123.7	131.8	94.7	120
IQ (or intelligence)	99	Normal	Normal	49	123
Short neck	−	+	+	+	+
Webbed neck	−	−	−	−	−
Shield chest	+	+	+	+	+
Cubitus valgus	+	+	+	+	+
Menstruation	−	−	−	−	−
Development of the breasts	−	−	+	−	−
Pubic and axillary hairs	−	Scanty	−	−	−
Small uterus	+	+	+	+	+
Gonadotropin (unit/day)	>32	>32	<32	<4	<4
17-KS (mg/day)	4.37	1.6	0.9	0.61	1.0

* Including two cells with two drumsticks.

Table 2. Dermatoglyphics of the patients

Case No.		Finger pattern types (I-V)*	Total ridge count	Palmar pattern formulae**	*atd* angle
3	Right	U. U. U. U. Lp	200	11.X.7.1−0−A^{r}.0.0.*l*.0	—
	Left	W. U. U. U. W		7.7.5″.1−0−A^{r}.0.0.0.L/L	—
4	Right	U. At. U. U. U	151	11.X.7.3−t−A^{u}.0.0.V.0	42°
	Left	U. R. Lp. Lp. U		9.7.5″.3−tt″−L^{u}V.V/V.0.0.V	t″ 78°, t 43°
5	Right	U. Ucp. Ucp. W. W	150	11.9.8.5′−t−V.V/V.0.L.0	45°
	Left	U. W. W. W. W		9.9.5″.1−tt″−W^{s}.V/V.0.L.V	t″ 75°, t 45°

* At; tented arch, W; whorl, R; radial loop, Ucp; ulnar central pocket loop, U; ulnar loop, Lp; lateral pocket loop.
** Cummins-Midlo's formula.

Thenar patterns as seen occasionally in patients with this syndrome were noticed in cases Nos. 4 and 5. In addition, all three patients had palmar transverse creases.

Further studies are required for the discrimination of some metrical characters genetically controlled by sex chromosomes.

Clinical Conditions of Patients with Apparently Normal Chromosomes, III

Hidetsune OISHI and Yasumoto KIKUCHI

Based on leucocyte cultures of peripheral blood, patients with various pathological conditions were found to have 46 chromosomes of apparently normal karyotype. Cases of some interest are listed below.

	Name	*Age*	*Legal sex*	*Clinical conditions*
a) Multiple deformations with mental retardation				
47	N.I.	3 years	F	Hypertelorism; antimongoloid slant eyes; low-set ears; webbing of the neck
48	M.O.	4 years	F	Microcephaly; exophthalmos; low-set ears
49	Y.M.	3 years	M	Microcephaly; hypertelorism; epicanthus; micrognathia ("Cri du chat" syndrome?)
50	S.K.	5 months	M	Exophthalmos; large deformed lobes of the ear; bifid tongue; high-arched palate
51	T.O.	2 years	M	Oblique palpebral fissures; low-set ears; polydactyly
52	A.O.	1 year	F	Congenital glaucoma; aphasia
53	Y.M.	1 year	F	Osteogenesis imperfecta; hydrocephalus; small deformed lobes of the ear; congenital heart disease
54	S.T.	2 years	M	Hypertelorism; antimongoloid slant eyes; hydrocephalus
55	Y.S.	5 months	F	Hypertelorism; syndactyly; antimongoloid slant eyes; epicanthus
56	H.T.	2 years	M	Hypertelorism; antimongoloid slant eyes
b) Sex anomalies				
57	H.S.	36 years	M	Slender body; funnel-shaped chest; small penis; cataract; low excretion of 17KS; high excretion of gonadotropin (Klinefelter's syndrome?)
58	E.M.	5 years	F	Female pseudohermaphroditism

59	—. S.	11 days	F	Female pseudohermaphroditism
60	K. K.	9 years	F	Primordial dwarfism; cubitus valgus (Turner's syndrome?)
61	A. G.	16 years	F	Male pseudohermaphroditism
62	Y. K.	6 years	F	Webbing of the neck; shield chest; short stature; hypertelorism (Turner's syndrome?)

c) Hereditary disease

63	T. I.	23 years	M	Recklinghausen's disease

Amino Acid Sequence Around Cystine Residues of a Lambda Type Human Bence-Jones Protein[1)]

Tomotaka SHINODA

A complete location of disulfide bridges of type L human Bence-Jones protein was carried out using a specimen, Sh. A peptic digest of Sh protein was purified several times by high voltage electrophoresis at pH 6.5 and 3.5. The cystine peptides were oxidized with performic acid vapour. Amino acid sequence of cystine peptides thus obtained was determined by the subtractive Edman degradation method. Three disulfide bridges have been found; Two of these were the intrachain bridges—a bridge which located in the variable part of the molecule and the other in the invariant part—, and the remainder formed an interchain bridge at the C-terminal, which showed why the Sh protein existed predominantly in the dimer

