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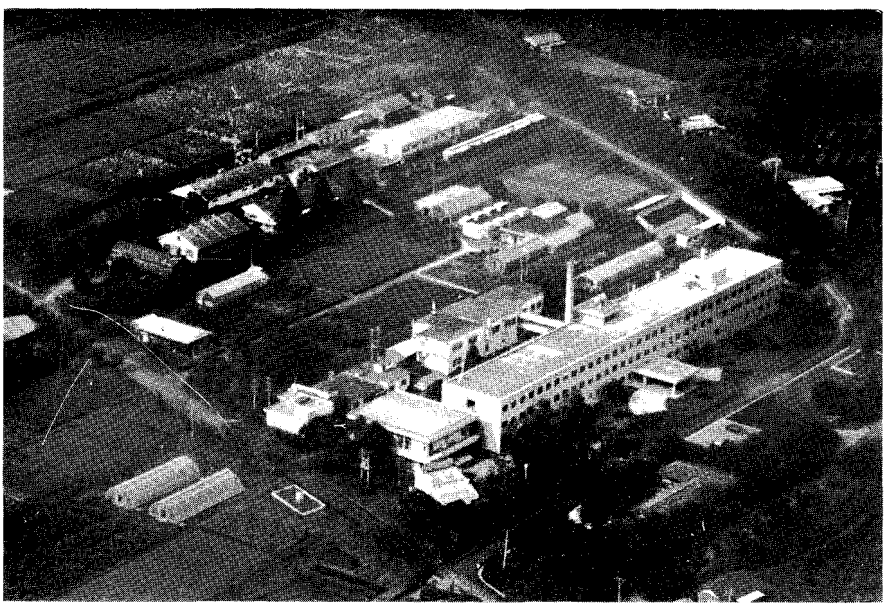
No. 18, 1967



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GENERAL STATEMENT

According to our expectation, the budget for the main building was allocated this year. The complete edifice is shown on the front page of this report. It took eight years to renew the old wooden structure with the present one built in concrete.

To our great regret, we have to announce that Dr. Seiji Matsumura, head of the Department of Induced Mutation died from leukemia on the 19th of February, 1967, at the age of 57.

Dr. Matsumura started his scientific career after graduating from Kyoto University, majoring in genetics in 1931. His main interests in his early days were directed to the cytogenetics of pentaploid wheat hybrids. He could establish seven nullisomics of the D-genome and he also found the principles governing the change of chromosome numbers in the offspring of those hybrids. With his fundamental study of triploid sugar beets a successful triploidy breeding of this crop was established.

He joined our Institute as a staff member after its establishment in 1949. He became the head of the Department of Induced Mutation in 1955, where he showed his ability as research worker as well as administrator. He succeeded in selecting good collaborators and equipping his laboratory with various instruments within a short time. His many valuable contributions were published in several series. Among others his efforts were directed to the study on the mechanisms of radiation injury and to the application of induced mutations to plant breeding.

He was also associated with the Kihara Institute for Biological Research as its secretary. Due to his efforts the institute could overcome the difficulties encountered during and after the War. With his untimely passing away the National Institute has lost an excellent scientist and administrator and the Kihara Institute deplores the loss of the efficient manager.

Our staff members are busy in the preparation for the coming XII International Congress of Genetics which will be held in Tokyo, 1968.

H. Kihara

RESEARCH MEMBER

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

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PROJECTS OF RESEARCH FOR 1967

Department of Morphological Genetics

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Repair processes in radiation mutagenesis (TAZIMA and ONIMARU)
Genetic studies of radiosensitivity in the silkworm (MURAKAMI)
Chemical mutagenesis in the silkworm (TAZIMA and ONIMARU)
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Genetical and biochemical studies of membrane proteins in the silkworm (SAKURAI and TSUJITA)
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Department of Applied Genetics

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Studies on competition in plants and animals (SAKAI, IYAMA, FUJISHIMA and NARISE, T.)
Estimation of genetic parameters in forest trees (SAKAI, HAYASHI and TOMITA)
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Biochemical studies on development of higher plants (SAKAI, NARISE, S. and HONDA)
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RESEARCHES CARRIED OUT IN 1967

I. CYTOGENETICS

The Use of Chromosomal Interchanges to Test for Crossing Over and Chromosome Segregation

Htoshi KIHARA and Minoru SHIMOTSUMA*

Tetrad pollen association was studied in the watermelon. It was found to continue for a short time after the dehiscence of the anthers. According to the number of normal pollen grains within a tetrad, these were classified into five types (Fig. 1).

Type-1: 4 normal pollen grains in a tetrad.
Type-2: 3 normal, 1 empty in a tetrad.
Type-3: 2 normal, 2 empty in a tetrad.
Type-4: 1 normal, 3 empty in a tetrad.
Type-5: 4 empty in a tetrad.

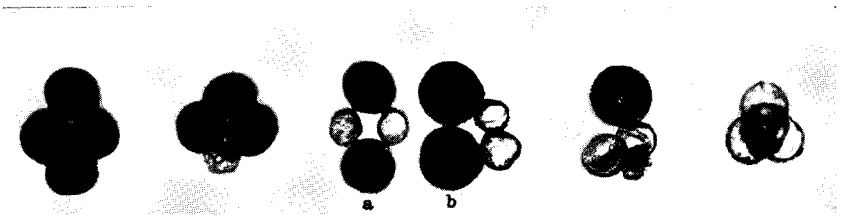

Fig. 1. Photomicrographs of 5 types of pollen tetrads in the chromosomal interchange heterozygotes (④+9_{II}).

All types except type-5 were found in the three normal varieties employed. Five types of pollen tetrads were observed in three interchange heterozygotes (④+9_{II}) obtained from γ-irradiation. Frequency of these five types was different in different strains. Type-1 tetrads are the result of alternate segregation of a ring of 4 chromosomes (④), while type-5 tetrads are obtained either from adjacent-1 or adjacent-2 segregation. Therefore the ratio of type-1 to type-5 tetrads represents the ratio of alternate to open segregation. Those ratios were respectively 1.0 : 1, 2.6 : 1 and 2.1 : 1 for the three interchange heterozygotes, MT-1, AT-1 and FT-1. The ratio of alternate, adjacent-1 and adjacent-2 segre-

* Kihara Institute for Biological Research, Yokohama.

Varieties	Strains	Alternate	Adjacent-1	Adjacent-2
Miyako No. 1	MT-1	2	1	1
Asahi-Yamato	AT-1	2	1	0
Fumin	FT-1	2	1	0

gation was estimated as follows: Type-3 pollen tetrads are the result of single crossing over between the interstitial segments. Therefore the percentage of type-3 tetrads corresponds roughly to the double number of single crossover. In the three interchange heterozygotes, MT-1, AT-1 and FT-1, the percentage was 14.5, 59.8 and 61.3, respectively. A fairly interesting detail of our work was the finding that from the distribution of normal and aborted pollen grains in a tetrad, we could recognize whether a single crossing over had occurred or not.

Characteristic Aggregate Formation of Mouse Plasma Tumor Cells with Different Chromosome Numbers[1)]

Yukiaki KURODA

A solid tumor of plasma cell neoplasm X5563, originally obtained from Dr. Michael Potter, National Cancer Institute, Bethesda, Maryland, and maintained in this Institute by serial subcutaneous transplantations in C3H mice, was examined for its specific aggregation pattern from dissociated single cells by rotation cultures.

It has previously been reported that this tumor had a modal chromosome number of 84 with one submedian metacentric and one subtelocentric chromosome as markers (Yosida *et al.*, 1966).

The solid tumor was dissociated into single cells by treatment with trypsin and cell suspensions each containing 10^6 cells in 3 ml culture medium were rotated on a gyratory shaker by the standard procedure. After 24 hours of rotation culture two aggregates were formed which were different in their morphology, namely one was large and compact with a rough surface and the other was small, loose and amorphous. Histologically the former consisted solely of degenerated cells in which the nuclei were faintly stained and some had completely lost their stainability with hematoxylin, whereas the latter consisted solely of active cells, some being in mitotic phase.

The ascites form of plasma cell tumor which developed in a BALB/c mouse after injection with complete Freund adjuvant was tested for its aggregation pattern in rotation cultures. Cell suspensions each contain-

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

ing 10^6 cells in 3 ml culture medium were rotated by the standard procedure. After 24 hours of rotation culture two or three spherical aggregates were formed. It was found that histologically in these aggregates active cells in the center were surrounded by degenerating peripheral cells.

From the results obtined from the rotation cultures of mouse plasma cell tumors, both solid and ascites forms, it is concluded that the degenerating cells become different in their surface properties from the actively proliferating cells of the same cell population and the former are sorted out from the latter in rotation cultures. The difference in aggregation pattern between solid and ascites plasma cell tumors, *i.e.*, solid tumors forming separate aggregates, either of active cells only and degenerating cells only, and ascites cell tumors segregating active cells from degenerating cells in the same aggregate, may reflect the difference in the *in vivo* conditions under which solid and ascites cell tumors were maintained in the mouse.

Two strains of the solid tumor of mouse plasma cell neoplasm developed by injection with complete Freund adjuvand and maintained for 14 generations of subcutaneous transplantations in mice were found to have diploid ($2s=40$) and hypotetraploid ($2s=73$) chromosome numbers.

The relationship between chromosome number and aggregation pattern in those two strains was examined. The solid tumors were dissociated into single cells by trypsinization and the cell suspensions were rotated as described above for 24 hours. The hypotetraploid tumor cells formed larger and more compact aggregates than did the diploid cells under identical conditions employed here. Since under the subcutaneous condition in the host mouse the former showed higher proliferating activity than the latter, the aggregate-forming activity of mouse plasma cell tumors may have some relation to the proliferating activity of tumor cells.

Selective Sorting-out Property of Mouse Plasma Cell Tumors for Specific Types of Normal Cells[1)]

Yukiaki KURODA

The solid tumors of mouse plasma cell neoplasm which have near-tetraploid chromosome number, were dissociated into single cells by trypsinization, intermingled with a variety of normal cells from chick

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

Table 1. Behavior of mouse plasma tumor cells intermingled with a variety of normal cells from chick embryos

	Embryonic chick cells		Behavior of mouse plasma tumor cells
	Type of cells	Age of embryo	
Ectodermal	Epidermis	7th day	Segregated
	Ectoderm of CAM*	7th day	
Mesodermal	Heart	8th day	Not-segregated
	Sclerotic cartilage	8th day	
	Mesonephros	7th day	
	Dermis	7th day	
	Mesoderm of CAM*	7th day	
	Mesenchyme	4th day	
Endodermal	Liver	8th day	Segregated
	Endoderm of CAM*	7th day	

* CAM: chorioallantoic membrane

embryos, and tested in rotation cultures for their selective sorting-out property in co-aggregates with normal cells from tissues of various embryonic origin. In these experiments, chick normal cells were of advantage as they could be distinguished by size and staining ability of their nuclei from mouse tumor cells in co-aggregates of both, considering that it was known that cells show their specific histogenetic behavior though they are derived from genetically remote animals.

When dissociated mouse plasma tumor cells were intermixed with a variety of normal cells from chick embryos and cultured in rotation, the former showed a selective sorting-out property for specific types of normal cells as shown in Table 1.

The results indicate that mouse plasma tumor cells became sorted out with embryonic chick cells of ectodermal and endodermal origin but formed chimaeric tissues interspersed with chick cells of mesodermal origin. Such selective affinity of tumor cells to mesodermal cells has been demonstrated by HeLa cells as reported previously by the author (Ann. Rep. **17**: 18, 1966). It is not known whether this selective affinity to mesodermal cells is the property restricted to some tumor cells, *i.e.*, mouse plasma cell tumors and HeLa cells, employed by the author or this character may be revealed by all tumor cells which are derived from mesodermal tissue. It is an interesting idea that the selective affinity of tumor cells for a specific type of normal cells might be connected with the selective mechanism by which the original neoplastic cells metastasize to some specific types of normal tissues or organs.

Cytogenetical and Biochemical Studies of 19 Primary Plasma Cell Neoplasms Induced in BALB/c Mice[1)]

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

Near-tetraploid karyotypes were observed almost in all tumor cells of transplantable mouse plasma cell neoplasms obtained from Dr. M. Potter (Yosida *et al.* 1964 and 1966, this report 14 : 42 and 16 : 45). In order to ascertain the chromosomal condition of the primary plasma cell tumors, we have already examined five of them (MSPC-1 to 5) (Yosida *et al.* 1967, this report 17 : 11). The modal chrosome number of one (MSPC-1)

Table 1. Plasma cell tumors (MSPC) induced by Freund adjuvant in BALB/c mice

Tumor line (MSPC)	Sex	Specific protein			Character of primary tumor	Chromosome number (mode)
		Ser.	As.	Ur.		
1*	♂	γA		−	Sol.	38～ 81 (40)
2	♀	γF		−	As.	39～ 93 (86)
3*	♀	γF		λ	Sol.+As.	35～ 94 (86)
4	♀	−		−	As.	39～168 (78)
5*	♂	γA		−	Sol.+As.	35～ 94 (44)
6	♂	−		−	As.	—
7	♂	+	+		As.	39～ 84 (79)
8	♂		−		Sol.+As.	75～164 (86)
9	♂	+			As.	83～ 90 (87)
10	♂	+	+		As.	40～ 79 (41)
11	♂				As.	40～ 90 (84)
12	♀	+	+		Sol.+As.	39～ 84 (40)
13	♂				As.	76～160 (80)
14	♀		+		As.	76～ 78 (82)
15	♂	+			Sol.	—
16	♀		−		As.	—
17	♀		−		As.	82～ 86 (85)
18	♀	−	−		As.	—
19*	♂	+	+		Sol.+As.	75～157 (78)

* Tumors transplanted successfully. Ser. = serum. As. = ascites. Ur. = urea. Sol. = solid tumor. + = γ-globulin detected. − = γ-globulin not detected.

1) This work was supported in part by a research grant from the National Cancer Institute (CA 07798-04), Public Health Service, U.S.A. and by a Grant in Aid for Foundament Scientific Research from the Ministry of Education in Japan.

was 40, of another (MSPC-5) 44, and the remaining three (MSPC-2, 3 and 4) had hyper- and hypotetraploid karyotypes. Recently 14 primary plasma cell tumors in BALB/c mice were obtained about half to one year after injection of Freund adjuvant, and we observed their character, γ-globulin, and chromosome constitution. All tumors obtained are shown in Table 1, in which 5 already published tumors are included. γ-globulin was checked by electrophoresis in serum and/or ascites of tumor bearing mice. Among the 19 tumors γ-globulin was found in serum of ascites of 11 mice, but was not found in 6 mice. γ-globulin of two mice was not examined. As to the type of tumor, 12 were found to be ascites tumors, two were solid tumors and the remaining 5 were solid tumors with ascites. It is interresting that transplantation of tumors was successful only when they have developed as solid tumors or solid with ascites and in tumors positive for γ-globulin production. In 15 primary tumors the chromosomes were successfully observed. Among them the modal chromosome number in two tumors was characterized by 40 chromosomes, two tumors had hyperdiploid chromosome numbers and the remaining 11 had near-tetraploid karyotypes. Relation between the frequency of polyploid cells and the latent period after injection of Freund adjuvant it given in Table 2. As the table shows, cells with diploid chromosome number were observed in tumors developed after a short latent period such as 6 to 8 months, while tumors developed

Table 2. Relation between polyploidization and latent period to tumor development after injection of Freund adjuvant

Latent period (in months)	Tumor line* (MSPC)	Ploidy			No. of cells observed
		$\pm 2n$	$\pm 4n$	$\pm 8n$	
6	7	14%	86	0	50
7	10	54.5	45.4	0	11
8	12	83.3	16.7	0	30
	11	6.7	93.3	0	30
	8	0	96.7	3.3	30
	9	0	100	0	30
10	13	0	91.7	8.3	12
12	19	0	90	10	30
	14	0	100	0	41
	17	0	100	0	30

* Same as in Table 1.

10 to 12 months after injection had only tetraploid or octoploid cells. Based on the above investigations, it is suggested that tumors can develop from cells with diploid chromosomes but they may change to tetraploid level by creating vigorous cell types.

Periodic Ploidy Changes in the Mouse Plasma Cell Tumor, MSPC-1

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

Besides the characteristic tetraploidization found in this tumor (Ann. Rep. 17 : 12), changes of tetraploid cell population to a diploid one were also observed. By these two repeatedly reversed alterations, the ploidy of this tumor altered periodically. Up to now, this tumor has been transplanted for more than two years, during which the periodic ploidy change has been repeated at least four times (Fig. 1).

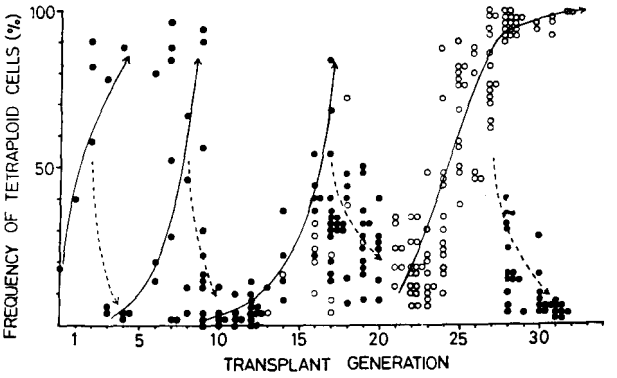

Fig. 1. Alteration in the frequency of tetraploid cells during serial transplantations of the mouse plasma cell tumor, MSPC-1
Open circle: Ascites form. Solid circle: Solid form.

It was suggested that the tetraploidization was mainly due to binucleate cell formation by a failure of cytokinesis (This Ann. Rep.). On the other hand the recovery of a diploid population seemed to be caused by a periodic activation of the proliferation of diploid cells which were present in a tetraploid cell population in minority. In most cases this recovery appeared when the cell population consisted of fifty or sixty percent of tetraploid cells. In ascites form tetraploidization proceeded irreversibly,

This work was supported in part by a Grant in Aid for Foundamental Scientific Research from the Ministry of Education in Japan and by a research grant from the National Cancer Institute (CA 07798-04), Public Health Service, U.S.A.

Table 1. The effect of γ-ray irradiation of the host mouse on the recovery of diploid cell populations in MSPC-1

Pretreatment of host	Tetraploids in inoculum	Tetraploids in grown tumor	t*
Control	62%	20.9±11.0%	—
200 R	62	17.6± 7.7	0.87<2.07 (5%, df=22)
300 R	62	15.7± 5.1	1.44<2.10 (5%, df=18)
400 R	62	13.2± 5.9	2.20>2.07 (5%, df=22)

* Each t value was calculated against control percentage.

whereas a remarkable recovery of diploid was observed when the tumor was transplanted subcutaneously in a solid form (See Fig. 1, transplant generation 28).

To obtain some information on the condition of the host mouse in connection with the recovery of diploid cells, ascites tumor cells (62 percent tetraploid cell population) were injected into mice subcutaneously after γ-ray (200, 300 and 400 R) irradiations. As shown in Table 1, a remarkable recovery of diploid cell population was observed in all grown tumors and no significant difference was found between control and experiments, except for a minor feature, namely the radiation in recovery rate was less in the experimental group. This finding might suggest that an immunological or some other physiological condition of the host mice does not allow for such an extensive recovery of diploid cells. It is interesting that there is a slightly significant increase in recovery at high dosage of γ-ray irradiation (400 R).

A clearer result was obtained in a simultaneous transplantation of 6 and 62 percent tetraploid tumor lines to the same host mouse (Table 2). As far as observed, the former diploid tumors did not change the mode of their diploid stem line, but in all latter, 62 percent tetraploid lines, recovery of diploid cells was observed. These experiments strongly suggest that the recovery mechanism of diploid cells in a tetraploid tumor line is an intrinsic character of the tumor cells.

Table 1. The effect of host mouse on the frequency of tetraploid cells in MSPC-1

Inoculation	Tetraploids in inoculum	Tetraploids in grown tumor
Subcutaneous, at left abdomen	62%	34.3±10.5%
Subcutaneous, at right abdomen	6%	3.8± 1.7%

Cytological Basis of Tetraploidization Mechanism Found in Mouse Plasma Cell Tumor, MSCP-1

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

MSPC-1 tumor which primarily had developed as a diploid line (s=40) has been characterized by a frequent occurrence of tetraploidization (Ann. Rep. 17 : 12). The diploid line has been maintained by strict selection and it had the tendency to change to tetraploidy. In the transient state from diploid to tetraploid, the tumor was composed of a mixed population of diploid and tetraploid cells. The ratio of tetraploid to diploid cells varied with the condition of host or tumor.

Three hypotheses are proposed for the mechanism of tetraploidization in this tumor line; namely, (1) endoreduplication, (2) cell fusion and (3) failure of cytokinesis. As far as observed, no mitotic figure showing endoreduplication was found. Therefore the first hypothesis seems to be untenable. On the other hand, there were frequently observed binucleate cells in this tumor (Fig. 1-B). The following evidences make the 3rd hypothesis more probable than the 2nd one. (1) Two nuclei in the binucleate cells were labeled simultaneously by H^3-thymidine. (2) Tetraploid cells had frequently a pair of marker chromosomes (metacentrics or submetacentrics) which suggests the occurrence of autopolyploidization. (3) Anaphase figures in which a cytokinesis seemed to have failed were observed.