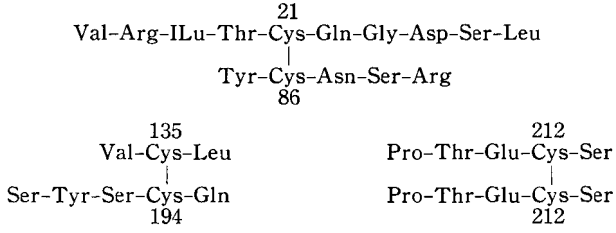

Fig. 1. Amino acid sequence around cystine residues.

form. The results are shown in Fig. 1.

It is suggested that the genetic conservation of secondary and tertiary structures may be reflected in the location of the disulfide bridges of light chains of immunoglobulin, and that a general manner of linkage may also be a common feature of the heterogeneous population of light chains.

1) This work was carried out at Indiana University, Bloomington, Indiana, U.S.A.

Cellulose Acetate Electrophoresis and α_2-Lipoprotein of Human Serum

Yoshito OGAWA

When electrophoretic analysis of human serum is carried out using cellulose acetate strips as a supporting medium, a fine new band often appears in the part of α_2-globulin pattern (Fig. 1, arrow). The nature of

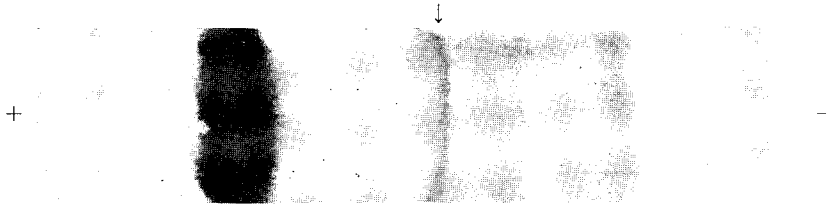

Fig. 1. Separation pattern of human serum on cellulose acetate strip (Separax).

this new fraction was not yet studied. The present writer has thoroughly examined this fraction and succeeded to confirm its identity with α_2-lipoprotein fraction because of its affinity to Sudan Black B (Swahn's method) and the results of immunochemical examination, immunoelectrophoresis and absorption test, applying anti α_2-lipoprotein rabbit serum (Behring-Werke).

Details of this works had been published in Physico-Chemical Biology Vol. 12.

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ABSTRACTS OF DIARY FOR 1966

January	21	141st Meeting of Misima Geneticist' Club
January	25	69th Biological Symposium
January	26	70th Biological Symposium
February	18	142nd Meeting of Misima Geneticist' Club
March	1	71st Biological Symposium
March	24	143rd Meeting of Misima Geneticist' Club
April	8	16th Meeting of the Society of Electrophoresis
April	20	72nd Biological Symposium
April	22	144th Meeting of Misima Geneticist' Club
May	20	145th Meeting of Misima Geneticist' Club
June	4	26th Meeting of the Board of Councillors
June	24	146th Meeting of Misima Geneticist' Club
July	18	73rd Biological Symposium
July	29	147th Meeting of Misima Geneticist' Club
September	12	4th Meeting of the Organizing Committee of the 12th International Congress of Genetics
September	30	148th Meeting of Misima Geneticist' Club
October	27, 28	Meeting of Directors of the National Institutes
November	2	74th Biological Symposium
November	4, 5	18th Meeting of the Tokai Branch of the Sericultural Society of Japan
November	10	75th Biological Symposium
November	11	Public Lectures on Genetics (at the Daiichi-Seimei Hall, Tokyo)
November	25	149th Meeting of Misima Geneticist' Club
December	16	150th Meeting of Misima Geneticist' Club

FOREIGN VISITORS IN 1966

Jan.	2	STROMBERGER, H. G., Pasadena High School and Junior College, Pasadena, California, U.S.A.
Jan.	24	TOKIN, B. P., Department of Zoology, University of Leningrad, Leningrad, U.S.S.R.
Feb.	28	NEWCOMBE, H. B., Biology Branch, Atomic Energy of Canada Limited, Chalk River, Ontario, Canada
Apr.	2	KANG, Y. H., Department of Botany, Yonsei University, Seoul, Korea
Apr.	16–21	SCHMIDT, J, W., Department of Agronomy, University of Nebraska, Lincoln, Nebraska, U.S.A.
Apr.	19	WAYLAND, H., Department of Engineering Science, California Institute of Technology, Pasadena, California, U.S.A.
May	4	JORDAN, H. O., Economics Development Institute, University of Nigeria, Enugu, East Nigeria
Jun.	30	TAKAHASHI, T., Department of Geology, University of Rochester, Rochester, New York, U.S.A.
Jul.	18	DEGENHARDT, K. H., Institut für Humangenetik und Vergleichende Erbpathologie der Universität Frankfurt am Main, Germany
Jul.	19	DAGG, C. P., The Jackson Laboratory, Bar Harbor, Maine, U.S.A.
Jul.	29	GAFFNEY, L., c/o A. A. Tegel Pty. Ltd., Leppington, New South Wales, Australia
Aug.	9	CARTER, O., Department of Agronomy, University of Sydney, New South Wales, Australia
Aug.	15	CHO, W. K., Department of Zoology, Seoul National University, Korea
Aug.	25	CHAKRAVARTTI, M. R., Indian Statistical Institute, Calcutta, India
		MAVALWALA, J., Department of Anthropology, University of California, U.S.A.
		SWAMINATHAN, M. S., Division of Botany, Indian Agricultural Research Institute, New Delhi, India
Aug.	28	KIM, Y. C., College of Agriculture, Korea University, Seoul, Korea
		SHAHI, B. B., Seed Testing Laboratory, Department of Agriculture, H. M. G., Kathmandu, Nepal
		YÜ, C. J., Department of Botany, College of Science, National Taiwan University, Taipei, Taiwan, China

Aug. 28 HOUSE, L. R., The Rockefeller Foundation, New Delhi, India
CHAKRABANDHU, M.C., Department of Agriculture, Bangkok, Thailand
CARANGAL, A. R. Jr., Department of Research and Development, San Miguel Corporation, Manila, Philippines
KU, Y., College of Agriculture, National Taiwan University, Taipei, Taiwan, China
CREECH, J. L., New Crops Research Branch, Beltsville, Maryland, U.S.A.
CALDECOTT, R. S., Department of Agronomy and Plant Genetics, University of Minnesota, Minnesota, U.S.A.
NATARAJAN, A. T., Institute of Radiobiology, Stockholm University, Stockholm, Sweden
SIGURBJORSSON, B., Plant Breeding and Genetics Section, I. A. E. A., Vienna, Austria

Aug. 29 CLAUSEN, J., Department of Plant Biology, Carnegie Institution of Washington, Stanford, California, U.S.A.

Aug. 30 LIDICKER, W. Z., Jr., Museum of Vertebrate Zoology, University of California, Berkeley, U.S.A.

Aug. 31 KANG, Y. S., Department of Zoology, Seoul National University, Seoul, Korea

Aug. 31—Sep. 1
BROCK, R. O., Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia
FRYXELL, P. A., Soil Crop Sciences Department, Texas A. and M. University (U.S. Department of Agriculture), Texas, U.S.A.
LEWIS, H., University of California, Los Angeles, California, U.S.A.
GRANT, W. F., Department of Genetics, McGill University, Montreal, Canada
ASTAUROV, B. L., Department of Experimental Embryology, A.N. Severtzov Institute of Animal Morphology, Moscow, U.S.S.R.

Sep. 1 CARSON, H. L., Department of Zoology, Washington University, St. Louis, Missouri, U.S.A.
LÖVE, A., and LÖVE, D., Department of Biology, University of Colorado, Boulder, Colorado, U.S.A.

Sep. 2-3 HUNZIKER, J. H,, Department of Biological Sciences, University of Buenos Aires, Buenos Aires, Argentina

Sep.	6	MEYER, G. F., Max-Plank-Institute für Biologie, Tübingen, Germany
Sep.	9	PAIK, Y. K., Department of Biology, School of Science, Yonsei University, Seoul, Korea
		GIESBRECHT, P., Molecular Biology Division, Robert Koch Institute, Berlin, West Germany
Sep.	20–21	BHATIA, C., Indian Agricultural Research Institute, New Delhi, India
Oct.	27	LEJEUNE, J., Institut de Progenèse, Faculté de Médecine, Université de Paris, Paris, France
Oct.	28–29	LAW, L. W., Carcinogenesis Section, National Cancer Institute,, N. I. H., Bethesda, Maryland, U.S.A.
		ROOSA, R. A., Wistar Institute, Philadelphia, Pennsylvania, U.S.A.
		STUDZINSKI, G., Jefferson Medical College, Philadelphia, Pennsylvania, U.S.A.
		PLEIBEL, N., Wistar Institute, Philadelphia, Pennsylvania, U.S.A.
Oct.	29–31	BORGHESE, E., Istituto di Anatomia Topografica, Dell' Univèrsità di Napoli, Napoli, Italy
Nov.	2	KOLLER, P. C., Department of Cytogenetics, Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, England
		POGGENDORFF, W. H. G., Department of Agriculture, Faner Place, Sydney, New South Wales, Australia
Nov.	10	BAPAT, C. V., and BHISEY, A. N., Biology Division, Indian Cancer Research Centre, Parel, Bombay, India
Nov.	11	GERHARTZ, H., Medizinische Klinik der Freien Universität, Berlin, Germany