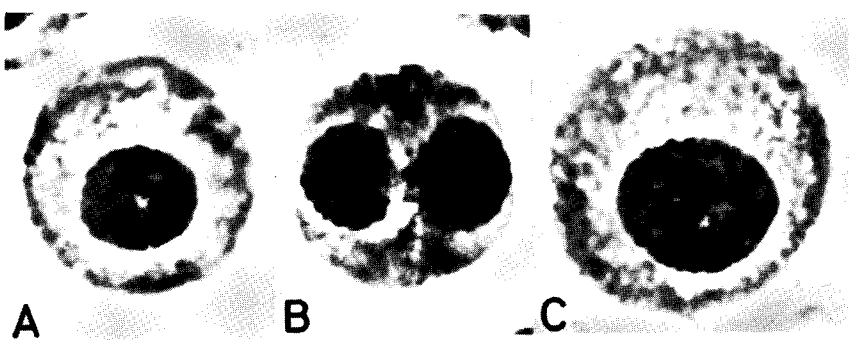

Fig. 1. Cells in the mixed cell population of mouse plasma cell tumor, MSPC-1.
1-A. Diploid cell. 1-B. Binucleate cell. 1-C. Tetraploid cell.

This work was supported in part by a Grant in Aid for Foundamental Scientific Research from the Ministry of Education in Japan and by a research grant from the National Cancer Institute (CA 07798-04), Public Health Service, U.S.A.

Table 1. Frequencies of binucleate cells of mouse plasma cell tumor, MSPC-1

Frequency of tetraploid cells	Frequency of binucleate cells	Total observed cells	Binucleate cells / Tetraploid cells
2%	1.23% (34)	2764	61.5%
15	3.43 (60)	2009	19.9
30	3.16 (133)	4212	10.5
38	3.78 (157)	4150	10.0
54	3.85 (41)	1065	7.2
68	2.89 (27)	934	4.3
84	0.63 (6)	946	0.8

Numbers in the parentheses represent observed cell numbers.

As shown in Table 1, the frequency of binucleate cells increased from one to four percent in parallel with the increase of the percentage of tetraploid cells. The maximum frequency of tetraploid cells was about 40 percent, but it decreased with the increase of diploid cells. As a synchronization of the mitotic phase of the two nuclei in binucleate cells appeared as a tetraploid figure at metaphase. The ratio of binucleate interphase cells against tetraploid metaphase was calculated (Table 1). The ratio was high at the stage of only a few tetraploid cells present and it decreased gradually following the increase of tetraploid cells. This result may indicate that at the start of tetraploidization, most of the tetraploid cells are represented by binucleate cells in which the two nuclei unite. One of the possible sequences of events leading to tetraploidization found in this tumor is considered to be as follows: Diploid cell (Fig. 1-A) → Karyokinesis → Failure of cytokinesis → Binucleate cell (Fig. 1-B) → Union of binuclei → Tetraploid cell (Fig. 1-C). The same alteration of nuclei as above were frequently observed in the other primary plasma cell tumors (Ann. Rep. 17 : 11).

Effect of Gene Dosage on the Rate of Protein Synthesis in the Mouse Plasma Cell Tumor, MSPC-1[1)]

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

A diploid plasma cell tumor, MSPC-1-D, has been induced by Freund

1) This work was supported in part by a Grant in Aid for Foundamental Scientific Research from the Ministry of Education in Japan and by a rasearch grant from the National Cancer Institute (CA 07798-04), Public Health Service, U.S.A.

Table 1. Comparison of the rates of total and myeloma protein syntheses, cell volume and protein content between diploid and hypotetraploid mouse plasma cell tumors.

	Tumor strains		T/D ratio	t
	MSPC-1-D	MSPC-1-T		
Ploidy (Mod. No.)	Diploid (40)	Hypotetraploid (73)	1.83	
Total protein synth. (cpm/10^6 cells/hr)	13.300±2.200	18,600±5,100	1.40	3.21>2.85 (1%, df=20)
Cell volume ($mm^3/10^6$ cells)	12.3±1.8	15.5±1.7	1.26	6.68>2.98 (1%, df=14)
Protein content ($\mu g/10^6$ cells)	72.0±23.8	107.0±32.6	1.50	3.41>2.80 (1%, df=24)
MP/TP ratio	0.0107±0.0012	0.0073±0.0007	0.68	5.13>3.36 (1%, df=8)

MP: Myeloma protein synthesis
TP: Total protein synthesis

adjuvant treatment of a BALB/c mouse in Misima, 1966. From the second transplant generation of this tumor, a hypotetraploid subline, MSPC-1-T, was derived. For the purpose of finding out whether the duplicated genes in the hypotetraploid cell have the same degree of expression as in the diploid cells, the two tumor lines were compared in terms of the rates of total and myeloma protein synthesis per cell.

The rate of total protein synthesis was measured by incorporating C^{14}-leucine into a TCA-insoluble fraction of tumor cells, and that of myeloma protein was estimated from the radioactivity of the myeloma protein secreted into the incubation medium and separated by DEAE cellulose column chromatography. As shown in Table 1, the rates of both protein syntheses, cell volume and protein content did not increase in parallel with the duplication of chromosome number. These results could suggest that the duplicated genes in the hypotetraploid cells might be partially suppressed including the gamma globulin locus.

In this case, however, another possibility can be considered, namely that the elimination of chromosomes at the formation of hypotetraploids from true tetraploids was accompanied by the loss of specific genes regulating the total and the myeloma protein synthesis, which might result in an apparent suppression of gene dosage effect. To rule out this possibility, the rates of the protein syntheses were determined employing the transient stage of the plasma cell tumor which had a mixture of various ratios of diploids and tetraploids but no hypotetraploids. The rate of total protein synthesis increased in parallel

with the increase of tetraploid cells until 40 percent, beyond which it reached a plateau (Fig. 1). Between the rate of myeloma protein synthesis and the percentage of tetraploid cells an almost similar relationship was demonstrated.

In the transient phase from diploids to tetraploids, a considerable number of binucleate cells was observed. Frequency of binuclei per

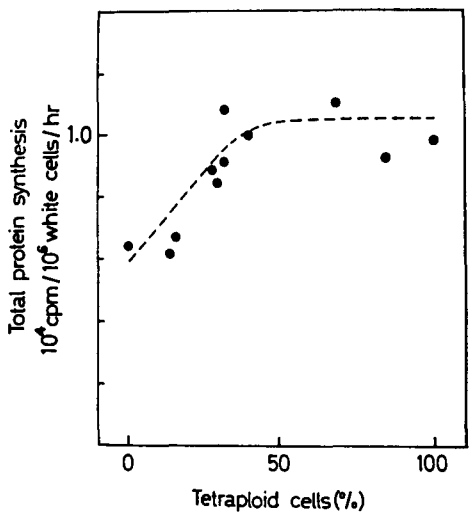

Fig. 1. Changes in the rate of total protein synthesis according to the increase of tetraploid celli in MSPC-1.

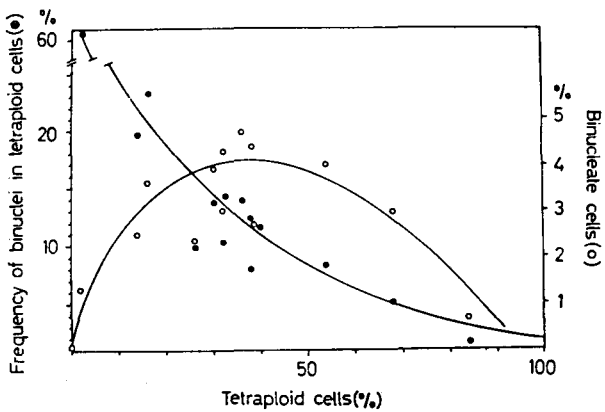

Fig. 2. Frequencies of binuclei per tetraploid cell and binucleate cells in MSPC-1 tumor during ploidy shift.

tetraploid cell was calculated. They showed the highest frequency simultaneously with the lowest percentage of tetraploid cells and became gradually fewer according to the increase of the tetraploid cells (Fig. 2).

Biochemical and cytological findings presented in this study may lead to the assumption that diploid cells in this plasma cell tumor can be changed to "mononucleate" tetraploid cells after passing through the transient stage of binucleate "tetraploid" cells and that the suppression of gene dose effect in the total and myeloma protein syntheses occurs only in "mononucleate" tetraploid cells, not in the binucleate "tetraploid" ones.

Chromosome Studies in 12 Malignant Hamster Cell Lines Transformed *in vitro* by Treatment with 4HAQO and 4NQO[1]

Tosihide H. YOSIDA, Hiroshi MASUJI and Toshio KUROKI[2]

The relation between chromosomal alteration and development of tumors is an important problm. The senior author presented in 1966 (Jap. Jour. Genet. 41 : 439) a hypothesis that a gradual change of karyotype by sequential events of mutation and selection is a causative process in the production of cells exhibiting vigorous malignant proliferation. In order to demonstrate the relationship between chromosomes and tumors, we observed the chromosomes of malignant cell lines of golden hamster embryos transformed *in vitro* at several stages of cultivation after treatment with 4 hydroxyaminoquinoline N-oxide (4HAQO) and 4-nitroquinoline 1-oxide(4NQO). Seven malignant transformed cell lines (HA-1, HA-2, HA-4, HA-6, HA-7, HA-8 and HA-15) had developed by treatment with 4HAQO *in vitro*, 4 lines (NQ-2 to NQ-5) by treatment with 4NQO and one line (ZEN-4) had developed spontaneously in the course of serial *in vitro* cultivations. The chemicals were used by the following techniques. The final concentrations of the carcinogens were 4×10^{-6} M of 4NQO and 10^{-5} M of 4HAQO, and Eagle's MEM medium containing the above drugs was added to a cell sheet after 7 day culture, kept for one day, and then changed to fresh medium. The treatment was repeated every other day two to 7 times.

[1] This work was supported in part by a research grant from the National Cancer Institute (CA 07798-04), Public Health Service, U.S.A., and by a Grant in Aid for Foundamental Scientific Research from the Ministry of Education in Japan.

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Normal somatic cells of the golden hamster embryos growing *in vitro* had 44 chromosomes. Among 20 cells analysed no cells with chromosome numbers deviating from normal diploid were found. Among 7 lines developed by treatment with 4HAQO, 4 lines (HA-1, HA-2, HA-4 and HA-7) showed a hypotetraploid chromosome number, while 3 lines (HA-6, HA-8 and HA-15) showed diploid number as a mode. Chromosomes in tumors of two lines (HA-1 and 8), which were transplanted successfully to hamsters were analysed. Chromosome number distribution in HA-1 tumor was almost the same as in HA-1 *in vitro* cells, but the number of cells at diploid level decreased in the tumor growing *in vivo*. In the HA-8 tumor line cells grown *in vivo*, hyperdiploid chromosome numbers increased more than in the cells growing *in vitro*. Among 4 lines developed by treatment with 4NQO, one line (NQ-2) showed a hypotetraploid mode (82 chromosomes), one line (NQ-5) a diploid mode (44), and the remaining two lines (NQ-3 and NQ-4) had bimodal distribution with one diploid and one tetraploid mode. In ZEN-4 transformed spontaneously *in vitro*, the modal number of chromosomes was 45.

All 8 tumor lines having hypotetraploid chromosome numbers strikingly deviated from karyotypes of normal somatic cells. Among 4 tumor lines having diploid chromosome numbers, two (HA-8 and NQ-5) had almost the same chromosome constitution as normal somatic cells, but the other 2 (HA-6 and HA-15) showed different karyotypes. It was noticeable that both tumors, HA-8 and NQ-5, with normal karyotypes have developed a short time after treatment with the chemical. The former developed 28 days after treatment with 4HAQO, and the latter about 10 days after treatment with 4NQO. In all other tumors, numerical aberrations such as trisomy, tetrasomy, monosomy and also structural shanges, such as breaks and translocation, were observed. The transformation of these tumors occurred a long time (50–300 days) after treatment with the chemicals. Based on the above investigation, it can be concluded that the majority of malignant cell lines transformed a long time after treatment with 4HAQO and 4NQO *in vitro* had altered karyotypes, but a few tumors developed a short time only after treatment had almost normal karyotype, but were easily changeable by transplantations to animals or serial cultivations *in vitro*. The results obtained from the present study confirm the hypothesis that chromosome alteration may play an important role in creating more vigorous and malignant cell types.

Chromosome Abnormalities in Bone Marrow Cells in Several Strains of Mice After Injection with 4-Nitroquinolin 1-oxide[1)]

Tosihide H. YOSIDA and Reiko TSURUTA

It is well known that 4-nitroquinolin 1-oxide (4NQO) is one of stronger carcinogenic agents. Leukemias have been induced in A strain mice by treatment with the chemical (Nishizuka and Nakakuki 1964, Gann 55 : 495). On the other hand, remarkable chromosome aberrations were induced in Yoshida sarcoma cells by injection of the chemical into peritomeal cavity of rats bearing the tumor (Yosida *et al.* 1965, Gann 56 : 253). The object of the present study was to ascertain whether this chemical can induce chromosome aberrations in bone marrow cells of mice injected by it subcutaneously, and, if so, whether its effect on the chromosomes differs by the strain employed.

0.2 ml of 10^{-3} M 4NQO was injected subcutaneously to newborn mice, and the chromosomes of bone marrow cells were observed 8, 10, 14, 18, 24, 48 and 72 hours after injection. Chromosome breaks and translocations were observed most frequently 10 hours after injection, and then the frequency gradually decreased, but was markedly reduced 18 hours after. Frequencies of chromosomal abnormalities in bone marrow cells

Tabel 1. Frequency of chromosome aberrations that occurred in bone marrow cells of various strains of mice injected with 4NQO

Strain of mice	Total aberrant cells (%)	% of cells with multiple breaks	Aberration per cell	No. of mice observed
C57BL/6	28.1	0	0.499	3
RF	23.7	0	0.305	2
AKR	38.2	9.25	+++*	2
A	40.3	0	0.719	2
DD	23.6	0	0.515	5
BALB/c	28.2	0.9	+++	1
C3H	47.0	4.6	+++	3
SWM	30.3	0	0.425	4

* +++ Calculation not possible due to multiple breaks.

1) This work was supported in part by a research grant from the National Cancer Institute (CA 07798-04), Public Health Service, U.S.A. and by a Grant in Aid for Foundamental Scientific Research from the Ministry of Education in Japan.

in 8 mouse strains are given in Table 1. As the table shows, the frequency of cells with abnormal chromosomes was 23 to 40 percent, and it did not vary much by the strain of mice used, but multiple breaks i.e. two or more breaks in one chromosome, were observed only in 3 strains, namely AKR and C3H at high frequency and BALB/c strain at low frequency. Frequency of aberrationa per cell increased almost linearly with increasing frequency of cells with chromosome aberration. This result suggests that chromosome aberration of bone marrow cells induced by treatment with 4NQO occur at random.

Karyological Studies of Three Species of Insectivora Collected in Japan

Tosihide H. YOSIDA, Yukuo MORIGUCHI and Jun SONODA

Karyotypes of three house shrews (*Suncus murinus*), dsinezumi shrew (*Crocidura dsinezumi*) and Horsfield's shrew (*Crocidura horsfieldii*) belonging to the Insectivora were analysed. The house shrews and Horsfield's shrews were collected in Okinoerabu island, Kagoshima-ken, and dsinezumi shrew in Misima, Sizuoka-Ken. Karyotypes of bone marrow cells were analysed by usual drying technique.

Table 1. Karyotype analysis of three Insectivora species

Species	No. of pairs of several autosome types				Type of sex-chromosomes		Total chromosome number
	A	ST	SM	M	X	Y	
Suncus murinus	14	1	2	2	SM	SM	40
Crocidura dsinezumi	13	3	2	1	ST	ST	40
Crocidura horsfieldii	0	2	4	6	M	SM	26

A=acrocentrics, ST=subtelocentrics, SM=submetacentrics, M=metacentrics.

The number of chromosomes in the house shrew, the dsinezumi shrew and the Horsfield's shrew was 40, 40 and 26, respectively. Karyotypic formulae of these species are shown in Table 1. As the table shows, karyotypes of house shrew and dsinezumi shrew are rather similar, but that of Horsfield's shrew is markedly different from those of two species. The chromosomes of Horsfield's shrew are markedly larger than those of house shrew and dsinesumi shrew. It is interesting to compare the karyotypes of house shrew and Horsfield's shrew, because if meta-, submeta-, and subtelocentric elements had been produced by

centromeric fusion of 14 acrocentric pairs of the house shrew, the chromosome number would be reduced to 26 and the karyotype would become very similar to that of Horsfield's shrew. From the above karyological investigations, it can be said that the karyotypes of dsinezumi shrew is similar to that of house shrew, but a morphological difference in a few chromosomes was found between them, and the karyotype of Horsfield's shrew is more closely related to that of the house shrew.

II. PHYSIOLOGICAL AND DEVELOPMENTAL GENETICS

Effect of Thermoshock and Virus Inoculation on Cytoplasmic Male Sterility in *Capsicum* and *Petunia*

Yasuo OHTA

A phenotypical similarity has been known between cytoplasmic and virus-induced male sterility in certain plants.

Cytoplasmic male sterility in *Capsicum annuum* L. is controlled by a sterile cytoplasm (*S*) and a male sterile gene (*ms*) (Peterson 1958). *S msms* plants are pollen sterile, *S Msms* or *S MsMs* plants are pollen fertile, and those having normal (*N*) cytoplasm are all pollen fertile. The normal counterparts, *N msms* plants, are called maintainers. *S Msms*×*N msms* and *N msms* selfed seeds of variety Fresno Chile were furnished by Dr. P. A. Peterson. The former produced male sterile (*S msms*) and male fertile (*S Msms*) plants in about 1 : 1 ratio. *S msms* plants were crossed with several Japanese varieties. A progeny test proved that Fushimiamanaga is a fertility restorer (*N MsMs*) and Ojishi is a maintainer (*N msms*) (Ohta 1961).

On the other hand, *S Msms* plants obtained from a cross *S msms* Freseno Chile×*N MsMs* Fushimiamanaga and *N MsMs* plants (Fushimiamanaga) were inoculated with an ordinary strain of cucumber mosaic virus (CMVo), two strains of tobacco mosaic virus, and tobacco ringspot virus (TRSV). *S Msms* plants inoculated with CMVo or TRSV showed reduced pollen fertility which was 64 to 68%. Selfing of those inoculated plants has been successfully made; the selfed progeny will be tested for pollen fertility in the coming season.

After exposing *S msms* seedlings (varieties Fresno Chile and Ojishi) for 15–120 minutes to 55–66°C temperatures, they were cultured as usual. Several of Ojishi produced some normal pollen grains. The stronger was the treatment the more normal pollen grains were produced, suggesting virus-like nature of a cytoplasmic agent.

Cytoplasmic male sterility in *Petunia hybrida* Vilm. is also controlled by a sterile cytoplasm (*S*) and a male sterile gene (*ms*) (Edwardson *et al.* 1967). Cytoplasmic male sterile (*S msms*) and normal counterpart (*N msms*) seeds were furnished by Dr. J. R. Edwardson. Leaf saps of both *S msms* and *N msms* plants were inoculated into indicator plants such as *Vigna sinensis*, *Phaseolus vulgaris*, *Nicotiana glutinosa*, *Chenopodium amaranticolor*, and *Cucurbita moschata*. It was found that the cytoplasmic agent was not infectious.

Zymographic Analysis of Cytoplasmic Male Sterile *Petunia* and *Zea*

Yasuo OHTA

Zymographic analysis during microsporogenesis (pre-meiosis to pre-anthesis) of cytoplasmic male sterile plants and their normal counterparts (maintainers) in *Petunia hybrida* Vilm. and *Zea mays* L. (F1a F6T and F1a F6) were made with starch gel electrophoresis in peroxidase, non-specific esterase, acid phosphatase, catechol oxidase, and several other enzymes. Certain differences in zymographic pattern were recognized between the cytoplasmic male steriles and their normal counterparts in peroxidase (*Zea*) and non-specific esterase (*Petunia* and *Zea*).

Histogenetic Aggregation of Dissociated Imaginal Disc Cells of *Drosophila melanogaster* Larvae in Rotation Culture

Yukiaki KURODA

To elucidate at a cellular level under strictly defined conditions the mechanism by which cells of identical genetic constituents show various phenotypic expressions in various organs and tissues, dissociated cells from various imaginal discs of *Drosophila melanogaster* were tested for their ability to form characteristic histogenetic aggregates in rotation culture.

Eye-antennal discs and wing discs were dissected from mature third-

Table 1. Components of medium K-10 for cultivation of *Drosophila* cells

Ingredients	mg/100 ml	Ingredients	mg/100 ml
NaCl	700	Thiamin·HCl	0.002
KCl	20	Riboflavin	0.002
$CaCl_2 \cdot 2H_2O$	2	Pyridoxine·HCl	0.002
$MgCl_2 \cdot 6H_2O$	10	Niaicn	0.002
$NaHCO_3$	5	Ca-pantothenate	0.002
$NaHPO_4 \cdot 2H_2O$	20	Biotin	0.001
Glucose	80	Folic acid	0.002
Sucrose	2,600	Choline·HCl	0.02
Lactalbumin hydrolyzate	1,280	Inosite	0.002
Tryptophan	100	*p*-Aminobenzoic acid	0.002

pH was adjusted to 6.6. The medium was sterilized through a Seitz filter and supplemented by 10% calf serum.

instar larvae (96 hours after hatching at 25°C) grown under sterile conditions. They were incubated in calcium- and magnesium-free salt solution for 15 minutes, then in 1 per cent trypsin solution for 15 minutes, and were dispersed in the culture medium into single cells by flushing the dissected materials through the tip of a fine pipette. Considerable effeorts have been made to improve and simplify the culture medium for *Drosophila* cells. Medium K-10 shown in Table 1 was better than other initial media.

One hundred eye-antennal discs isolated from Oregon-R larvae yielded about 4×10^5 single cells, whereas one hundred wing discs from the same strain produced about 4.5×10^5 cells. One hundred eye-antennal discs from *Bar* larvae yielded about 3×10^5 cells by the same procedure.