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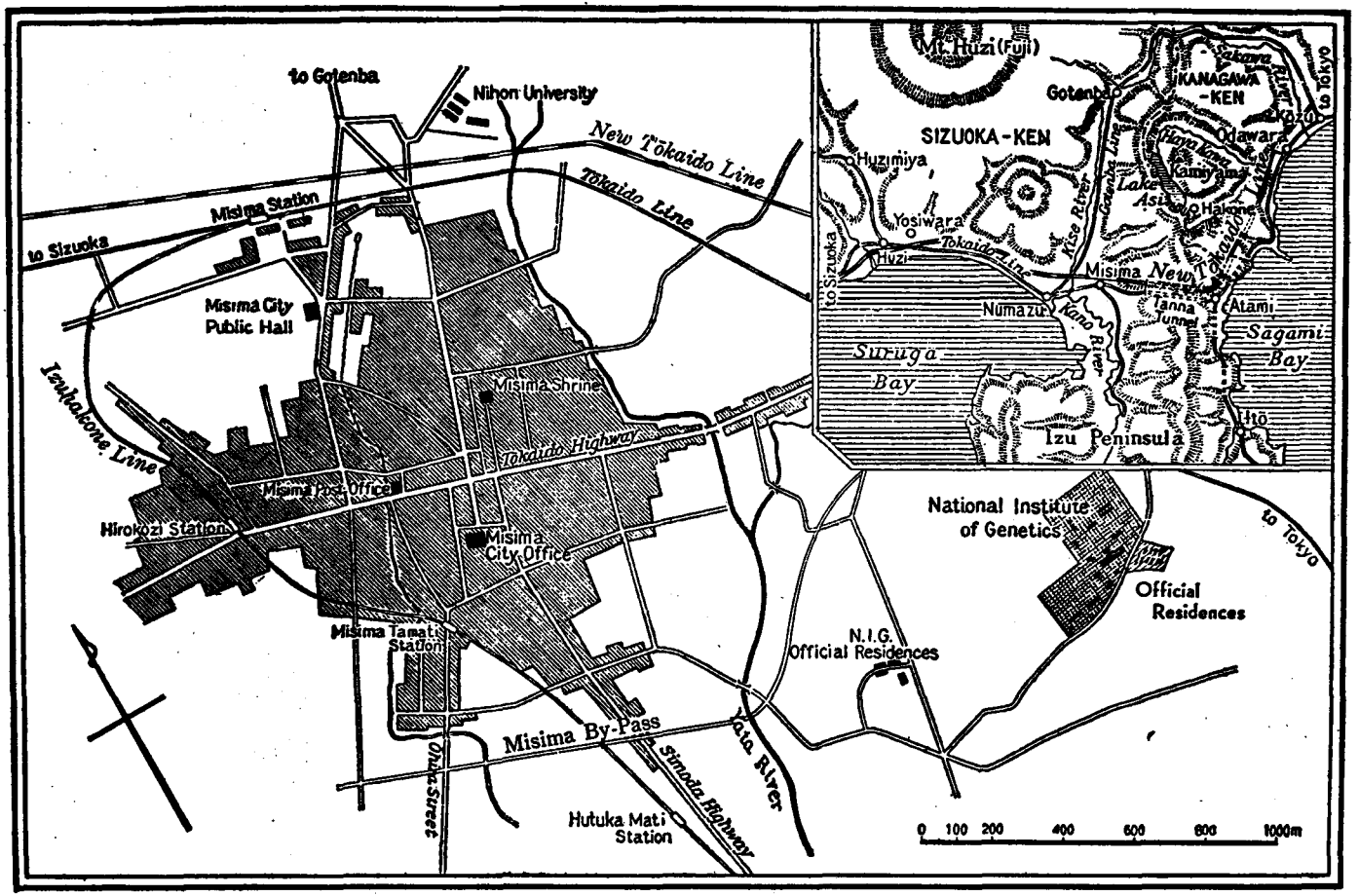
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