When single cell suspensions each containing 10^5 cells in 0.3 ml medium were introduced into tightly covered micro-beakers, rotated on a gyratory shaker for 24 hours at 100 rpm at 28°C, tissue-like cell aggregates were reconstituted in the center of the micro-beakers. Cell-aggregates obtained from eye-antennal disc cells of the Oregon-R strain had an average diameter of 0.6 mm, whereas eye-antennal disc cells from the *Bar* strain formed smaller and looser aggregates of an average diameter of 0.4 mm. Wing disc cells from the Oregon-R strain formed after 24 hours of rotation aggregates of 0.5 mm in diameter which showed a characteristic structure different from that of eye-antennal disc cells.

The differences in histogenesis and organogenesis of different imaginal discs are now further studied.

Differentiation of Imaginal Discs of *Drosophila melanogaster* Cultured in Synthetic Medium and Effects of Some Hormonal Substances

Yukiaki KURODA and Kiyoshi MINATO

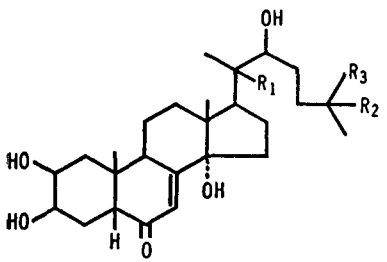
Eye-antennal discs were dissected from third-instar larvae of *Drosophila melanogaster* grown under sterile conditions and were cultured in hanging drops of synthetic medium K-10 at 28°C.

In the medium without any supplementations of hormonal substances eye-antenal discs showed a pronounced increase in the eye-forming and antenna-forming portions after 24 hours of cultivation. Folded area of eye disc extended and flattened out, but no distinct differentiation of the ommatidia was observed.

When cephalic complexes involving the brain hemispheres, ventral ganglion, and ring gland of third-instar larvae were put into the medium in which eye-antennal discs were cultured, a marked differentiation of

ommatidia was observed. Several cells which would develop into an ommatidium formed after 24 hours of cultivation single cell clusters which were clearly separated from each other. The eye-forming portion was distinguished from other portion of the eye disc.

Recently four steroids having ecdysone activity have been isolated from a plant, *Podocarpus nakaii* Hay by Nakanishi *et al.* (1966) and named ponasterone A, B, C, and D. Takemoto *et al.* (1967) have also isolated two kinds of steroids, inokosterone and iso-inokosterone (ecdysterone) having ecdysone activity from the roots of *Achyranthes fauriei.* The chemical structures of ecdysone and other steroids having ecdysone activity are shown in Fig. 1.



	R_1	R_2	R_3
Ecdysone	H	OH	CH_3
Ecdysterone	OH	OH	CH_3
Ponasterone A	OH	H	CH_3
Inokosterone	OH	H	CH_2OH

Fig. 1. Chemical structures of ecdysone and related compounds

When these ecdysone analogues of plant origin were added to the medium in which eye-antennal discs were cultured, a pronounced differentiation of ommatidia was observed. The formation of distinguishable cell clusters was found like in the cultures of eye-antennal discs cultured together with cephalic complexes.

It was also found that dodecyl methyl ether, a syethetic substance having juvenile hormone activity, accelerated slightly the outgrowth of eye-antennal discs which were explanted in TD-7 flasks. Phytol, which is a terpene having juvenile hormone activity, was tested for its activity in accelerating the outgrowth of eye-antennal discs in culture. Slight acceleration was found when phytol was added to the medium. Glycerol, cholesterol and Raben-type hormone had no effect on eye-antennal discs in culture.

The artificial controls of differentiation and proliferation of imaginal disc cells by adding ecdysone and juvenile hormone, under *in vitro* conditions will be studied.

Distribution of Auxin Protectors and Their Kinetic Study in Japanese Morning Glory*

Yoshiaki YONEDA and Tom STONIER**

The existence of substances which inhibit the enzymatic destruction of auxin in shoots of a normal strain of Japanese morning glory (*Pharbitis nil*) was confirmed, as was the fact that these substances are distributed in a gradient diminishing from apex to base in a manner indicating their regulatory role in internode elongation and tissue maturation.

In addition to the two auxin protector substances reported previously (protector-I (Pr-I), molecular weight 5,000 to 10,000 and protector-II (Pr-II), molecular weight 1,500 to 5,000) which appeared to account for most of the inhibition of enzymatic auxin destruction in young elongating stem tissue, a third substance, designated as protector-A (Pr-A), has been found to be highly active in seeds, and stem tips of mature plants. In germinating seeds, no activity of Pr-I or II was observed; in stem tips, no activity of Pr-II and only a slight one of Pr-I was observed. In contrast, old tissue contained no detectable amounts of Pr-A, but did contain Pr-I and Pr-II. Between these extremes along the shoot axis, mixtures of the three substances were found. This result indicates that Pr-A is degraded into Pr-I and II and perhaps translocated in this form. Gel filtration studies indicate that Pr-A has a molecular weight exceeding 200,000 gm/mol.

In vitro studies on the mechanisms of auxin protection by Pr-I indicate that 1) the Pr-I -induced characteristic lag preceding auxin destruction by peroxidase is completely eliminated by a strong oxidant such as H_2O_2; 2) strong organic reductants such as glutathione mimick the Pr-I-induced lag and 3) the length of the lag varies inversely with the reaction rate. These results are best explained on the basis that Pr-I acts as a strong reductant to protect IAA from the enzymatic oxidation.

Distribution of IAA Oxidase and Peroxidase in Japanese Morning Glory Stem

Yoshiaki YONEDA

Distribution of auxin protector substances in the shoot of the Japanese

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morning glory suggests their important implication in the elongation and maturation processes of internodes. A kinetic study of auxin protection revealed that the protector-induced lag was reduced by anything that speeds up the auxin oxidation reaction. One of such factors is the quantity of enzyme. So far, the efficiency of protectors was determined by the volume of extract required to get a lag of certain duration preceding IAA destruction in the presence of a constant quantity of horseradish peroxidase, without consideration of the endogeneous enzyme in the extract.

From this view point, the quantitative aspects of endogeneous enzymes were examined in detail. Stem internodes of young, vegetatively growing plants of a normal strain, TKS, were homogenized and the resultant water extracts were assayed for enzyme activity. IAA oxidase activity was determined by the increase of optical density at 247 mμ in the reaction mixture consisting of IAA (substrate), dichlorophenol and $MnCl_2$ (co-factors) plus 0.04 mM H_2O_2, which was used to inactivate the protectors. Peroxidase activity was measured photometrically by guaiacol test.

The results indicated that distribution of both peroxidase and IAA oxidase in stem internodes from base to stem tip was rather uniform: in other words, the overall variations of these enzyme activities among internodes were rather small, though the young terminal internode had the highest activities of both enzymes. Both enzymes varied parallel with each other. When H_2O_2 was omitted from the reaction mixture of IAA oxidase, a protector-induced lag was observed, which had a gradient of high activity in the young elongating internodes and low activity in the non-elongating ones, as already reported.

III. BIOCHEMICAL GENETICS

Genetic Effects of DNA in *Ephestia*

Saburo NAWA and Masa-Aki YAMADA

It was already reported that when larvae or eggs of *a*/*a* (red-eyed) *Ephestia* were treated with DNA prepared from wild-type adults (black-eyed), black-eyed mutants were found in the treated generation and in subsequent generations obtained by backcrosses to untreated *a*/*a* animals, although the breeding behaviors of some of the mutants were unusual (this Annual Report, No. 17). All of the black-eyed mutants, which were obtained in the experiments either by injection of larvae or treatment of eggs and appeared either in the treated or subsequent generations, were back-crossed to *a*/*a* successively until segregation into black- and red-eyed occurred. After the segregation had occurred, the black-eyed animals produced always black-eyed and red-eyed progeny in the proportion of 1 : 1 in backcrosses and 3 : 1 in sister-brother matings in all cases. Furthermore, in successive sister-brother matings, black-eyed animals were bred to each other and have been kept as mutant lines ever since. In these lines, no contribution of the a^+ allele had taken place other than that made by a^+-DNA. To date no red-eyed individual has appeared for more than ten generations. These results indicate that whatever the nature of the effect of DNA was in the first generations it behaves ultimately as a chromosomal gene. There have been many cases of non-specific mutations induced by DNA in *Drosophila* after treatment by feeding or injection. The induced mutations were in some cases restricted to particular segments of the chromosome and usually consisted of deletions. If DNA were so mutagenic in our experiments as to cause an exchange of nucleotides or a small deletion, such mutations at most positions on the a^+-locus may have resulted in the appearance of *a*-phenotype when the a^+-gene was affected by DNA. If this were the case, frequency of the change of wild to *a*-phenotype should have been much higher than that of *a* to wild-phenotype. The experiments where a^+/a or a^+/a^+ eggs were treated with *a*-DNA or a^+-DNA, gave no such indication (cf. Nawa and Yamada (1968), Genetics *58*).

In control experiments, larvae or eggs of *a*/*a* were treated either with DNA prepared from *a*/*a* adults or with sonically disrupted wild type DNA. No black-eyed mutant was obtained. Production of black-eyed mutants in overall experiments to date is given in Table 1. The appearance of mutants behaving like a^+/a^+ homozygotes in the progeny derived from crosses of treated but phenotypically unchanged animals with

Table 1. Production of black-eyed mutants by treatment of *a*/*a* larvae or eggs with DNA

DNA	Treated generation		B_1	
	No. of adults examined	Black-eyed mutants	No. of adults examined	Black-eyed mutants
wild	7,913	5	111,678	18
a/*a*	2,572	0	25,172	0
sonicated wild	4,607	0	43,164	0
heated wild	1,637	0	29,284	1*
saline	600	0	16,823	0

* produced only red-eyed progeny in the cross to *a*/*a*.

untreated *a*/*a* was reported in the previous paper. Additional experiments have confirmed this to be a not rare phenomenon.

Evidence of Uptake of DNA by *Ephestia* Eggs

Masa-Aki YAMADA and Saburo NAWA

It has been undoubtedly demonstrated that hereditary changes could be induced in *Ephestia* either by injection of DNA into larvae or by immersion of eggs in DNA solution (see the preceding paper). In connection with this result, the purpose of the study presented here was to determine whether donor DNA penetrates the recipient cells without degradation. The ^{3}H-labelled DNA (1.5×10^6 cpm/μg) was prepared from a thymine-less *Salmonella* mutant cultivated on a medium with added ^{3}H-thymidine. Eggs (0.1—0.2 g) ranging from 0 to 16 hours after spontaneous egg-laying were dechorionated by immersing in a solution of sodium hypochlorite. After washing with saline, the eggs were immersed in the ^{3}H-labelled DNA solution for 2 hours and then washed with saline. In order to remove DNA adhering to the surface of eggs, they were treated with DNase and washed thoroughly with saline. DNA was extracted from the treated eggs by SDS-phenol method. The DNA was submitted to fractionation by centrifuge chromatography on columns of DEAE-cellulose paper pulp, which could give us estimations about the degree of polymerization of the foreign DNA incorporated into eggs. An elution property of the extracted radioactivity (bound with DNA) is given in Table 1. Most of the radioactivity was found in fractions of high molecular weight, indicating that the foreign DNA had been incorporated into the eggs without significant depolymerization.

Table 1. Fractionation of the DNA extracted from eggs treated with ^{3}H-DNA of *Salmonella* on centrifuge DEAE-cellulose column

Fraction No.	(Average molecular weight)	*Salmonella* DNA used in treatment		DNA extracted from eggs treated with ^{3}H-DNA		
		Radioactivity (cpm)	%	Radioactivity (cpm)	(O.D)	%
1.	(5×10^{2})	0	0	2	(0.13)	2
2.	(2×10^{3})	0	0	2	(0.28)	2
3.	(5×10^{4})	16	1	32	(1.83)	32
4.	(5×10^{5})	195	12.5	54	(0.34)	54
5.	(1×10^{6})	1220	78.7	6	(0.85)	6
6.	(5×10^{6})	119	7.7	5	(0.33)	5

Ribonucleic Acid Content in the Hypodermis of the Silkworm

Kunihiro KOJIMA, Mitsuo TTUJITA and SUSUMU SAKURAI

Using larvae from the 4th moulting stage to the 5th day of 5th instar of normal strains, i.e. C-124, Daizo and w-c, as materials, nucleic acid was extracted by phenol method from hypodermis and pteridine granules and analysed by methylated albumin column chromatography. The experimental results are as follows.

1. The amount of s-RNA and r-RNA per 1g of fresh hypodermis was measured throughout the period from the 4th moulting stage to the 5th day of 5th instar. A large amount of s-RNA an dr-RNA was detected at the 4th moulting stage.

2. The amount of those two kinds of RNA, s-RNA and r-RNA, was markedly decreased on the 1st day of 5th instar as compared with the 4th moulting stage, but it increased gradually again and attained the maximum on the 3rd day of 5th instar, thereupon decreasing from its 4th to 5th day.

3. The largest amount of s-RNA and r-RNA per 1g of fresh hypodermis was found on the 3rd day of 5th instar, suggesting the highest synthetic protein activity at this stage.

4. Nucleic acid fractions extracted from the hypodermis on the 1st and 3rd day of 5th instar by phenol method consisted of s-RNA, r-RNA and DNA. Nucleic acid fractions extracted from pteridine granules on the 3rd day of 5th instar also consisted of s-RNA, r-RNA, and DNA-like substance. Thus it become clear that numerous minute particles surrounding the pteridine granules were ribosomal particles.

5. Ribosomal RNA was not detected in the chromatographic profiles of the nucleic acid of pteridine granules isolated from larval hypodermis on the 5th day of 5th instar. It seems that this lack of r-RNA is due to disintegration of ribosomal particles surrounding the pteridine granules and to their detachment from the granules at this stage.

Incorporation of ^{14}C-amino Acid into Pteridine Granules

Susumu SAKURAI and Mitsuo TSUJITA

In oder to define pteridine granule function in hypodermal cells, ^{14}C-alanine, ^{14}C-serine and ^{14}C-tyrosine were injected into the the body fluid of silkworm larvae on the 3rd day of 5th instar. Afer 2 hours, fresh cytoplasm of hypodermal cells was suspended in 0.25 M sucrose solution and centrifuged at 900 g, 5,000 g, 12,000 g, 35,000 g and 78,000 g, and radioactivity of resultant particles was counted.

^{14}C was rapidly incorporated into pteridine granules as it could be expected from our previous experiments with incorporation of ^{14}C-glycine. The specific radioactivity of premature granules sedimented at 78,000 g was higher than that of mature granules.

Pteridine granules were dissolved in 2 per cent solution of sodium deoxycholate. The solution was eluted through a Sephadex G50 column and separated into polypeptide and membrane protein fractions, and then the radioactivity of each fraction was determined.

^{14}C was incorporated into polypeptides and membrane protein of both mature and pre-mature granules. However, polypeptides and membrane protein from premature granules showed higher incorporation activity of ^{14}C amino acid than the mature granules.

Morphology of pteridine granules during the developmental process was already reported. The electron micrographs of mature granules showed that the maturation process caused the detachment of ribosomes from their membrane and transformation from rough surface to smooth surface type.

It may be suggested from those experimental results that ribosomes attached to the granule membrane participate in the synthesis of polypeptides which are secreted and stored within the granular vesicles.

Significance of the Pteridine Granule Development in Larval Skin Cells of the Silkworm

Mitsuo TSUJITA, Kunihiro KOJIMA and Susumu SAKURAI

Pteridine granules produced in hypodermal cells of normal silkworm

larvae consist each of a granule membrane and content, i. e. polypeptide-pteridine pigment and polypeptide-uric acid complexes, with which the granules are filled.

In order to make clear the mechanism by which pteridine metabolic products are accumulated into pteridine granules, the activity of enzymes concerned with pteridine metabolism such as pterine dehydrogenase or pteridine reductase in adipose tissue and hypodermis of the larvae was examined.

Normal (C-124) and E-lem(*E*/*E*; *lem*/*lem*) larvae in 5th instar were used as materials. As we were afraid that the larval hypodermis used in previous experiments (Tsujita 1963) had contained near the skin adipose tissue, in the present experiments the larval skin was as thoroughly as possible freed from adipose tissue which was used for the preparation of crude enzyme solution.

The experimental results indicate that the activity of those enzymes was very strong in adipose tissue but weak or lacking in the hypodermis of normal larvae. In lemon larvae weak activity of pteridine reductase was found in adipose tissue but it was scarcely detected in hypodermis. Nevertheless, products of pteridine metabolism such as isoxanthopterin and sepiapterin were abundantly accumulated in hypodermal cells but could hardly be detected in adipose tissue.

It was described in our previous paper that the developmental process of pteridine granules follows the sequential phases of endoplasmic reticulum, and that their complete development into mature granules is due to normal development of granule membrane to which the ribosomes are attached. In addition, our experimental results concerning incorporation of labeled amino acid into pteridine granules suggest that polypeptides are synthesized by the ribosomes attached to the granule membrane.

According to the general belief, it seems that the larval skin cells of the silkworm have the ability to absorb uric acid from the body fluid and to store it within the cells.

On the basis of our experimental results the following hypothesis is presented for the formation of pteridine granules.

The hypodermis of the silkworm has the ability to absorb uric acid as well as pteridine compounds through the body fluid from other organs in which those compounds are produced, and, combining with polypeptides produced around the granules, are secreted into granule vesicles where they are stored. In the hypodermal cells of normal larvae the granule vesicles have complete or sufficient ability to secrete or store those products and thus abundant pteridine granules of high density are yielded. Normal larvae are opaque owing to the presence of those

granules in hypodermal cells. However, in the hypodermal cells of mutant larvae homozygous for w^{ox}, *w-a*, or w^{ol}, the granular development is defective and no mature pteridine granules or a small amount of minute granules is produced, resulting in their highly transparent skin.

Thus, it is concluded that although endoplasmic reticulum can be in general observed in various tissue cells of the silkworm the coarse surface endoplasmic reticulum found in larval skin cells shows a special differentiation according to its cellular function, namely production of pteridine granules.

Reconstitution of Vesicular Membrane of Pteridine Granules from Degraded Subunits

Mitsuo TSUJITA and Susumu SAKURAI

Purified mature pteridine granules dissolved in 0.2 mol phosphate buffer (pH 8.0) were heated at 95°C for 3 minutes. Non-degraded granules were sedimented by refrigerated centrifugation at 20,000 g for 60 minutes,

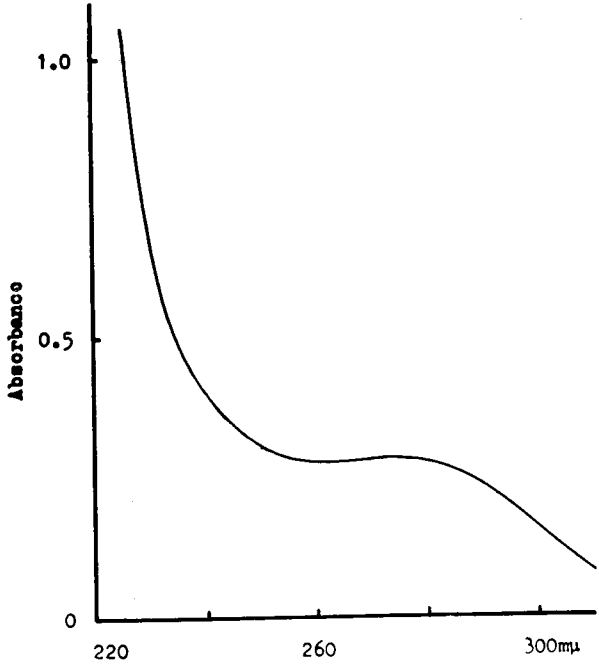

Fig. 1. Ultraviolet absorption spectrum of membrane protein from pteridine granules.

and the supernatant was overnight dialysed against distiled water. For the reconstitution of granule membranes from degraded subunits, 1 volume of dissolved membrane preparation was mixed with 1 volume of 0.156 mol sodium chloride solution containing 0.01 mol magnesium chloride. Fibrous substances could be by electron microscope observed in the sediment obtained from the mixture.

At first, pH of the mixture was about 8, it was converted to pH 5 by adding acetic acid and the preparation was kept at 5°C for 12 hours, the white sediments produced were separated by centrifugation at 20,000 g for 60 minutes and studied by electron microscope. It was found that a number of granule vesicles surrounded by thin membranes were reconstituted from self-assembling membrane subunits. Although those reconstituted vesicular membranes were of smooth surface type without attached ribosomal particles, rarely rough surface type vesicular membrane with a small number of attached ribosome-like particles were found. Thin sections of reconstituted membrane material showed the same pattern as the original membranes of pteridine granules. For the reconstitution of membrane materials, the presence of divalent cation ($MgCl_2$)

Table 1. Amino acid composition of membrane protein from pteridine granules

Amino acids	Micro moles
Aspartic acid	1.36
Threonine	0.97
Serine	0.82
Proline	0.18
Glutamic acid	1.92
Glycine	1.13
Alanine	1.29
Valine	0.91
Cystine	0.06
Methionine	0.27
Isoleucine	0.68
Leucine	1.10
Tyrosine	0.36
Phenylalanine	0.43
Ammonia	0.97
Lysine	0.70
Histidine	0.14
Arginine	0.79

gave the best results, but $CaCl_2$ was as effective.

The granule membrane subunits from which the lipid had been extracted by treatment with chloroform and methanol no longer showed a membranous structure; reintroduction of lipid into the extracted subunits restored the property of vesicular formation.

Absorption spectrum of the membrane protein solution resembles that of a mixture of tryptophan, tyrosine. cysteine and phenylalanine (Fig. 1). The membrane protein purified from pteridine granules separated from the larval hypodermis of a normal strain (YD_4) was hydrolysed by 6N HCl at 110°C for 20 hours and the amino acid constitution was analysed using Hitachi amino acid analyser KLA 3. The experimental results are given in Table 1.

Formation of Vesicle Membranes of Pteridine Granules from Repeated Particles

Mitsuo TSUJITA and Susumu SAKURAI

Using pteridine granules separated from the hypodermis of larvae of a normal silkworm strain (C-124) the following electron-microscopic study was carried out.

After heating a suspension of granules in phosphate buffer (pH 8.0) at 95°C for 3 minutes the degrading granules were centrifuged and the sediments were examined by electron microscope. Fibrous substances (called "strings") about 40 Å in thickness, sometimes bundles of such substances which protruded from broken parts of granule membranes were observed. In positively stained granule sections, fibrous substances showing linear arrangement of 30-40Å thick particles could be observed in the granule membranes.

The fibrous structure was confirmed in the reconstitution experiments of vesicular membranes; fibrous substances about 40 Å thick, 0.5-1.0 μ long, running closely and almost parallel to each other in one layer were observed in newly formed membranes. Furthermore, in each of the fibres linearly arranged particles, 30-50Å in diameter, were observed.

It seems from above-mentioned observations that the membrane of pteridine granules is made up of strings running almost parallel in one layer, each string being composed of particles arranged linearly.

As described in our previous paper, various abnormally shaped and defective granules are produced in the hypodermis of larvae homozygous for *w*-3 multi-allelic genes, i. e. *w*-3, *w-a*, *w-b*, w^{oz} or *ol*. Especially, in the hypodermis of larvae homozygous for *ol* granules having abnormally thick membranes in which sparse short fibrous substances, 0.1-0.2 μ long,

were entangled were observed. Small amounts of secretion products were stored within the granules and from some of them all contents were lost leaving behind abnormal membranes having sparse fibrous structure. In addition, aggregates of short fibrous substances, 0.1-0.2 μ in length, composed of linearly arranged particles were scattered here and there around the granules. They seemed to be masses of defective fibrous substances which failed to form vesicle membranes.

It was reported that protein and lipid are the principal components of pteridine granule membrane (Tsujita and Sakurai 1966) and that their membrane protein consists of a homogeneous single protein (unit membrane protein) (Sakurai and Tsujita 1967).

Green and Perdue (1966) define the membranes as vesicular or tubular systems, the continuum being made up of lipoprotein-repeating units (one layer thick) which are the only structural elements. As it is considered that their repeating units correspond to particles linearly arranged in strings in the pteridine granule membrane, the following diagrammatic representation of their vesicular membrane construction is shown in Fig. 1.

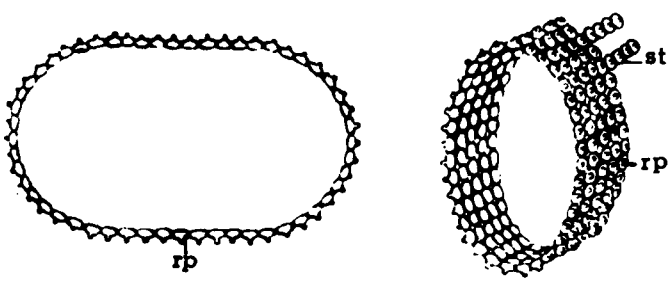

Fig. 1. Diagrammatic representation of the membrane of pteridine granules as a fused continuum of repeating particles. A particle consists of a base, a stalk and a detachable head (omitted in the figure). rp: repeating particle. st: string.

Vesicular membrane formation from subunits confirmed by our reconstitution experiments may be regarded as a case of vesicularization by repetition of units, and strings may be an intermediate form.

Generally speaking, organelles have two or more component membranes which have entirely different repeating units and functional attributes and are rather systems of membranes than individual membranes. However, it seems from our observations that the membrane of pteridine granules produced in larval hypodermis of the normal silkworm is a monolayer membrane. Rarely, partly double membranes which are per-

haps overlapping parts of the monolayer could be seen. It is highly probable that the membrane of pteridine granules is an example of a single membrane structure.

A Staining Procedure of Human Serum Lipoproteins on Cellulose Acetate Strips

Yoshito OGAWA

The purpose of this report is to present a rapid and simpler high-resolution technique for the fractionation of human serum lipoproteins on cellulose acetate strips. It is essentially the same as the Straus and Wurm's method reported in 1958 for paper electrophoresis[1]. But the analytical result of the present technique has a remarkable advantage over the original one due to the superior character of cellulose acetate as the supporting medium of electrophoresis (Fig. 1).

Standard barbital buffer of pH 8.6 and ionic strength from 0.05 to 0.07 was used and a constant current of 0.6 mA was passed per one cm. width through the strips at room temperature. Serum should be applied in volum of approximetely 1.2 μl per one cm. width of a strip. A staining

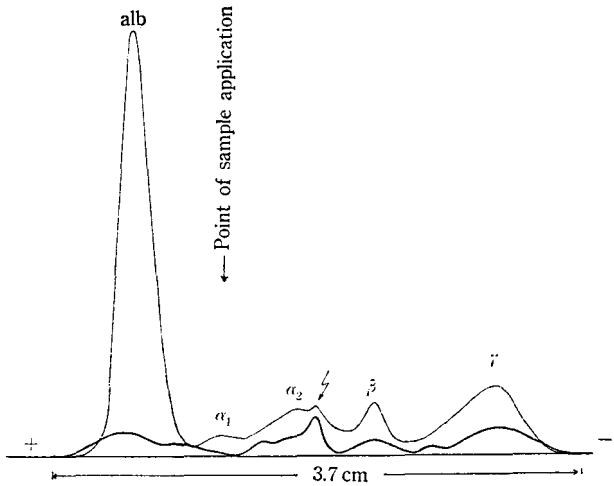

Fig. 1. Curves obtained by scanning a Fat red 7B (thick line) and a Ponceau 3R (fine line) stained Separax (1×6 cm.) cleared with paraffin oil by using 0.5×8.0 mm slit adapter and 500 mμ wavelength. Arrow: α_2-lipoprotein fraction.

1) Straus, R. and Wurm, M.: Am. J. Clin. Path., 29: 581 (1958).

solution was prepared by addition of 0.1 gm of Fat red 7B (Chroma 10725) and 7.5 gm of trichloroacetic acid to 175 ml of methanol, which was brought to a boil under a condenser so that less of the solvent was minimized. After that 75 ml of hot distilled water of about 60°C was added and the mixture was kept at 40°C.

Immediately after the removal from the electrophoresis chamber, the strips were immersed in the dye solution for a period of two hours at 40°C and then transferred into a decolorizing bath, consisting of a one per cent solution of 5 per cent commercial sodium hydrochloride in two per cent acetic acid, for 4 or 6 minutes at room temperature until the separated patterns had clearly developed. After rinsing in tap water, the strips were dried between paper toweling.

The above staining procedure showed satisfactory results on Membranfolin (Germany) and Serometrics (U. S. A.) as well as on Separax (Japanese), but was not practicable on Millipore (U. S. A.), Sepraphore III (U. S. A.), Oxoid (England), Sartorius (Germany) and Cellogel (Italy). The relation between the analytical results of lipoprotein fractions stained by Fat red 7B and serum protein fractions stained by Ponceau 3R obtained under the same experimental condition on Separax is shown in Fig. 1 and the normal values of serum lipoprotein fraction in the Japanese population age ranging from 30 to 40 years are given in Table 1.

Toble 1. Normal values of Japanese serum lipoproteins analysed on Separax (Ogawa & Sakamoto 1968)

Blood donors	Albumin	Globulin			
		α_1	α_2	β	γ
Male					
10 persons	17.48±3.18	9.98±1.38	27.52±6.22	15.79±2.06	29.23±3.50
(% range)	(10.64–25.00)	(6.67–12.77)	(10.00–40.43)	(11.11–20.00)	(20.59–38.00)
Female					
10 persons	16.90±4.26	9.33±2.96	25.63±3.58	18.41±3.24	29.73±5.36
(% range)	(5.56–25.52)	(2.64–16.67)	(19.95–30.80)	(10.53–24.00)	(18.00–41.66)
Technical error*	21.47±3.64	7.57±1.42	25.17±4.37	13.00±2.27	32.79±3.20

* Calculated from the results of five time analysis of the same sample

Zymographic Analyses of Peroxidase-Indoleacetate Oxidase Isozymes in Several Plants

Toru ENDO

Many authors have considered that both peroxidase and indoleacetate

oxidase activities are due to the same enzyme species. This was, in this study, zymographically proved by a new staining method developed for indoleacetate oxidase activity. The experimental results indicate that some, but not all, peroxidase isozymes have an indoleacetate oxidase activity in several plants on starch gel zymograms.

Zymographic examinations were carried out of a commercial peroxidase preparation of horseradish as well as extracts from several plant materials regarding dual catalytic preperties of single isozymes. A reacting mixture for the oxidase stain contained 1 to 5 mM indoleacetate, 0.5 mM trichlorophenol, 2 mg per ml of Fast Blue BB salt and 0.2 μ acetate buffer, pH 5.0, at final concentrations. An unidentified intermediate resulting from the enzymatic indoleacetate degradation was shown to be coupled with diazonium salt and to form an insoluble complex. The starch gel was sliced transversely; the top half was subjected to oxidase stain and the bottom half to peroxidase stain. At least four cathodal and four anodal peroxidase bands were detected in the horseradish preparation, the former appearing as dark oxidase bands and the latter as very faint bands.

In morning glory root, several anodal peroxidase bands were observed as dark oxidase bands, which appeared rapidly during the staining treatment, but one sharp cathodal peroxidase band was faintly stained as an oxidase band. A similar result was also obtained with sweet potato root. One anodal band was located as very weak peroxidase band but quickly stained as a strong oxidase band, while one faint cathodal peroxidase band did not appear as an oxidase band. In both turnip and radish, a heavy anodal peroxidase band was shown as an appreciably stained oxidase band.

The indoleacetate oxidase zymograms were obtained in the absence of manganese ion. It was noted, however, that many of rice and maize peroxidase isozymes required around 10 mM manganese ion at a final concentration of the reacting mixture for oxidase stain. The results clearly show that the requirement for the metal ion is considerably different in the oxidases of plant materials. Also, intensity of peroxidase bands was not always correlated with that of the corresponding oxidase bands on zymograms.

Peroxidase and indoleacetate Oxidase Isozymes in Japanese Morning Glory

Yoshiaki YONEDA and Toru ENDO

The evidences that indoleacetate oxidase plays a significant role in the developmental processes in plants, directly or indirectly through an auxin

protector system, are being accumulated (Yoneda & Stonier, 1967). Our interest has been focused on the relation of auxin destroying enzyme and auxin protector system to the elongation of stem internodes in morning glory. In this study, we have started to analyze such enzymes that could control tissue differentiation.

We have developed a staining method for the detection of indoleacetate oxidase isozymes separated electrophoretically on starch gel zymograms (Endo, in press). By this method, the oxidase isozymes were compared for identification with peroxidase isozymes. The crude extracts from various organs of a normal strain, TKS, were zymographically analysed, using borate buffer, pH 8.5, system.

In root, at least 5 isozyme bands of peroxidase moving towards the anode (they were located as 1A to 5A numbering from the start line) were identified. TKS strain is divided into two substrains concerning a cathodal sharp band, 1C; it appeared in T016 but not in T036 substrain. This band was distributed in most of the organs irrespective of their age. In hypocotyl, 3A and 4A bands were usually found. Very young hypocotyls had 3A and $2A_1$, $2A_2$ bands (numbered by their location between 2A and 3A). Band 5A appeared in older hypocotyls. The isozyme patterns of stem are similar to those of hypocotyl, and the terminal, young elongating internodes had $2A_1$ and $2A_2$ bands. Whereas the basal, old internodes had 5A band in addition to 3A and 4A. Cotyledons and leaf blades had only 3A band, but in very old stages, 5A, 1A and 2A bands appeared.

Weak bands, $2A_1$ and $2A_2$, appeared both in very young hypocotyl and stem internodes. In contrast, 5A band was always detected when the tissues became older. Such an age-specific change of peroxidase and indoleacetate oxidase patterns suggests a special role of some isozymes in tissue differentiation, in addition to organ-specific distribution of isozymes.

In relation to the auxin protector system, effects of hydrogen peroxide were zymographically examined. Peroxidase bands, 3A to 5A, took intense indoleacetate oxidase stain, whereas peroxidase bands, 1A and 2A, appeared as very weak or almost negative oxidase bands. These oxidase bands, however, became strongly stained ones in the presence of a very small amount of hydrogen peroxide. This indicates that hydrogen peroxide inactivates the natural auxin protectors which migrate to the regions of 1A and 2A bands on zymogram.

Changes of Peroxidase Isozyme Patterns by *in vitro* Culture of Japanese Morning Glory Tissues

Yoshiaki YONEDA

In order to investigate the relation of peroxidase isozymes to morphological tisssue differentiation, the tissues from three different organs (root, hypocotyl and cotyledon) in a normal strain, T036, were cultured *in vitro* on four different media (Linsmaier & Skoog's basal medium, and this basal medium plus 10 ppm indoleacetic acid (IAA) or 5 ppm kinetin (KT) singly or in combination). After one month culture in the dark, each tissue or callus was frozen, macerated and the exudate, absorbed in a filter paper, was run on starch gel electrophoretically to obtain zymograms of peroxidase, which was detected by staining with benzidine-H_2O_2.

Tremendous callus growth was observed on KT and IAA-KT media in all three kinds of tissue, and IAA promoted a small callus growth in hypocotyl and root tissue, whereas no signs of callus formation were seen in cotyledonary tissue. IAA stimulated root formation, on the contary, suppressed by kinetin. Callus growth on basal medium was almost nil and only a few roots elongated strongly.

As described elswhere, each tissue used in this study had a charcacteristic pattern of peroxidase isozymes *in vivo*; namely, cotyledon had only 3A band, hypocotyl had 3A and 4A, and root had 5 bands (1A to 5A).

The most fundamental change of the isozyme pattern observed was the appearance of 1A and 2A bands and a very intense 5A band in all tissues regardless of the medium, though 1A and 2A, induced on KT or IAA-KT media were generally much more intense than on basal and IAA media. Another interesting change was that while the tissues on basal medium, producing no callus, retained their original bands of 3A and 4A, they generally became weak or faded away along with callus growth. The third change observed was that in connection with callus growth, one or two new bands appeared between 2A and 3A, which were named $2A_1$ and $2A_2$ by their location.

Briefly, the disappearance or reduction of tissue specific isozymes and appearance of new bands seem to correlate with callus growth or loss of specific morphological structure, in addition to the basic change due to *in vitro* culture. Thus, the patterns of peroxidase isozymes in calli (on KT and IAA-KT media) became almost the same, whatever organ they derived from. These results suggest a close correlation of peroxidase isozymes with tissue differentiation, caused by hormone treatment.

Effect of Exogenous RNA on Protein Synthesis in L-M Cell Culture[1]

Kazuo MORIWAKI

In this study an attempt was made to transfer the ability of specific protein synthesis from a mouse plasma cell tumor, X-5563[2], which can produce a specific gamma glubulin, to L-m cell culture[3], using X-5563 tumor RNA.

L-M cells were cultured in 199 peptone medium and treated with 0.01 μg/ml actinomycin D for 12 hours, prior to incubation for 28 hours with 5 μc/ml C^{14}-valine and 0.8 mg/ml RNA extracted from X-5563 tumer or C3H mouse liver by hot SDS-phenol method and used with 30 μg/ml

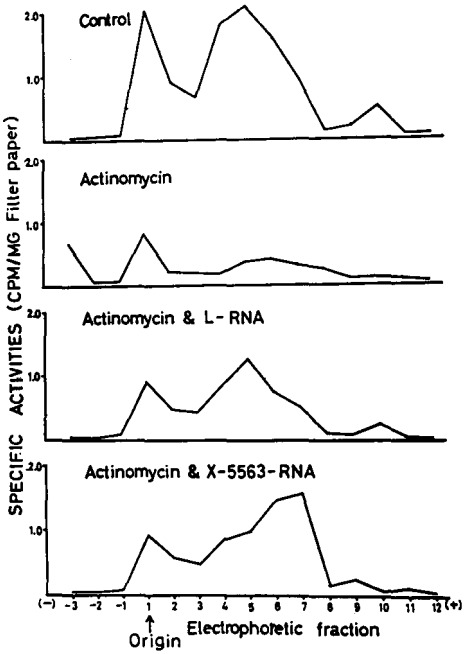

Fig. 1. Induction of new protein synthesis following treatment with exogenous RNA in L-M cell culture.

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1) This work was conducted in Mammalian Genetics Center, Department of Zoology, The University of Michigan, Ann Arbor, Michigan, U. S. A. and was supported by a PHS Grant 5 TI GM 71-08.

2) Kindly furnished by Dr. M. Potter, National Cancer Institute, U. S. A.

3) Generously supplied by Dr. D. J. Merchant, Microbiology Department, The University of Michigan Medical School.

protamine sulfate. After RNA treatment, the soluble fraction of the cultured cells was extracted by homogenization and centrifugation. This fraction was mixed with the serum of X-5563 bearing mouse serving as a carrier and was submitted to paper electrophoresis. Amino black 10 B staining of the paper was followed by cutting off each protein fraction of the paper and weighing and counting the radioactivity.

As shown in Fig. 1, the depressed level of protein synthesis in L-M cells by actinomycin D could be restored to control level with either liver RNA or X-5563 RNA treatment. Especially, the X-5563 RNA was capable of inducing new fractions of protein synthesis different from the endogenous spectrum of L-M cells on the pattern of electrophoretic paper.

In order to ascertain whether or not this newly induced proteins contain a specific globulin (myeloma protein), the radioactivity of the above mentioned soluble fraction was assayed by a serological method. As far as the ratio of myeloma protein synthesis to total soluble protein synthesis is concerned, the X-5563 RNA treatment fairly accerelated the synthesis of myeloma protein in L-M cells (Table 1), though the mechanism of the function of the used RNA remains unknown.

Table 1. Effect of X-5563 RNA on myeloma protein synthesis in L-M cell culture

Treatments	C^{14}-val. incorporated, cpm/10^6 cells/40 hrs.		T.P./T.S.P. ratio
	M.P.	T.S.P.	
Control (199P medium)	395	14,600	0.027
Act-D (0.018 μg/ml)	—	2,540	—
Act-D & X-5563 RNA (0.8 mg/ml)	257	6,220	0.042
Act-D & Liver RNA (0.8 mg/ml)	186	6,650	0.028

M.P.: Myeloma protein, T.S.P.: Total soluble protein.

Inhibitory Effect of Homologous RNA on Protein Synthesis in Cell-Free System[1)]

Kazuo MORIWAKI

The present study deals with the inhibition of total protein synthesis in a cell-free system by the addition of RNA extracted from the same

1) This work was conducted in Mammalian Genetics Center, Department of Zoology, The University of Michigan, Ann Arbor, Michigan, U. S. A. and was supported by a PHS Grant 5 TI GM 71-08.

Table 1. Components of the cell-free protein synthesis system

This buffer, pH 7.0	80 mM	PEP-kinase	15 μg/ml
NH_4-acetate	50 〃	Valine-H^3	5 μc
Mg-acetate	8 〃	S-RNA (Bovine liver)	100 μg
KCl	50 〃	CTP	0.03 mM
Mercaptoethanol	9 〃	UTP	0.03 〃
17 amino acids (-Val.)	0.075 〃 ea.	GTP	0.2 〃
K-ATP	2.5 〃	Liver, Ehrlich tumor or X5563 S-30*	0.4 ml (10 mg Protein Eq.)
K-PET	2.5 〃		
		Total volume	2.0 ml

* S-30: A supernatant fraction obtained from 0.25 M sucrose homogenate of each tissue by a centrifugation at 30,000 rpm for 30 min.

Table 2. Inhibitory effect of homologous RNA in cell-free protein synthesis systems

	H^3-Val. incorporation (cpm)/19 mg protein eq. S-30 Cell-free systems				
	Liver	Ehrlich tumor		X-5563 tumor	
Experimental No.	1	1	2	1	2
NO	4,750	750	688	2,266	283
Liver RNA (2 mg)	1,310	—	1,825	4,365	763
Ehrlich tumor RNA (2mg)	—	171	258	3,028	1,098
X-5563 tumor RNA (2mg)	5,500	729	1,158	2,732	335

kind of tissue as that from which the cell-free system was prepared. Mouse liver, Ehrich ascites tumor and mouse plasma cell tumor (X-5563) were employed as the materials for RNA extraction and the preparation of a cell-free protein synthesis system. RNA extraction was performed by hot SDS-phenol method. Each component contained in the system is enumerated in Table 1. After incubation at 37°C for one hour, the reaction was stopped by adding TCA to a final concentration of 5%. The protein fraction was spun down and washed twice by 5% cold TCA with 1% valine, followed by washing with 70% ethanol, 100% ethanol and 3 : 1 ethanol-ether. The radioactivity of H^3-valine in this precipitate was markedly reduced by addition of a homologous RNA into the system (Table 2). This tendency was strongly exhibited in the combination of liver RNA with the liver cell-free system and also in the combination of

Ehrlich tumor RNA with Ehrlich tumor cell-free system, suggesting some tissue specificity in the reaction of RNA with the cell-free protein synthesis system. A hot TCA treatment of the protein precipitate seemed to abolish the inhibitory action of homologous RNA, though the precise mechanism has not been studied yet.

Myeloma Proein Synthesis in a Cell-Free System Prepared from Mouse Plasma Cell Tumor, X-5563[1)]

Kazuo MORIWAKI

Myeloma protein synthesis of this tumor in a cell-free system had been once before undertaken by Askonas (1961) who obtained a poor yield of this protein. In the present study several factors concerning the efficiency of a cell-free system were examined using the system prepared from X-5563 tumor. The components of this system were enumerated elsewhere (This Ann. Rep. p. 48).

First, 0.8 mg/ml RNA extracted from X-5563 tumor or normal liver by hot SDS-phenol method, was supplemented to the cell-free system. Although the synthesis of total soluble protein was considerably enhanced either by X-5563 RNA or normal liver RNA, both RNA's resulted in no significant increase of myeloma protein synthesis.

As it is already known that liver cell sap contains a potent inhibitor of RNase (Roth, 1958), the supernatant (S-100) fraction obtained from 0.25 M sucrose homogenate of mouse liver by centrifugation at 100,000 g for 30 minutes was used as a RNase inhibitor throughout the preparation of the cell-free system in the second experiment. No increased myeloma protein synthesis, however, was observed in the system prepared with the S-100 fraction, suggesting that RNase contained in the system is not a major cause of the poor yield of myeloma protein in the present experiment.

Thirdly, the process of cell disruption was carefully re-examined. Tissues of the tumor and mouse liver as a control were minced and ground in the cold by Potter-Elvehjem glass homogenizer. As shown in Table 1, the homogenization after ten rounds of hand operation gave a cell-free extract having a much higher activity for myeloma protein synthesis, whereas operation by motor for one minute only seemed almost to destory it. This result allows to assume that there exists a delicate

1) This work was conducted in Mammalian Genetics Center, Department of Zoology, The University of Michigan, Ann Arbor, Michigan, U. S. A. and was supported by a PHS Grant 5 TI GM 71-08.

mechanism in tumor cells concerned with specific pretein synthesis.

Table 1. Effect of homogenization procedures on myeloma protein sythesis in X-5563 cell-free system

Process of homogenization	Cell-free extract	H^3-Val. incorporated		cpm/19 mg protein equivalent S-30
		M.P	T.S.P.	M.P./T.S.P. ratio
Motor operation for 1 min.	X-5563 S-30	32	2,300	0.014
		35	2,750	0.013
		42	2,630	0.016
	Liver S-30	16	1,310	0.012
		27	1,910	0.014
Hand operation 10 rounds	X-5556 S-30	615	1,305	0.47
		698	1,316	0.53
		824	1,442	0.57
	Liver S-30	33	1,390	0.024
		32	1,200	0.027

Abbreviations: S-30; supernatant fraction obtained from 0.25 M sucrose homogenate of the tissues by centrifugation at 30,000 g for 30 minutes. M.P.; Myeloma protein. T.S.P.; Total soluble protein.

IV. EVOLUTIONARY GENETICS

Local differences in frequencies of No. 1 chromosomal polymorphism of *Rattus rattus*

Tosihide H. YOSIDA[1)]

Differences due to different locality in the frequencies of the polymorphic largest No. 1 chromosome pair, namely a telocentric homomorphic (*T*/*T*), a telocentric and subtelocentric heteromorphic (*T*/*S*) and a subtelocentric homomorphic pair (*S*/*S*), were already reported (Yosida et al. 1967, this Annual Report 17 : 61). We newly obtained the animals 3 localities in Japan, namely Tottori, Ooizumi (Gunma) and Okinoerabu (Kagoshima) and also added to our collection animals from Niigata and Seoul (Korea). The frequenceies of the three chromosomal types in No. 1 pair are shown in Table 1. As the table shows, the rats collected in Tottori were of all of *T*/*T* type, while the population collected in Ooizumi showed *T*/*S* and *S*/*S* types only. The population in Okinoerabu was similar to those in other southern parts of Japan.

A population map of chromosomal polymorphism of animals collected in Japan and Korea is shown in Fig. 1. As the figure shows, animals collected in Sapporo, Niigata, Obama and Tottori which are located in the north and north-west of Japan were of *T*/*T* type, those collected in Sugadaira, Hiratsuka, Misima, Hamamatsu, Ube, Nagasaki and Okinoerabu located in the east and south showed about 20 to 30 percent *T*/*S* animals. The population of Ooizumi (Gunma), located in the east, however, was markedly different from the other populations, in having *T*/*S* and *S*/*S* types. Frequency in the Korean population was also slightly

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

Karyotypes	Locality				
	Tottori	Ooizumi (Gunma)	Okinoerabu (Kagoshima)	Niigata	Seoul (Korea)
T/*T*	24	0	18(81.8)	43	18(46.1)
T/*S*	0	9(81.8)%	4(18.1)	0	17(43.5)
S/*S*	0	2(18.1)		0	4(10.3)
Total	24	11(99.9)	22(99.9)	43	39(99.9)

1) The author wishes to thank to Dr. Y. S. Kang for collection of animals in Korea.

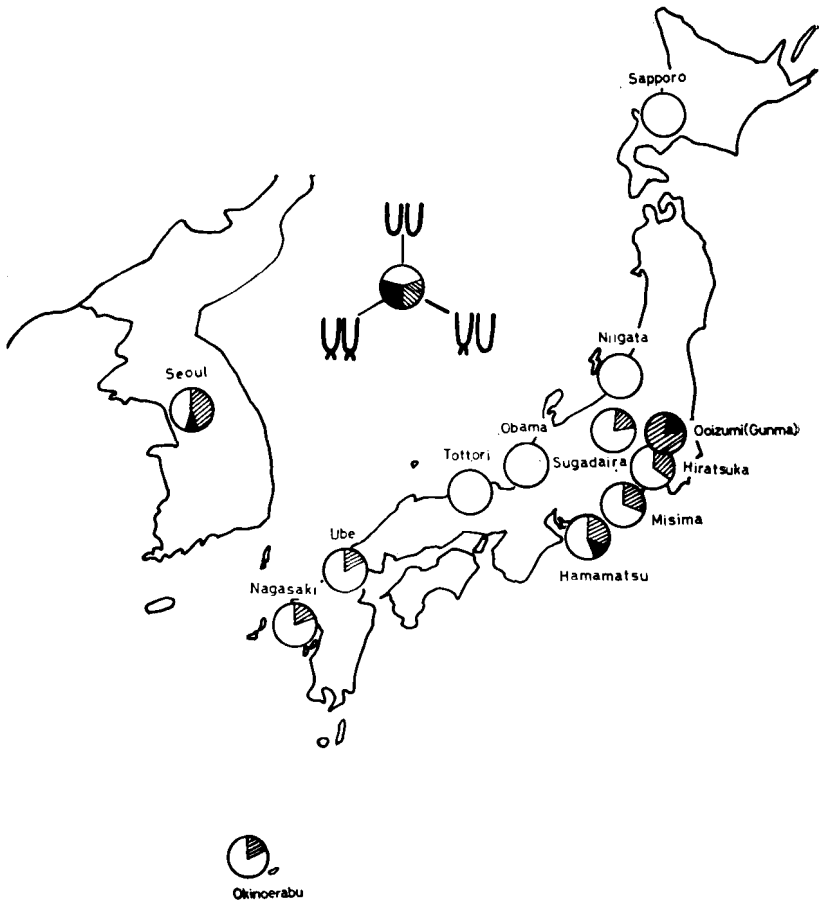


Fig. 1. Population map of the frequency of chromosomal polymorphism in *Rattus rattus* collected in Japan and Korea. Localities collected the animals were show by circle, in which white, shaded and black areas show the frequency of animals with *T*/*T*, *T*/*S* and *S*/*S* chromosome pairs, respectively.

different from those of Japan; namely, it was characterized by considerably high frequencies of *T*/*S* and *S*/*S* types. It is diffecult to conclude at present, why the chromosomal polymorphism is maintained in this species, how and where did the polymorphism develop, and what do the local differences in frequencies of the polymorphism mean for the differentiation of this species. These problems will be further studied cy-

togenetically and statistically when a larger material collected at more different localities is available.

Further studies on segregation of three chromosome types of *Rattus rattus* in the laboratory

Tosihide H. YOSIDA

Segregation of three chromosome types (T/T, T/S and S/S) of No. 1 chromosome in *Rattus rattus* bred in the laboratory was already reported (Yosida et al., 1967. this report, No. 17 : 61) and the deta on F_1 hybrids $T/T \times T/T$, $T/T \times T/S$, $T/S \times T/S$ and $T/S \times S/S$ were given. Newly we obtained F_1 hybrids $T/S \times T/T$, $TS/ \times S/S$, $S/S \times T/T$, $S/S \times S/T$ and $S/S \times S/S$, and we wish to add the new data and correct the old ones published last year. Scheme of crosses and data on segregation of the three chromosome types are shown in Table 1.

Table 1. Segregation of three chromosome types in *Rattus rattus* bred by several combination of parents and average litter size of the hybrids

Combination of parents	Segregation			Total no. of litters	Total no. of animals	Average litter size	X^{2*}
	T/T	T/S	S/S				
$T/T \times T/T$	89 (69)**	0	0	17	89	5.3	
$T/T \times T/S$	36 (39)	42 (39)	0	13	78	5.6	0.46 P>0.3
$T/S \times T/T$	28 (36.5)	45 (36.5)	0	14	73	5.2	6.5 P<0.02
$T/S \times T/S$	29 (25)	54 (50)	17 (25)	20	100	5.0	3.52 P>0.1
$T/S \times S/S$	0	30 (26.5)	23 (26.5)	9	53	5.8	0.46 P>0.92
$S/S \times T/T$	0	12 (12)	0	3	12	4.0	
$S/S \times T/S$	0	5 (5)	5 (5)	3	10	3.3	
$S/S \times S/S$	0	0	8 (9)	2	8	4.0	

* X^2 value was calculated for the theoretical segregation.

** Figures in parenthese show theoretical segregation.

As the table shows, *S/S* males and *S/S* females are fertile in the laboratory. Average litter size of hybrids $T/T \times T/S(♂)$ and $T/S(♀) \times T/T$ is similar (5.6 and 5.2). This means that fertility of *T/S* males is not different from that of *T/S* females. When *S/S* females were crossed to *T/T*, *T/S* and other *S/S* males, the litter size of the hybrids decreased to 3.3–4.0, while the litter size of the hybrids $T/S(♀) \times S/S(♂)$ was very high (5.8). From the above results it is suggested that females with *S/S* chromosomes have lower productivity than those with *T/S* and *T/T* chromosomes. In the case of $T/S \times T/S$, the ratio of segregation of the three chromosome types did not deviate significantly from the theoretical 1 : 2 : 1 ratio, although *S/S* animals are somewhat few. On the other, hand, in all crosses more animals with *T/S* heteromorphic chromosome pair were usually obtained than expected from the segregation ratio. Hybrid vigour may be involved.

Serum Transferrin Polymorphism in *Rattus rattus*

Kazuo MORIWAKI

An electrophoretic survey by starch gel of serum protein polymorphism in natural population of *Rattus rattus* in Japan has revealed three types of transferrin variants, *R*-, *N*- and *RN*-type. *R* type was characterized by two fast moving bands, *N* type by two slowly moving bands and *RN* type by four bands as a mixture of both types, though all of those bands migrated into the diffused region between slow α_1-globulin and fast α_1-globulin. Electrophoretic patterns of those three types and that of *Rattus norvegicus* serum as a reference are schematically shown in Figure 1.

The starch gel electrophoresis was carried out under the following conditions: Hydrolyzed starch powder was prepared from potato starch (Wako Chemicals) by hydrolysis in 0.12 N HCl at 37°C for 135 minutes. Starch gel for electrophoresis contained 12.7% (*W/W*) hydrolyzed starch in glycine-borate-NaOH buffer (25 mM glycine, 5 mM boric acid and 5.3 mM NaOH, pH 8.6). Borate buffer (300 mM boric acid and 50 mM NaOH, pH 8.3) was used for electrode vessels. Gel tray was laid horizontally in the refrigerator and connected with a constant current-power supply for 14 hours at 3.6 mA/cm^2. Staining of the proteins on the gel was performed by amino-black 10 B.

Ninety four rats which were collected from five localities in Japan, Sizuoka Pref. (21), Gunma Pref. (10), Niigata Pref. (20), Tottori Pref. (21) and Okinoerabu Island (22), consisted of 70.3% *R* type (66), 26.4% *RN* type (24) and 4.3% *N* type (4). No marked difference in the frequencies of these three types was observed among those localities. When a Hardy-

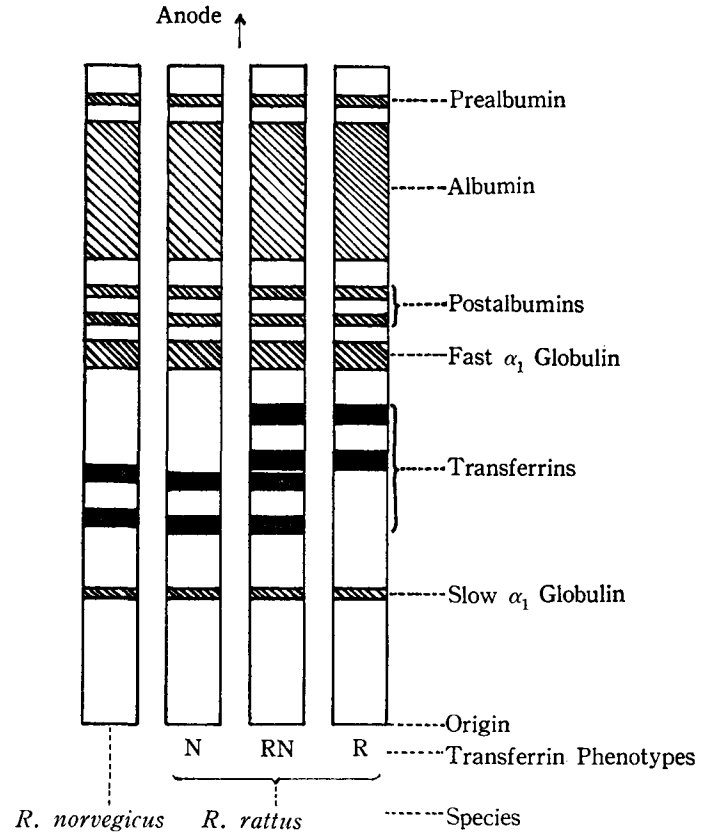


Fig. 1. Schematic patterns of serum electrophoresis in transferrin variants of *Rattus rattus*.

Table 1. Progeny test of serum transferrin variants in *Rattus rattus*.

Parent	No of litter	Progenies				Litter size	Theor. ratio			Chi-square
		R	*RN*	*N*	Total		*R*	*RN*	*N*	
R×*R*	28	141	0	0	141	5.1	1	0	0	—
R×*N*	5	0	23	0	23	4.6	0	1	0	—
N×*N*	1	0	0	5	5	5.0	0	0	1	—
R×*RN*	18	54	50	0	104	5.8	1	1	0	0.15(df=1, $P>0.5$)
RN×*RN*	18	29	55	18	102	5.7	1	2	1	3.01(df=2, $P>0.2$)

Weinberg test was applied to this material, no significant departure from numerical expectations was obtained ($X^2=2.35$, df=2, $P>0.3$).

Distribution of transferrin phenotypes among progeny from the laboratory crosses of *R*-, *RN*- and *N*-type is summarized in Table 1. These data are consistent with the hypothesis that the two transferrin types, *R* and *N*, are controlled by two codominant alleles at a single locus.

Migratory behavior of vestigial strains of *Drosophila melanogaster*

Takashi NARISE

In the series of experiments on migration with wild and vestigial strains of *Drosophila melanogaster*, it was found that the migratory activity of a vestigial strain was accelerated by a coexisting wild strain. The extent of acceleration seemed to be dependent upon the similarity in genetic constitution between the vestigial and the mixed wild strain. To make the point clear, an experiment was conducted with four wild strains, MS-1, Niihama, Oshoro and Tosu, their substituted vg strains (see the last issue of this Annual Report) and the F_1 hybrids between MS-1 and the remaining three wild strains. These wild type strains were mixed with the substituted ones in a certain combination and the effect of genetic closeness on the change in migratory activity of the substituted flies was examined. The experimental results are presented in Table 1.

Table 1. Migratory activity of substituted vg strains

	Migratory activity of vestigial strains			
	MS-1(vg)	Niihama(vg)	Oshoro(vg)	Tosu(vg)
MS-1	17.2	19.6	24.4	16.4
Niihama × MS-1	17.8	16.0		
Oshoro × MS-1	22.0		20.0	
Tosu × MS-1	20.4			14.0
Niihama	18.0	8.0		
Oshoro	24.8		13.6	
Tosu	23.6			11.6

As shown in Table 1, the migratory activity of MS-1(vg) was 17.2% in the mixture with MS-1, while the activity increased to 20.4% and 23.6% respectively in the mixture with F_1 (Tosu × MS-1) and Tosu strain.

From the experimental results, it is concluded that the vestigial flies become active in migration if the coexisting flies are different from them in genetic constitution. In other words, the overall difference in genotypes between the coexistants is capable of accelerating migration.

Three Intergeneric Amphidiploids of the *Tribe Triticeae*

Sadao SAKAMOTO

Applying 0.5% colchicine solution to the basal parts of tillering clones of various intergeneric hybrids of the tribe Triticeae, the following three intergeneric amphidiploids were produced: (1) *Eremopyrum orientale* Jaub. et Spach (2n=28; Iran) × *Agropyron tsukushiense* var. *transiens* Ohwi (2n=42; Japan), (2) *Er. orientale* × *Henrardia persica* var. *glaberrima* (Hausskn.) C.E. Hubbard (2n=14; Iran) and (3) *Er. buonapartis* (Spreng.) Nevski var. *buonapartis* (2n=14; Iran) × *Hordeum* sp. (2n=28; material "No. 66w" was received from the All-Union Institute of Plant Industry, Leningrad, 1964).

As to morphological characteristics (1) was of *Agropyron* type, (2) was of elongated *Eremopyrum* type and (3) was of *Hordeum* type, having in addition two florets with two, three or four empty glumes at each spikelet node of the spike.

Average chromosome paring at MI of PMCs and seed fertility of the amphidiploids were in the order of above enumeration (1) $29.4_{II}+11.2_{I}$; 5.5%, (2) $20.2_{II}+2.2_{I}$; 42.8% and (3) $0.2_{IV}+0.3_{III}+17.8_{II}+4.9_{I}$; 34%. Low seed fertility of (1) resulted from unexpected high frequency of univalent formation at MI.

Phenetic Variation and Phyletic Relationship of Strains of *Oryza perennis*, Estimated by Numerical Taxonomic Method

Hiroko MORISHIMA

Last year (Ann. Rep. 17, p. 59–61), we reported about an analysis of variations among strains of *Oryza perennis* Moench made from the "phenetic" (overall similarity between existing organisms) standpoint. This year, the same set of data (24 characters × 65 strains) were analysed not only phenetically, but also from a "phyletic" (phylogenic) standpoint. In some parts of the computation, F_1 sterility data of the strains were treated together with the data for other 24 characters. For phenetic studies, the technique of "cluster analysis" (Sokal and Sneath 1963) and "pattern analysis" (Hayashi 1956) were employed. The results consistently showed that the four geographical groups of strains, Asian, African, American

and Oceanian, formed separate clusters on a phenetic plane. Then, "cladograms" showing evolutionary branching sequences of the strains by a tree-like diagram were computed by Camin and Sokal's (1965) method, which is based on the hypothesis of minimum evolution. It was attempted, further, to introduce a time axis into the cladograms. The time elapsing between two given branching points in a cladogram was considered proportional to their phenetic distance and inversely proportional to the relative evolutionary rate of the plants, and the latter was estimated from breeding characteristics, *i.e.*, the duration of life cycle, seed dispersing ability, and the rate of autogamy.

The derived cladograms suggested that the geographical groups might have evolved independently from one another, and that in the Asian group various advanced forms might have differentiated with a high evolutionary rate. It was postulated that geographical isolation between continents and variation in the breeding system might have been the major factors conditioning phylogenic differentiation of this plant group. The main part of this study was done at the Computation Center of the University of Kansas, where the reporter worked under Dr. R. R. Sokal for two months supported by the Rockefeller Foundation.

Occasional Escape from the Isolating Barrier Surrounding *Oryza perennis* subsp. *barthii*

Yaw-En CHU and Hiko-Ichi OKA

The African race of *Oryza perennis*, called *barthii*, is partly isolated from its wild and cultivated relatives (other races of *O. perennis*, *O. sativa* *O. graberrima* and *O. breviligulata*) by a barrier that hinders the development of F_1 embryos with a certain frequency. Observations of the deterioration process were reported before (Ann. Rep. 16, p. 71). This, year, an analysis of the genes controlling this embryo deterioration was attempted. In triple crosses of the *sativa*×(*sativa*×*barthii*) design, the cross-pollinated seeds were found to segregate into normal and shrunken ones, the ratio being 1 : 1. This suggests that a set of complementary dominant lethals controls the deterioration of F_1 embryos. The penetrance of the gene effect may not be complete so that a few germinable F_1 seeds are sometimes obtained.

Plants of a *barthii* population may also have different combinations of the lethal genes. Those with double recessive combination map produce normal F_1 seeds with *sativa* or *glaberrima*. In Africa, *barthii* often grows in the fields of *sativa* and *glaberrima*. Some *barthii* plants, raised from seeds collected from natural habitats, due to some particular characters,

were suspected to be hybrids with *sativa* or *glaberrima*. Having crossed them with true *barthii* and with *sativa*, they proved to have the lethal gene combination either of the *barthii* or the *sativa* parent. These *barthii* plants, we call "Obake", seem to be slipping out of the isolating barrier by spontaneous successful hybridization. Their peroxidase isozymes showed bands peculiar to *sativa* and to *glaberrima*, indicating that the "Obake" may be of a complex hybrid origin. This work was supported by a grant of The International Rice Research Institute.

Partial Self-incompatibillity Found in *Oryza perennis* subsp. *barthii*

Yaw-En CHU and Hiko-Ichi OKA

It was suggested by Jachuck and Sampath (1966, Oryza 3: 49-57) that the African strains of *O. prennis*, called *barthii*, could be self-incompatible, as the panicles when bagged set only a few seeds. An investigation on this point was made in three plants, collected from Guinea (Af9) and Mali (ML36-4 and ML36-6, belonging to the same population). The plants grown in pots were, after heading, separated by a distance of about 20 m, and self- and cross-pollinations by hand were compared with natural self-pollination. Bagging was not made. Cross-pollination brought about an apparently higher percentage of seed set (65% to 83%) than selfed, the pollen tubes penetrating into the stigma tended to have a swollen tip, resulting in a failure to fertilize. This work was supported by a grant of The International Rice Research Institute.

Comparison of Variations in Peroxidase Isozymes between *Perennis-Sativa* and *Breviligulata-Glaberrima* Series of *Oryza*

Yaw-En CHU and Hiko-Ichi OKA

The two cultivated rice species, *O. sativa* and *O. glaberrima*, are thought to have independently evolved from an Asian form of *O. perennis* and African *O. breviligulata*, respectively. The isozyme variations in leaf-blade peroxidase were investigated in strains of *O. breviligulata* and *O. glaberrima*, and were compared with those previously observed in *O. perennis* and *O. sativa*. The *breviligulata* and *glaberrima* strains showed only three zymogram types, and appeared to be much less variable than the *perennis-sativa* series that showed eleven different zymogram types. Also the within-populational variability in isozymes was quite small in

O. breviligulata, in contrast to the polymorphic populations of *O. perennis*. The zymogram variations suggested the evolutionary paths of the plants. Observations of *sativa-glaberrima* F_1 hybrids and back-cross progenies indicated that the two species might have basically the same genes for peroxidase isozymes, showing differences in the presence or absence of certain bands. This work was supported by a grant of The International Rice Ressarch Institute.

V. MATHEMATICAL AND STATISTICAL STUDIES ON POPULATION GENETICS

Gene frequency cline in a habitat of finite length

Motoo KIMURA and Takeo MARUYAMA

The previous report (Annual Report, No. 15, pp. 95-97, 1965), was about a study by the senior author on the gene frequency cline in a linear habitat of finite length. Using the method of differential equation, Kimura derived a series solution for the case of genic selection assuming that the length of the habitat is relatively short.

When the length of the habitat is longer, the analytical solution of the differential equation seems to be very hard to obtain, but the solution may be tabulated by applying numerical integration.

In the present note, we will consider a case in which the habitat extends from $x=l_1(<0)$ to $x=l_2(>0)$ and in which the equation giving the gene frequency $p(x)$ at point x satisfies the differential equation

$$\frac{d^2p}{dx^2}=xp(1-p)\{1+h(1-2p)\}\,, \qquad (1)$$

with boundary conditions

$$\frac{dp}{dx}=0 \quad \text{at} \quad x=l_1 \quad \text{and} \quad x=l_2\,. \qquad (2)$$

With the help of a high speed computer, we are carrying out an extensive tabulation of the solution of the above equation for various values of the

Table. Comparison of values obtained from power series (3) and numerical integration

		from power series	from numerical integration
$l=0.5$	P(l)	0.489586	0.489686
	P′(0)	0.03124	0.03125
$l=1.0$	P(l)	0.418432	0.418420
	P′(0)	0.12270	0.12270
$l=1.5$	P(l)	0.25	0.267274
	P′(0)	0.24992	0.23900
$l=5.0$	P(l)	———	0.00045
	P′(0)	———	0.32340
$l=\infty$	P(l)	———	0
	P′(0)	———	0.3302

parameter h. So far, we have nearly completed tabulation for the cases of $h=0$ and $h=1$.

The accompanying table shows some results of the numerical integration for the case of $h=0$ (no dominance) and $-l_1=l_2=l$. Also, in the table, the corresponding values obtained by the series solution of Kimura, namely

$$p(x)=0.5-Bx+\frac{x^3}{24}-\frac{B^2x^5}{20}+\cdots, \qquad (3)$$

where $B=l^2/8-11l^8/4608$, are listed for l up to 1.5.

On the special functions of population genetics

Takeo MARUYAMA

The spectral representation of the operator (1), acting in a suitable Hilbert space, and the elementary solution for the parabolic partial differential equation (2),

$$A=(1-x^2)\frac{d^2}{dx^2}-[4x+sh+(1-2h)sx]\ \frac{d}{dx}-\left[\frac{(1-x^2)(1-2h)s}{2}-shx-\frac{x(1+x)(1-2h)s}{2}+c(x)\right] \qquad (1)$$

and

$$\frac{\partial\phi(t,\ x)}{\partial t}=A\phi(t,\ x) \qquad (2)$$

have been studied by M. Kimura. Taking first the case $s=0$, and then the case $h=0.5$, though assuming $c(x)=0$ in both cases, he has obtained the explicit forms of Green's functions which seem to have an increased importance in population genetics because various kinds of information concerning the stochastic behavior of a population may be obtained from them. However for cases other than those treated by Kimura, no comparable works have been done. Therefore it is desired to construct the spectral representation of the operator (1) and the elementary solution for (2), for general cases. Since the spectral set of the operator (1) is countable real numbers such that $0>\alpha_1>\alpha_2>,\cdots,\ \alpha_n>,\cdots-\infty$, the representation has a simple form. The proof of this fact will be given elsewhere when an extensive tabulation of the functions are given. However it should also be mentioned that if we add two more terms, $(1-\mu)(1-x)/2$ and $\nu(1+x)/2$, which represent the reversible mutations, to the second term of (1), the spectrum is still discrete countable real numbers

but includes zero as the largest eigen-value.

If we change (1) into an appropriate self-adjoint form, it is easy to show that the eigenfunctions of (1) are mutually orthogonal with respect to the weight function $w(x)=(1-x^2)\exp[-s(1-x)(2h+1-x+2hx)/8]$. For we can verify the equality

$$\alpha_i\int_{-1}^{1} w(x)E_i(x)E_j(x)dx=\alpha_j\int_{-1}^{1} w(x)E_i(x)E_j(x)dx$$

where $E_i(x)$ and $E_j(x)$ are two eigenfunctions associated with eigenvalues α_i and α_j, respectively. Since the first part of the operator (1) admits the Gegenbauer polynomials as eigenfunctions and the spectrum is a set of countable real numbers, it is natural to expand an eigenfuction of (1) in the form

$$E(x)=\sum_{n=0}^{\infty} c_n T_n^{(1)}(x) \tag{3}$$

where $T's$ are the Gegenbauer polynomials and are the bounded solutions of $(1-x^2)T''(x)-4xT'(x)+n(n+3)T(x)=0$. Now we want to determine the coefficients $c's$ in (3) so that $E(x)$ satisfies the equation

$$AE(x)=\alpha E(x)$$

and is finite at both singular points, $x=1$ and $x=-1$, that is equivalent to the condition that $E(x)$ is a square integrable function with respect to the weight $w(x)$ mentioned above, over the interval $(-1, 1)$. Upon substitution of (3) into (1) and making use of the following recurrence relationships

$$xT_n^{(1)}=[(n+1)T_{n+1}^{(1)}+(n+2)T_{n-1}^{(1)}]/(2n+3)$$

and

$$(1-x^2)\frac{dT_n^{(1)}}{dx}=(n+1)T_{n+1}^{(1)}-(n+3)T_n^{(1)}$$

we have a system of equations that the coefficients $c's$ must satisfy;

$$A_{n+2}c_{n+2}+B_{n+1}c_{n+1}+D_nc_n+E_{n-1}c_{n-1}+F_{n-2}c_{n-2}=0 \tag{4}$$

where the capital letters of the alphabet are functions of n, s, h and $c(x)$ in the operator (1), and in each equation only D contains α, the separation constant. Although there is a simpler way to determine α and $c's$ simultaneously, it works only when s is small. Here a method of continued fraction developed from (4) will be used in actual computations, because it has a great advantage when a high speed computer is used and more importantly it works up to large values of s. We can develop the continued fractions into two directions; one in descending order of

n and the other in ascending order of n. Then we choose α such that one fraction converges to the reciprocal value of the other fraction. As soon as a computer becomes available in our institute, extensive tabulations of these special functions will be carried out.

Development of temporary overdominance associated with neutral alleles

Takeo MARUYAMA and Motoo KIMURA

Universality of overdominance has long been a subject of controversy among geneticists. It is an attractive hypothesis for explaining the maintenance of genetic variation in populations and there seems to be little doubt that some variations are due to overdominance. However it is too naive to assume that most of the variations are maintained by overdominance. In a finite population, 2Ns, that is twice the population size times the selection coefficient, has to be considerably greater than unity to make overdominance effective for maintaining the variation. Moreover, if we assume that most of the existing variations are due to overdominance, the number of offspring produced by a single female, in some organisms, is not sufficient to overcome the genetic death that would be caused by segregations.

In the present study, we intend to show that in a finite population

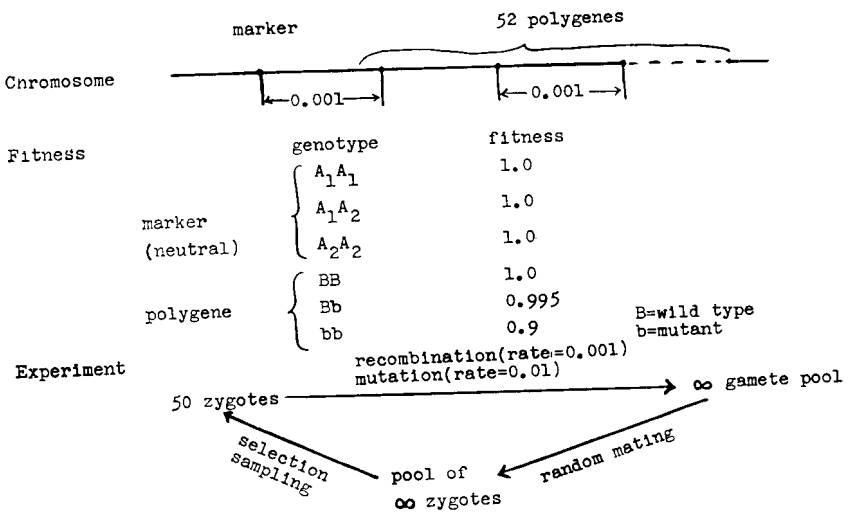


Fig. 1. Scheme of Monte Carlo Experiment.

temporary overdominance is produced when a marker locus with neutral alleles is linked with other loci affecting fitness, even though there are no loci having heterozygous advantage by themselves. For this purpose, we carried out the following computer simulation experiments. We assumed a diploid organism having one pair of homologous chromosomes on which the marker locus with neutral alleles A_1 and A_2, and 52 polygene loci affecting fitness were located at 0.001 crossover distance between adjacent loci. In an experiment performed, population size was 50, mutation rates between alleles were 0.001 (same for all loci), and we assumed that at each polygenic locus the mutant gene reduces fitness by 10% in homozygous and 0.5% in heterozygous condition. The scheme of the experiment is illustrated in Figure 1. In the starting population, the marker alleles A_1 and A_2 were set with equal frequencies, and at each polygene locus, the mutant gene and the wild type gene were set according to Wright's distribution formula, but independently of other loci.

Table I. Result of Monte Carlo Experiment.

Generation		0	5	10	15	20	25	30	35	40	45	50
Fitness	A_1A_1	0.577	0.605	0.693	0.595	0.686	0.718	0.733	0.697	0.637	0.664	0.680
	A_1A_2	0.608	0.723	0.763	0.770	0.771	0.788	0.756	0.732	0.703	0.733	0.685
	A_2A_2	0.611	0.670	0.621	0.640	0.623	0.575	0.597	0.641	0.596	0.714	0.616

Generation		55	60	65	70	75	80	85	90	95	100
Fitness	A_1A_1	0.638	0.565	0.654	0.471	0.580	0.471	0.349	0.468	0.409	0.440
	A_1A_2	0.736	0.745	0.733	0.768	0.767	0.778	0.734	0.753	0.755	0.787
	A_2A_2	0.394	0.558	0.597	0.605	0.598	0.571	0.593	0.669	0.650	0.669

The results of an experiment that was continued over 100 generations using this scheme are summarized as follows: The heterozygotes for the marker, A_1A_2, showed about 15% higher fitness than the homozygotes, A_1A_1 or A_2A_2. Thus we have demonstrated the occurrence of apparent overdominance at a locus with neutral alleles on a chromosome segment where no loci are showing heterosis by themselves. Details of the results are given in Table 1. More extensive investigations of the above phenomenon are under porgress.

Effect of epistasis on the probability of joint fixation in 2 loci

Motoo KIMURA and Tomoko OHTA

Let us consider a haploid population of effective size N_e and assume

that fitnesses and frequencies are as follows:

genotype	fitness	frequency
AB	$1+s_1+s_2+\varepsilon$	pq
Ab	$1+s_1$	$p(1-q)$
aB	$1+s_2$	$q(1-p)$
ab	1	$(1-p)(1-q)$

Here, p and q stand for the frequencies of A and B respectively. If u is the probability of ultimate fixation, then u satisfies the following partial differential equation which is a steady state form of the Kolmogorov backward equation.

$$p(1-p)\frac{\partial^2 u}{\partial p^2}+q(1-q)\frac{\partial^2 u}{\partial q^2}+2N_e p(1-p)(s_1+\varepsilon q)\frac{\partial u}{\partial p}$$
$$+2N_e q(1-q)(s_2+\varepsilon p)\frac{\partial u}{\partial q}=0\,. \qquad (1)$$

For the special case of $s_1=s_2=0$, Kimura obtained, from the above equation, the following formula for $u(AB)$, i.e., the probability of joint fixation of A and B,

$$u(AB)=\frac{1-e^{-2N_e\varepsilon pq}}{1-e^{-2N_e\varepsilon}}\,. \qquad (2)$$

It is interesting to note that this formula coincides with that for the fixation probability of a single locus case in which a single allele denoted by AB has selective advantage ε and initial frequency pq. By Monte Carlo experiments, Ohta found that the formula (2) is valid. The fixation probability was also studied by Monte Carlo simulation; assuming various recombination values it was found that intensity of linkage has not much influence on $u(AB)$ with initial linkage equilibrium.

Kimura also showed that when $|N_e s_1|$, $|N_e s_2|$ and $|N_e\varepsilon|$ are all small, the approximate solution of (1) is,

$$u(AB)=pq\{1+N_e s_1(1-p)+N_e s_2(1-q)+N_e\varepsilon(1-pq)+\cdots\}\,.$$

However, the general solution of (1) is not known at present. For the cases in which the above parameters are not small, Monte Carlo studies were also carried out. Fixation probability of A is very much affected by the frequency of B for large ε and it was found that, even with 50% recombination, $u(AB)>u(A)\cdot u(B)$ for positive ε. (submitted to Züchter)

Effects of initial linkage disequilibrium on fixation probabilities for 2 loci

Tomoko OHTA

Probabilities of joint fixation for 2 linked loci were studied by the method of Kolmogorov backward equation and also by Monte Carlo simulation with special reference to initial linkage disequilibrium. Such a study may particularly be important when selection starts with a population produced by crossing 2 strains in breeding experiments.

When there was no selection or the selection coefficients were small, the probabilities were obtained by solving steady state form of the Kolomogorov backward equation. Let us assume 2 pairs of genes, A and a in the first and, B and b in the second locus. Let the initial frequencies of gametes, AB, Ab, aB and ab be g_1, g_2, g_3 and g_4 respectively. Also, let N_e be the effective size of the population, c the recombination fraction between A and B loci, and let u be the fixation probability of a gamete type. Then u satisfies following partial differential equation which is a steady state form of a Kolmogorov backward equation.

$$\begin{aligned}
&\frac{1}{4}g_1(1-g_1)u_{11}''+\frac{1}{4}g_2(1-g_2)u_{22}''+\frac{1}{4}g_3(1-g_3)u_{33}''\\
&-\frac{1}{2}g_1g_2u_{12}''-\frac{1}{2}g_1g_3u_{13}''-\frac{1}{2}g_2g_3u_{23}''\\
&+\frac{1}{2}N_es_1\{g_1(1-g_1-g_2)u_1'+g_2(1-g_1-g_2)u_2'-g_3(g_1+g_2)u_3'\}\\
&+\frac{1}{2}N_es_2\{g_1(1-g_1-g_2)u_1'-g_2(g_1+g_3)u_2'+g_3(1-g_1-g_3)u_3'\}\\
&-N_ecD\{u_1'-u_2'-u_3'\}=0\,, \qquad (1)
\end{aligned}$$

where s_1 and s_2 are respectively the selective advantages of A and B over a and b measured as the difference between 2 homozygotes (assuming no epistasis). In the above equation, u_{ij}'' is the second derivative of u with respect to g_i and g_j, and, u_i' is the first derivative of u with respect to g_i.

The general solution of the above formula has not been obtained, but when $|N_es_1|$ and $|N_es_2|$ are small, the approximate solution for the fixation probabilities was obtained. They are:

$$u(AB)\doteqdot g_1-d+N_es_1(1-g_1-g_2)(g_1-d)+N_es_2(1-g_1-g_3)(g_1-d)$$

$$u(Ab)\doteqdot g_2+d+N_es_1(1-g_1-g_2)(g_2+d)+N_es_2\left\{\frac{d}{2N_ec}-(1-g_1-g_3)(g_1-d)\right\}$$

$$u(aB)\fallingdotseq g_3+d+N_es_1\left\{\frac{d}{2N_ec}-(1-g_1-g_2)(g_1-d)\right\}+N_es_2(1-g_1-g_3)(g_3+d)$$
$$u(ab)=1-u(AB)-u(Ab)-u(ab) \qquad (2)$$

where $d=2N_ecD/(2N_ec+1)$.

When there is no selection, i.e., $s_1=s_2=0$, the above formulas reduce to,

$$\begin{aligned} u(AB)&=g_1-d \\ u(Ab)&=g_2+d \\ u(aB)&=g_3+d \\ u(ab)&=g_4-d \end{aligned} \qquad (3)$$

which are exact solutions of formula (1) for the case of no selection. The results agree with the probabilities obtained by Kimura (1963) by a different approach. However, these results are slightly different from those obtained by Karlin and McGregor who used the method of probability transition matrix. The difference is due to the models used.

When $|N_es_1|$ and $|N_es_2|$ are large, the solution has not been obtained, but Monte Carlo experiments were carried out. From the results of Monte Carlo study with various degrees of initial linkage disequilibrium and $|N_ec|$, it was concluded that the effects of disequilibrium for the case of large $|N_es_1|$ and $|N_es_2|$ are usually larger than the selectively neutral case because of the rapid approach to fixation. Therefore, in some cases, in order to reduce disequilibrium, keeping a population as a large random mating one before starting selection may be recommended in breeding experiments. (submitted to Züchter)

Distribution of matrimonial distance in Mishima district

Norikazu YASUDA

Distribution of matrimonial distance, that is, the distance between the birthplaces of spouses, is important in the study of the pattern of human migration and geographical range of marriages. It also supplies a basic information for the evaluation of the probability of consanguineous marriages, effective size and heterogeneity of a population, and also for the estimation of the inbreeding coefficient.

Let $m(x, y)$ be the probability density that a male born in (0, 0) marries a female born at (x, y), where x and y are two coordinates in a Cartesian system. Under isotropic migration, the distribution of matrimonial distance $r=\sqrt{x^2+y^2}$ may be expressed in a polar coordinate system; namely,

$$m(x, y)dxdy=2\pi m'(r)rdr\ .$$

Nine hundred and forty-four couples who were born (both husband and wife) in Mishima and its neighboring towns were sampled from *koseki* or household records in which addresses of birthplaces are given. The birthplaces were identified on maps (scale 1/3,000~1/25,000) with the aid of *khozu* or terrier. Distances were measured on the map along the straight line between birthplaces of husband and wife, and the angle (θ) between the line and the longitude was also measured for a survey of isotropic migration.

The observed distribution is given in Figure 1. Among the observed couples two showed the same address of their birthplaces, but in one of them, it was found that husband and wife were actually born in two neighbor houses. This is a significantly different finding from that of the previous studies. The present result is due to a more precise evaluation of short distances. In order to find a mathematical form of $m'(r)$, the observed number at a given distance was divided by the corresponding distance, and the resulting values were plotted on a log-square root coordinate system (Figure 2). The result fits remarkably well to a straight line. That is, $\log [m'(r)r/r]=C'-k\sqrt{r}$, or

$$m'(r)r=Cre^{-k\sqrt{r}} \qquad (C=e^{C'})$$

where $C=k^4/24\pi$ and k is obtainable from the slope, giving $2.3\times 1.24=2.85$/km. The maximum likelihood estimate for k and its standard error

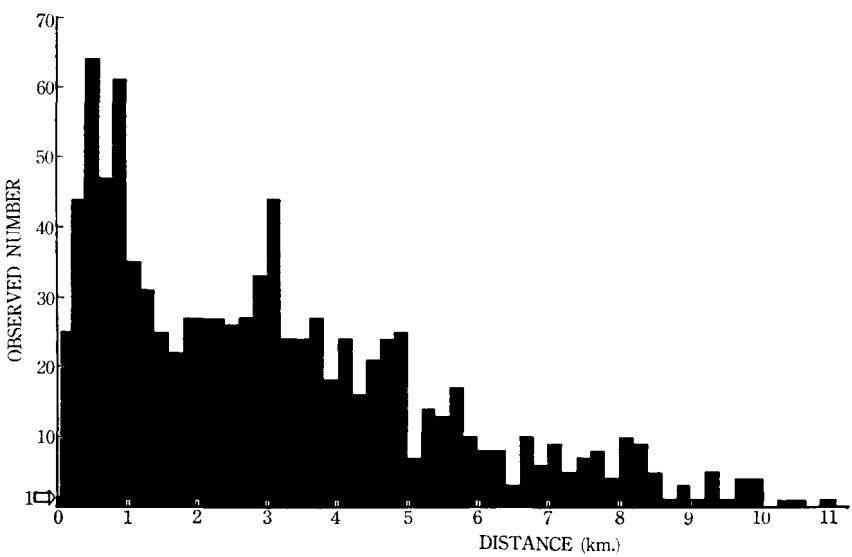


Fig. 1. Distribution of matrimonial distance in Mishima district ($T=944$).

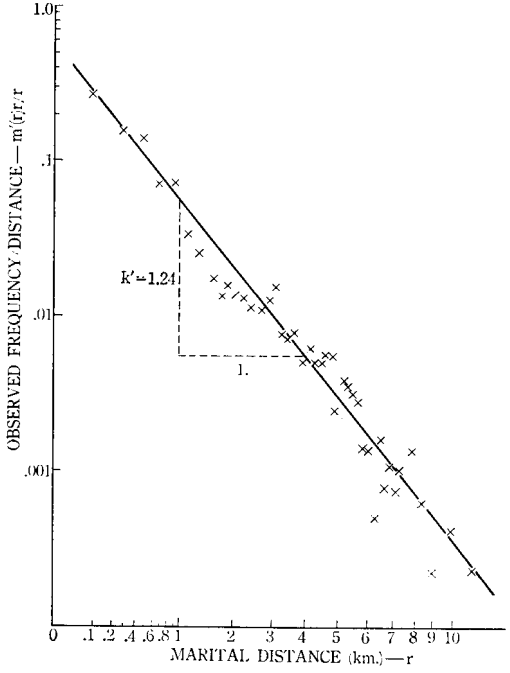


Figure 2. Fitting of function $Cre^{-k\sqrt{r}}$ to the distribution of marital distance in Mishima district.

are $k=4T/\sum\sqrt{r}$ and $s=\sqrt{4T}/\sum\sqrt{r}$, respectively, in which T is the total number of couples. The present data gave $k=2.52\pm0.04$/km. The estimated mean ($\bar{r}$) and standard deviation (σ) of the distance were $\bar{r}=3.16\pm0.26$ km and $\sigma=3.31\pm0.27$ km, respectively.

For the above consideration, we assumed an isotropic nature of migration. This was examined using the variance of angle, which is expected to be $\pi^2/3=3.2899$ (in radians). If the dimensionality is defined by $d=1+$(observed v.)/(expected v.), then we obtain $d=1.98$, since the observed variance is $[\sum\theta^2-(\sum\theta)^2/T]/(T-1)=3.2405$. However, migration may be one-directional for long distances. This hypothesis was also tested by using the dimentionality for a given distance interval, and we found that the d-values did not deviate significantly from two for the distance less than 7 km. This covers 91.6% of the sample. It is therefore concluded that migration is isotropic in Mishima.

The project will be continued to clarify the above points.

The use of surname for estimating Wright's F-statistics in man

Norikazu YASUDA

Isonymous marriages, that is marriages between persons having the same surname, take place either due to mere chance or to consanguineous marriages. In the latter case, a quarter will be isonymous (1). Let I be the proportion of observed isonymous marriages, k the expected isonymy frequency due to inbreeding, q_m the male proportion with a certain surname and q_f the corresponding female proportion. Then we have

$$I=\sum q_m q_f+(1-\sum q_m q_f)k$$

where $\sum q_m q_f$ is the contribution due to random matches, and $(1-\sum q_m q_f)k$ to non-random marriages. Sicne the population is divided into subgroups by surname, $\sum q_m q_f$ and k would correspond to the inbreeding coefficients F_{ST} and F_{IS}, respectively. F_{ST} is the coefficient for an individual A whose common ancestor was a person having a different surname from A, while if the surname of ancestor was the same as that of A, we have F_{IS}.

Therefore, as shown by Crow and Mange (1), the total inbreeding coefficient F_{IT} of the population can be obtained from the relation (2):

$$F_{IT}=F_{ST}+(1-F_{ST})F_{IS}$$

where

$$F_{ST}=\sum q_m q_f/4$$

and

$$F_{IS}=(I-\sum q_m q_f)/4(1-\sum q_m q_f)\,.$$

At *Nakazato* village of Mishima district, 1464 couples were sampled from *koseki* or household records, in which his or her parents' surnames as well as his or her own are recorded. Among them, 1063 males and 614 females were born in the area in question during the last hundred years.

Table 1. Proportion of isonymous marriages and estimated F-statistics at *Nakazato* and in *Tokyo*

	Isonymy (I)	F_{IS}	F_{ST}	F_{IT}	α	α/F_{IT}
Nakazato	9/430 (0.0209)	0.00456	0.00276	0.00731	0.0044*	0.60
Tokyo	10,116/363,797 (0.0278)	0.00641	0.00051	0.00695	0.0029**	0.45

* Shizuoka study (4).

** $\alpha=0.049/16$, first cousin only. Furusho, personal communication.

Their main occupation is farming. Table 1 summarizes the result, including a study in Tokyo (3). We have found that 95 pairs were isonymous marriages according to koseki, but only nine of them were truly isonymous. In this connection, we define the proper surname of an individual as his or her father's surname when he or she was born. The above phenomenon is mainly due to *Muko-Yoshi-Engumi*, a Japanese custom for maintaining the family name through generations. When a couple does not have a son but has a daughter, a bridegroom-to-be is adopted just before the marriage takes place. Consequently, this results in an increase of isonymous marriages in the koseki.

It is still too early to attribute any significance to the figures reported in Table 1, but they suggest that F_{ST} caused by random genetic drift would be much higher in rural than in urban societies, although it is interesting to note that the total inbreeding coefficients (F_{IT}) are almost the same. The mean inbreeding coefficient (α) from pedigree study would be F_{IT} if the ascertainment of pedigrees is complete, but it is only about one half of F_{IT} in both areas.

Sampling bias on F_{ST} may be checked by a comparison of the quantities $\sum q_m^2$, $\sum q_f^2$ and $\sum q_{m+f}^2$: each corresponding to the proportion of isonymous pairs when two males are drawn at random, to the corresponding proportion in females, and to the frequency of isonymous pairs irrespective of sex. Table 2 shows the results in terms of F_{ST}. The difference between the second and the third columns may be due to sample size with regard to sex, since koseki is filed by *hittosha* or family representative who is usually a husband; only in sixty of the 1464 couples the wife was the family representative. The last column is taken from

Table 2. Random component (F_{ST}) estimated from various procedures

	$\sum q_m q_f/4$	$\sum q_f^2/4$	$\sum q_m^2/4$	$\sum q_{m+f}^2/4$	$\sum q^2/4$
F_{ST}	0.00276	0.00294	0.00444	0.00346	0.00492
N	1677	614	1063	1677	5928

N=sample size

Table 3. The relationship between the proportion of isonymous marriages and matrimonial distance in Mishima

Marital distance (r) (km)	0–0.4	0.4–0.8	0.8–1.2	1.2–2.8	2.8–
# isonymous	5	4	2	3	0
# couples	70	111	96	212	455
Frequency (I)	0.0714	0.0360	0.0208	0.014	0.0000

a telephone directory which covers the studied area, and it can be compared with the fourth column obtained from koseki.

An attempt was made to study isolation by distance with a relationship between the frequency (I) of isonymous marriages and the matrimonial distances (r). Adding some other data in Mishima to the above survey, we found that the proportion of isonymous marriages decreases with distance (Table 3). A mathematical form of the relationship is tentatively $I=\exp(-kr^{1/4})$, where $k=2.37$/km.

The study is still continued in order to clarify.

1. Crow, J. F. and Mange, A. P., 1965. *Eugen. Quat.* 12: 199.
2. Wright, S., 1951. *Ann. Eugen.* 15: 323.
3. Kamizaki, M. 1954. *Seibutu-Tokei* 2: 292. (In Japanese).
4. Tanaka, K. 1963. In *The Genetics of Migrant and Isolated populations* (Ed. E. Goldschmidt). p. 148.

A gene counting method of maximum likelihood for estimating gene frequency

Norikazu YASUDA

Let us suppose that the observed number of the i-th phenotypic class and the corresponding expected number are respectively n_i ($\sum_{i=1}^{k} n_i=N$) and $N\theta_i$ ($\sum_{i=1}^{k} \theta_i=1$), where θ_i is a function of gene frequency p_j ($j=1,\cdots, m$ in which m is the number of alleles). The log-likelihood of observation is

$$\ln L=\text{const.}+\sum_{i=1}^{k} n_i \ln\theta_i .$$

Defining score U_j as the first derivative of $\ln L$ with respect to p_j, the number of the representatives of the j-th allele in the population may be given by

$$E_j=p_jU_j=p_j\sum_{i=1}^{k} n_i\left[\frac{1}{\theta_i}\frac{\partial\theta_i}{\partial p_j}\right] \quad (j=1,\cdots, m) . \tag{1}$$

The gene frequency is thus calculated from

$$p_j=E_j/\sum_{r=1}^{m} E_r \qquad (j=1,\cdots, m) , \tag{2}$$

where the denominator is the number of identified genes in the population. Since E_j may be expressed in terms of n_i and proportion of a certain genotype within a given phenotype, h, the gene frequency to be estimated may be obtained by iteration starting from a rough value of p or h. The final value obtained by this method is the solution of

maximum likelihood equations which are $U_i=U_j$ $(i,\ j=1,\cdots,\ m)$ in the present terminology, because for all j we have $U_j=\sum_{r=1}^{m} E_r$ from (1) and (2).

Example. If individuals are unselectively sampled, the total number of genes, $\sum_{r=1}^{m} E_r$ is independent from p; that is $2N$. Thus, for instance, suppose that the MNSs blood group system is described by the following notations:

phenotype	genotype	observed no. (n_i)	expected freq. (θ_i)
MS	MS/MS	n_1	p_1^2
MSs	MS/Ms	n_2	$2p_1p_2$
Ms	Ms/Ms	n_3	p_2^2
MNS	MS/NS	n_4	$2p_1p_3$
MNSs	MS/Ns, Ms/NS	n_5	$2p_1p_4+2p_2p_3$
MNs	Ms/Ns	n_6	$2p_2p_4$
NS	NS/NS	n_7	p_3^2
NSs	NS/Ns	n_8	$2p_3p_4$
Ns	Ns/Ns	n_9	p_4^2
Total		N	1

We have then simple formulae:

gene	gene frequency
MS	$p_1=\alpha_1+\beta h$
Ms	$p_2=\alpha_2-\beta h$
NS	$p_3=\alpha_3-\beta h$
Ns	$p_4=\alpha_4+\beta h$,

where α's and β are constant so that

$$\alpha_1=(2n_1+n_2+n_4)/2N\ ,\qquad \alpha_2=(n_2+2n_3+n_6+n_5)/2N\ ,$$
$$\alpha_3=(n_4+2n_7+n_8+n_5)/2N\qquad \alpha_4=(n_6+n_8+2n_9)/2N$$

and $\beta=n_5/2N$.

The probability, h, that an individual with phenotype MNSs has genotype MS/Ns can be determined as a solution $(0\leqq h\leqq 1)$ of a cubic equation:

$$f(h)=h^3+ah^2+bh+c=0$$

in which

$$a=[(\alpha_1+\alpha_4)-(\alpha_2+\alpha_3)-\beta]/2\beta$$
$$b=[\alpha_1\alpha_4+\alpha_2\alpha_3-\beta(\alpha_1+\alpha_4)]/2\beta^2$$

and $$c=-\alpha_1\alpha_4/2\beta^2\ .$$

We may choose $-c/b$ as a trial value of h. Further improvement may be made through iteration using $h_{i+1}=h_i-f(h_i)/f'(h_i)$, where $f'(h)=3h^2+2ah+b$.

The variances of estimates are obtained after some calculations as follows:

$$V(p_1)=\frac{p_1}{2N}\left[\frac{p_1(p_3+p_4)}{p_1+p_2}+\frac{p_2\{D-p_1p_2(p_3+p_4)\}}{D-p_1p_2(p_3+p_4)-p_3p_4(p_1+p_2)}\right],$$

$$V(p_2)=\frac{p_2}{2N}\left[\frac{p_2(p_3+p_4)}{p_1+p_2}+\frac{p_1\{D-p_1p_2(p_3+p_4)\}}{D-p_1p_2(p_3+p_4)-p_3p_4(p_1+p_2)}\right],$$

$$V(p_3)=\frac{p_3}{2N}\left[\frac{p_3(p_1+p_2)}{p_3+p_4}+\frac{p_4\{D-p_3p_4(p_1+p_2)\}}{D-p_1p_2(p_3+p_4)-p_3p_4(p_1+p_2)}\right],$$

and

$$V(p_4)=\frac{p_4}{2N}\left[\frac{p_4(p_1+p_2)}{p_3+p_4}+\frac{p_3\{D-p_3p_4(p_1+p_2)\}}{D-p_1p_2(p_3+p_4)-p_3p_4(p_1+p_2)}\right].$$

where $D=p_1+2p_2p_3-(p_1+p_2)(p_1+p_3)$.

The above technique may be applied to any type of data that are complicated by sampling procedure and blood-relation among individuals. For detail, see Yasuda, N. and M. Kimura, 1968. *Ann. Hum. Genet.* 31: 409-420, and Yasuda, N. 1968. *Jap. J. Hum. Genet.* 12: 226-245.

VI. EXPERIMENTAL STUDIES ON POPULATION GENETICS

Allelic Rate between Lethal Genes Extracted from Japanese Natural Populations of *Drosophila melanogaster*

Chozo OSHIMA and Takao K. WATANABE

During the past seven years, a total of 2886 second chromosomes were extracted by *Cy-Pm* technique each from a different male fly collected from several natural populations of *D. melanogaster*, distributed at Kofu and Katsunuma. About fifteen per cent contained recessive lethal genes and most of the lethal chromosomes have been maintained by the *Cy* balanced condition in our laboratory. Allelism tests between these lethal genes isolated simultaneously and in successive years were performed and the results are shown in Table 1.

All allelic rates except that of 1959, were quite high, because many groups of allelic lethal genes where isolated simultaneously and some of them have persisted for a long time as shown in Table 2.

As two kinds of lethal genes, namely *l* 204 and *l* 207, had been extracted in 1959, they have persisted at least 7 years in the natural

Table 1. Results of allelism tests between lethal genes

Year	1959	1963	1964	1965	1966	Total
No. of extracted second chhromosomes	144	688	826	905	(323+α)	2563
No. of lethal chromosomes	21	114	100	140		375
Frequency (%)	14.58	16.57	12.11	15.47		14.63
No. of lethal chromosomes tested for allelism	21	114	83	132	61	411
No. of crosses (half diallel)	210	6441	3403	8646	1830	20530
No. of allelic crosses	0	170	143	169	37	519
Allelic rate (%)	0	2.64	4.20	1.95	2.02	2.53
No. of lethal chromosomes tested for allelism		97 81	79 132	124 61		
No. of crosses (diallel)		7857	10428	7564		25849
No. of allelic crosses		184	284	85		518
Allelic rate (%)		2.35	2.38	1.12		2.00

Table 2. Allelic groups found in lethal genes

Year	1963	1964	1965	1966
No. of lethal chromosomes	114	83	132	61
No. of non-allelic lethal chromosomes	68	39	81	40
No. of allelic groups	13	14	16	10
No. of appearance	13	9	10	*l* 204- 5
	8	9	9	4
	l 204- 6	*l* 204- 7	8	4
	6	6	7	*l* 207- 3
	5	6	*l* 207- 6	3
	l 207- 4	3	5	3
	4	3	*l* 204- 4	3
	3	3	2	2
	2	*l* 207- 2	2	2
	2	2	2	2
	2	2	2	
	2	2	2	
	2	2	2	
		2	2	
			2	
			2	

populations. Another two kinds of lethal genes, namely *l* 201 and *l* 215, have been obtained every year since 1963. The loci of these long term persistent lethal genes, *l* 215, *l* 204, *l* 201 and *l* 207, were determined at 1.9, 32.1, 47.9 and 58.4 on the second chromosome, respectively.

Viability of Lethal Heterozygotes under Fluctuating Environment

Chozo OSHIMA and Takao K. WATANABE

Several factors have been assumed to be involved in the persistence of some recessive lethal genes such as linkage with an epistatic gene complex, association with a heterotic inversion and linkage with SD gene (Oshima 1965, 1967). Another factor confirmed in our recent experiment is dealt with in this report. 323 second chromosomes were isolated each from a different male fly collected simultaneously from

natural populations at Kofu and Katsunuma at the end of October 1966. The viability of homozygotes for those chromosomes was examined individually and thereafter 9 D_i/D_j heterozygotes, 89 D/N heterozygotes and 225 N_i/N_j heterozygotes were obtained by random combinations of those chromosomes. (D: lethal or semilethal chromosomes, N: subvital or normal chromosomes). Viability of all those heterozygotes was estimated by *Cy-Pm* technique under both constant and fluctuating temperature environments. Under the former environment a temperature of 25°C was constantly kept in the cultural room and under the latter it was fluctuating twice within 24 hours between 20 and 30°C in an "Insectoron" having a controller. The results are given in Table 3.

Table 3. Viabilities of three genotypes under constant and fluctuating environments

Genotype	No. of lines	Constant (25°C)		Fluctuating (20–30°C)	
		No. of counted flies	Relative viability	No. of counted flies	Relative viability
N_i/N_j	225	59148	0.9940±0.01395	46088	1.0137±0.01245
N/D	89	22958	0·9794±0.01844	18054	1.0431±0.01616**
D_i/D_j	9	2005	0.8775±0.08520	1682	0.9884±0.09371
Total	323	84111	0.9869±0.01123	65824	1.0211±0.01007*

The mean viability of *Cy/Pm*, *Cy* and *Pm* flies is standard (1.0000)

The viability of all three genotypes increased under the fluctuating environment as compared with the constant one, but the viability of lethal or semilethal heterozygotes increased significantly and became better than that of normal heterozygotes under the fluctuating environment.

Table 4. Viabilities of lethal heterozygotes under constant and fluctuating environments

Genotype	No. of lines	Constant (25°C)		Fluctuating (20–30°C)	
		No. of counted flies	Relative viability	No. of counted flies	Relative viability
L/N	128	89790	1.0068±0.00864	70664	1.0353±0.01118 *

* 5% level significance ** 1% level significance

Similar experiments were performed with lethal and normal chromosomes extracted from flies collected from natural Viennese population by Dr. D. SPERLICH. The same tendency as our above experiment was recognized in the results. Especially the viability of lethal heterozygotes increased significantly under the fluctuating environment as shown in Table 4. From those results, the lethal heterozygotes showed the highest response to the fluctuating environment. This could be a contributing factor to the presence of some lethal genes in natural populations. The delicate effect of lethal genes on viability should be repeatedly examined in fluctuating environments.

Persistence of a Lethal Gene Associated with SD in Cage Populations of *Drosophila melanogaster*

Takao K. WATANABE

It was previously reported that a lethal gene *l*401 (=*l*201) has been maintained in natural populations associated with SD gene and that the SD action was not always effective when the SD chromosomes were paired with many second chromosomes isolated from the natural populations. The segregation ratio *k* (*k*=SD-progeny/total-progeny) varied from 1.0 (SD-sensitive) to 0.5 (SD-resistant).

Two large experimental populations containing SD and lethal chromosomes started with the following initial genetic constitutions: 100% SD-*l*201/SD-sensitive heterozygotes in S population and 100% SD-*l*201/SD-resistant heterozygotes in R population. About 150 females were sampled from each population several times during about 250 days, and *l*201 gene frequency and SD frequency was examined. The mean

Table 5. Lethal frequencies, SD frequencies and mean *k* values of chromosomes extracted from cage populations

Sampling	Days after construction	S population				R population			
		No. of flies	Lethal freq.	SD freq.	Mean *k* value	No. of flies	Lethal freq.	SD freq.	Mean *k* value
	0	800	50.0	50.0	0.996	800	50.0	50.0	0.496
I.	20	126	57.9	58.7	0.839	118	29.7	29.7	0.570
II.	92	114	31.6	29.0	0.815	134	16.4	15.7	0.567
III.	142	143	30.1	33.6	0.928	129	13.2	14.7	0.522
IV.	190	132	25.0	28.0	0.853	133	13.5	12.1	0.522
V.	247	91	27.5	—	—	—	—	—	—

k value was also determined. The results are given in Table 5.

The frequency of lethals decreased from 50 % to 25–30 % in the S population and about 13 % in the R population. These frequencies seem to have reached in both cases an equilibrium state. The frequency of SD gene decreased with the lethal gene frequency in both populations. Mean k value in the S population was 0.996 at the initial state. But it decreased to 0.853. In the R population the initial mean k was 0.496 and was fixed at 0.522 after about 140 days.

Interference by a Foreign Species in the Genetic Constitution of a *Drosophila melanogaster* Population

Takashi NARISE

It was already known that the gene frequency of a half-pint milk bottle population of *Drosophila melanogaster* might be affected by constantly invading flies of another species, *D. simulans*. The rate of change was more or less proportionate to the number of the invading flies. The melanogaster fly is a strong competitor against simulans. It is of interest to find how a small number of melanogaster flies that initially invaded into a simulans population could propagate at the cost of simulans, and what would occur as to its gene frequency. This is a report of the results of an experiment dealing with this problem.

A population consisting of some 500 flies of simulans was grown in a milk bottle. One or two pairs of heterozygous melanogaster flies, ss/ss^a, were every two weeks repeatedly introduced into a simulans population. The experiment was conducted with three replications.

As was expected, the melanogaster flies soon took the place of simulans flies in the next generation when two pairs of melanogaster were introduced and in the third generation by introduction of one pair of melanogaster flies.

The frequency of ss^a gene of melanogaster flies was lowered to 0.33 when initiated from one pair or 0.39 when initiated from two pairs of melanogaster instead of expected 0.45 by the effect of simulans flies. After the extinction of simulans, however, the frequency of ss^a gene recovered to 0.45 which was the equilibrial point for the heterogeneous population of *Drosophila melanogaster*.

Aggressiveness of Race 21B of Wheat Leaf Rust

Keizo KATSUYA

The aggressiveness of races 1 A and 21 B of wheat leaf rust, *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Erikss., was estimated in the condition of a growth chamber. About the same number of seedlings of a wheat variety, Norin No. 16, inoculated singly with urediospores of races 1 A and 21 B were kept in a growth chamber. The growth chamber was maintained at the temperature of 20°C and relative humidity of about 60 %. Light was supplied for 16 hours per day by cool white fluorescent tubes. Three months later, new seedlings of Norin No. 16 planted in clay pots were placed in the growth chamber. The seedlings were naturally infected with urediospores from the initially inoculated plants. Six months after the experiment started, new seedlings were again used which were also naturally infected in the growth chamber. Thereafter randomly selected nine uredia from the naturally infected plants were isolated and multiplied on seedlings of Norin No. 16 for making subcultures. The sub-cultures were tested on differential varieties for identification of races 1 A and 21 B. The result show that all sub-cultures tested belonged to race 21 B. At the same time, three mass-cultures originated from urediospores on naturally infected plants in the growth chamber were also examined on differential varieties for race identification. One of the mass-cultures was race 21 B and the two others were mixtures of races 1 A and 21 B. But the proportion of race 1 A in the mixtures was very small. The proportion of each race in the mixtures was determined on Norin No. 55 by counting the number of resistant (race 1 A) and susceptible infections (race 21 B). From the results, it seems that under the condition of the growth chamber, as to sporulating ability and propagating ability of urediospores race 21 B may be superior to race 1 A. The conclusion is that race 21 B of wheat leaf rust is more aggressive than race 1 A under the condition employed.

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN ANIMALS

Comparison of repair ability of premutational damages among different radiation sensitivity strains of the silkworm

Yataro TAZIMA and Kimiharu ONIMARU

As reported previously (this Annual Report No. 17, pp. 91-92) a direct evidence of repair of premutational damages was obtained by split-dose irradiation to silkworm spermatids. When 1,000 R γ-rays were administered in two fractions to full-grown larvae of strain C108 with 6 and 12 hour intervals a decrease in induced mutation frequency was clearly observed.

After confirming the results, comparisons were made among different sensitivity strains with respect to the effect of split-dose irradiation on mutation frequency. The strains used were rb (the most sensitive), Ascoli (intermediate) and Kansen (the most resistant) in Experiment 671 and Kojiki (sensitive), Aojuku (intermediate) and Kansen in Experiment

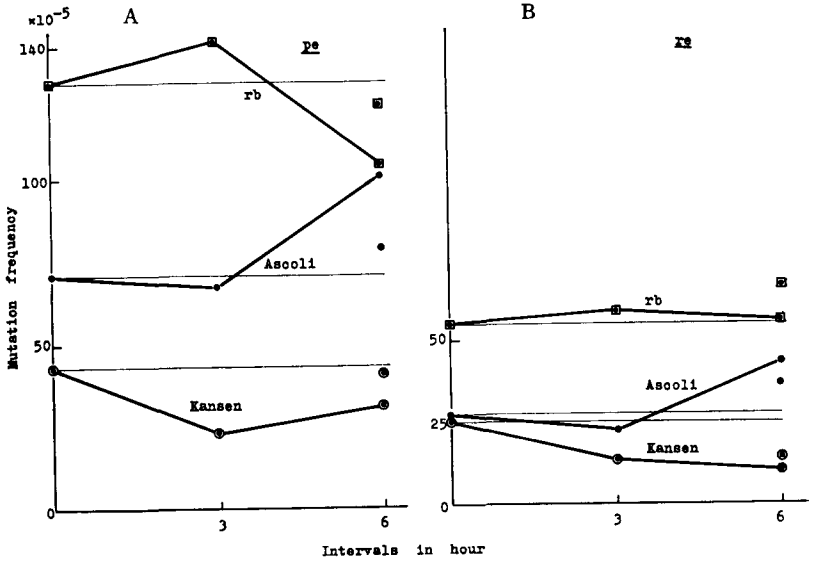


Fig. 1. Comparison of the effect of split-dose irradiation on induced mutation frequency among three strains having different radiation sensitivity. A: Mutation at *pe* locus, B: Mutation at *re* locus.

672. γ-rays were administered to full-grown larvae in two fractions, 500 R+500 R, with intervals of 0, 3, and 6 hours. The method used for estimation of mutation frequency was similar to that reported previously.

The results of the Experiment 671 are shown in Fig. 1.

Throughout Experiments 671 and 672, a decrease in mutation frequency by split-dose irradiation was observed only in the resistant strain Kansen.

These results, together with those obtained with strain C108, seem to indicate that only resistant strains are capable to repair radiation induced premutational damages, while sensitive strains lack this ability.

Aerobic and anoxic X-irradiation to spermatids in different radiation sensitivity strains of the silkworm

Yataro TAZIMA, Kimiharu ONIMARU and Akio ONUMA

While we were engaged in applying pre- and post-irradiation treatments with nitrogen, it was soon noticed that a dose modifying effect toward a decrease was more pronounced in irradiation in N_2 gas than post-treatment with the same gas. Mutation frequency obtained after irradiation in N_2 was 0.46 times as high as that obtained after irradiation in the air.

Based on these observations, irradiation experiments both in aerobic and anoxic atmospheres were carried out in combination with post-treatment with O_2 and N_2 atmospheres using strains with different radiation sensitivity. The method used for the detection of mutations was the same as that reported previously. 1,000 R X-rays were administered to full-grown larvae in specially designed flat irradiation chambers, which were rotating during irradiation. Dose reduction factor thus obtained was 0.48 for Kansen, the most resistant strain, while it was 0.64 for rb, a highly sensitive strain.

These findings seem to suggest that some of the mutations in Kansen induced in anoxic atmosphere were reparable, and the amount of repair was estimated to be approximately 0.16 (0.64-0.48). An alternative explanation may be that the protectiveness of nitrogen gas from mutation induction is lower for strain rb than for strain Kansen.

Furthermore, anoxic irradiation of strain rb markedly increased the proportion of whole body mutants to mosaics, whereas anoxic irradiation of strain Kansen kept this proportion almost at the same level as obtained from aerobic irradiation. In the former case it seemed as if a fairly large part of mosaic mutants had been converted into whole body mutants. Since nitrogen is a well known protector from radiation injury, the observed high incidence of whole body mutants cannot be interpreted by a "lethal-hit" hypothesis, which asserts that a proportion of mosaics

is transformed into whole body mutants by a concomitant lethal hit on the complementary strand. It could be rather explained by assuming incapability of rb strain to restrict, under anoxic atmosphere, the transmission of a lesion from one strand of DNA to the complementary strand.

Relation between character of diapausing and mutation response to γ-radiations observed in the silkworm

Yataro TAZIMA, Piere TEULADE and Kimiharu ONIMARU

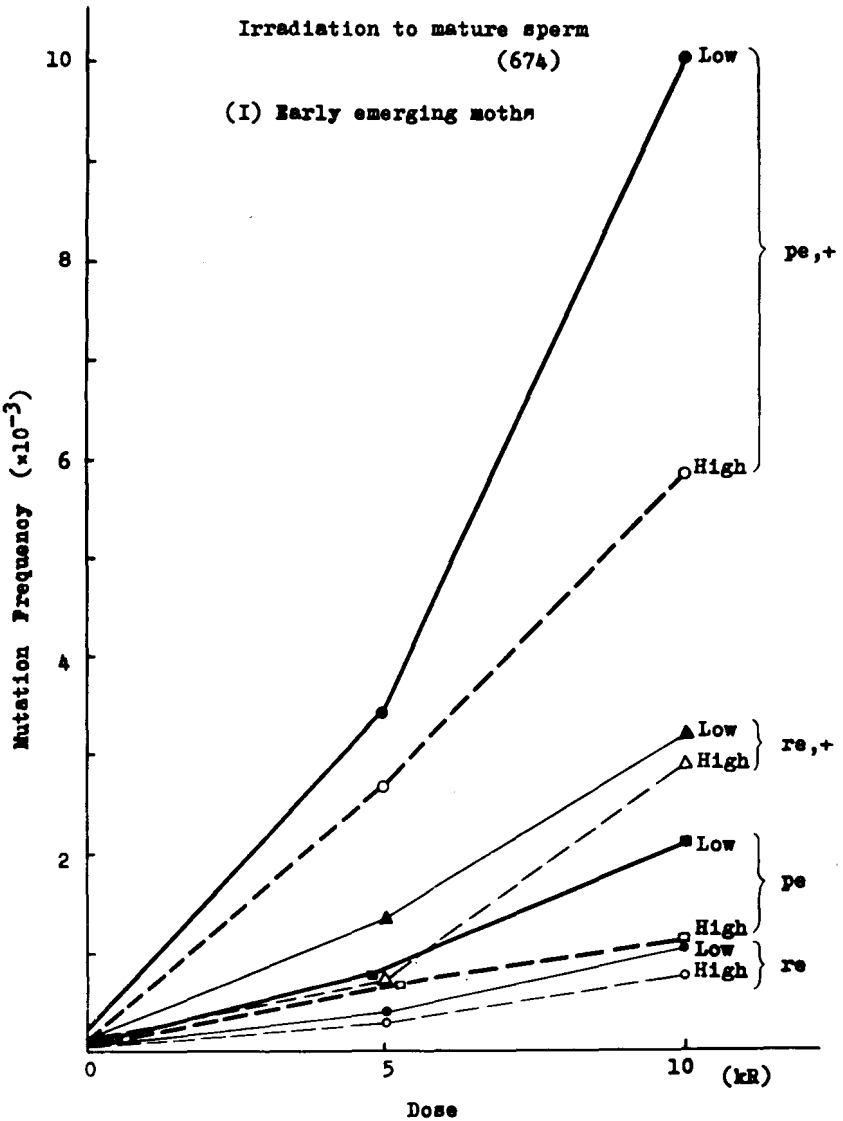
It was assumed in our previous paper (Tazima, 1964) that radiation induced mutation frequencies depend on the metabolic conditions of irradiated cells. With the purpose of obtaining supporting evidences of this assumption, experiments have been carried out to examine the effect of the diapause hormone upon mutation induction.

The extraction of the hormone was performed following Hasegawa's method from ca. 3,000 suboesophageal ganglia extirpated from the pupae of strain C108, which had been incubated during the embryonic stage at 25°C under light condition. The extract was injected as a saline solution into the body cavity of the full-grown larvae. However, all injected individuals died out though every precaution was used with respect to extraction and injection.

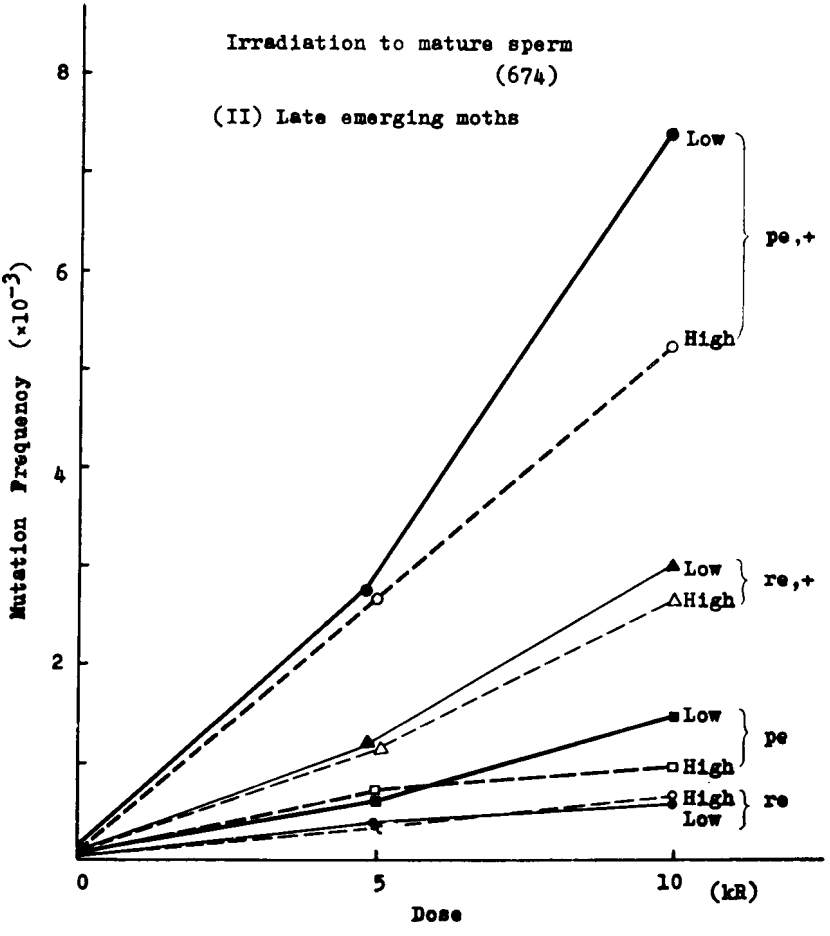
Under those circumstances we were obliged to investigate the effect of a modified diapausing character upon mutation induction by applying low and high temperatures during incubation. It is well known that incubation at low temperature produces non-diapausing egg layers, while high temperature produces diapausing egg layers. Five egg-batches of standard type strain C108 were each divided into two; one group of the half batches was incubated under 25°C (high temperature) and the other under 20°C (low temperature). Both groups were exposed to γ-radiation simultaneously at two developmental stages; i.e. immediately after hatching and after emergence of the moths. Mutation frequencies induced in spermatogonia and mature sperm were estimated by specific loci method.

The group incubated at high temperature produced 100% diapausing egg layers among females, while in the low temperature group 32.2% of females were non-diapausing egg layers. In the latter group moths which emerged at early dates were almost all non-diapausing egg layers and those which emerged at later dates were mostly diapausing egg layers. With the assumption that a similar tendency holds true also in the male, male moths were divided into two groups according to the date of emergence; early emerging and late emerging.

Mutation frequencies obtained for both groups are shown in Fig. 1. A


Irradiation to mature sperm
(674)
(I) Early emerging moths
Mutation Frequency (×10⁻³)
10
8
6
4
2
0
5
10
(kR)
Dose
Low
High
pe,+
Low
High
re,+
Low
pe
High
Low
High
re

(A)



(B)

Fig. 1. Relation between incubation temperature and induced mutation frequency after γ-irradiation to mature sperm in male moths. A: Early emerging moths and B: Late emerging moths.

and B.

As clearly seen from the figures, radiation induced mutation frequencies were higher in the group incubated under low temperature and in moths emerged early which might have been non-diapausing egg layers if they had been female.

These results seem to agree with the observation of one of the authors,

Teulade, that eggs are resistant to radiation during oestivation, but they become sensitive after hibernation.

Difference in the proportion of mosaics among mutants induced by 14 MeV neutrons, γ-rays and some chemical mutagens in silkworm spermatogenic cells

Yataro TAZIMA, Kimiharu ONIMARU and Yosoji FUKASE

In the previous note (this Annual Report No. 17, pp. 97-98) we reported that mutation response of silkworm spermatogenic cells to γ-radiation varies with the progress of development and that the incidence of mosaics in relation to whole body mutations is very low before miotic metaphase, reaches to the unity around V-4.5 (instar V day 4.5) and then increases very rapidly with the progress of spermiogenesis up to 6-10 in mature sperm.

Since mosaics and whole body mutations were assumed to be mutations, the former occurring in one of the double helices of DNA and the latter occurring in both helices, it was considered to be of interest to compare the above values with those obtained with densely ionizing radiations supposed to produce fairly large lesions and those obtained with some chemical mutagens which were known to act on DNA within a very small molecular range. 14.1 MeV neutrons, EMS and mitomycin-C were chosen for our pourpose. Materials and method used for the detection of mutations were similar to those reported previously.

14.1 MeV neutron beams, generated from Cockroft-Walton type machine, were irradiated to the insects at V-1 (germ cells being just before meiotic metaphase) and mid-pupal stages (most of the germ cells being at the stage of young sperm). The irradiation doses were 500 and 1,000 rad.

The ratios of mosaics to whole body mutations thus obtained were 0.1-0.5 at V-1 and 2.3-2.7 at mid-pupal stage, both being more or less lower than those obtained for γ-rays. EMS and mitomycin-C were administered to the insects by injection at stages of IV-3, V-1, mature larvae and early pupae. The doses (concentration and volume) of the injected chemical solutions varied according to the developmental stage of the insect. The incidence of mosaics was surprisingly high regardless of the injected stage. For instance, injection at IV-3, when germ cells are at the stage of late spermatocytes, gave mosaic ratios of 4-8 for EMS and 11-16 for mitomycin-C. By injection at stages later than V-1, the values became much higher. Namely, mutations induced by both chemicals at later stages appeared as mosaics and very few were whole body mutants.

Those observations were in good accord with our expectation that the larger is the mutation lesion the smaller should become the ratio of mosaics to whole body mutations and that the chemical mutagens such as EMS and mitomycin-C will attack selectively single helices of DNA of cells at non-dividing phase.

Stage of γ-irradiation to the germ cells and survival of discovered mutants in the silkworm

Yataro TAZIMA and Kimiharu ONIMARU

Since egg color genes are used in our study as markers for the detection of mutation, induced mutants are discovered at an early embryonic stage. Using this advantage, comparisons have been made with respect to the survival rate among mutants induced at different developmental stages of the germ cell.

The materials used in this experiment were obtained from our study on the variation in mutation response in accordance with the development of germ cells (this Annual Report No. 17, pp. 97-98). The stages compared were III-1, V-4, fully grown larvae and moths. Male larvae of wild type strain C108 were irradiated with 1,000 R γ-rays, while irradiation to male moths was performed with 5,000 R γ-rays. For the detection of induced mutations specific loci method using *pe* and *re* genes was applied. Irradiation experiments were carried out in 1966; in spring irradiation was given to fullgrown larvae, in summer to III-1, and V-4 larvae and in autumn to moths. Mutant *pe* and *re* eggs were collected in the winter of 1966 and were submitted to survival tests in the spring of 1967.

Table 1. Relation between the stage of applied γ-irradiation to the germ cells and survival of the discovered mutants (Expt. 671)

Mutation at the locus	Plot No.	Irrad. stage	Dose	No. of eggs tested	Hatched eggs		Fully grown larvae		Pupae	
					No.	%	No.	%	No.	%
pe	pe-1	III-1	γ-1000 R	164	41	25.0	32	19.5	23	14.0
	pe-2	V-4	〃	609	236	38.8	178	29.2	165	27.1
	pe-3	Full G.	〃	274	148	54.0	135	49.3	134	48.9
	pe-4	Moth	γ-5000 R	250	119	47.6	110	44.0	108	43.2
re	re-1	III-1	γ-1000 R	39	14	35.9	10	25.6	8	20.5
	re-2	V-4	〃	278	138	49.6	89	32.0	81	29.1
	re-3	Full G.	〃	111	75	67.6	72	64.9	71	64.0
	re-4	Moth	γ-5000 R	80	66	82.5	62	77.5	61	76.3

The survival was checked at the following three stages: i.e. at hatching, mounting of fully grown larvae and pupation. The data are given in Table 1.

As can be seen from the Table, 1) hatchability of *pe* mutants was lower than that of *re* mutants, 2) mutants induced at later developmental stages had higher hatchability than those induced at earlier stages, and 3) failure to survive occurred mainly during the embryonic stage of the mutants. The finding (3) seems to suggest that they comprise a fairly large part of gross chromosomal aberrations in addition to point mutations induced by radiations. The finding (2) does not appear to be compatible with our expectation that gross chromosomal aberrations would be unable to pass through moisis. Instead, mutations induced at earlier gemetogenic stages, especially those induced at premeiotic stage showed lower survival rate. Presumablly some change in physical property of genetic material may be responsible for this difference as mentioned in our previous note.

Studies on strain differences in radiosensitivity of the silkworm
III. Comparison of radiosensitivity of a sensitive and a resistant strain during early cleavage cycle

Akio MURAKAMI

Differences in sensitivity to X-rays were examined in about 70 silkworm strains. After X-irradiation to overwintered eggs at an early embryonic stage, their sensitivity with respect to the killing effect was determined in terms of hatchability. The average LD_{50} for 70 strains examined was approximately 1,000 R. LD_{50} of the most sensitive strain, *Kojiki* (170 R), was one-tenth of that of the most resistant strain, *Tenmon* (1,710 R). Since it is known that the radiosensitivity of silkworm embryo is markedly different according to the developmental stage of the embryo (HIROBE and OHI), one must carefully avoid this source of error. In this experiment the sensitivity was compared within one cell cycle at very early cleavage of eggs.

Expt. 1. Eggs from females of a resistant strain *Kansen* (LD_{50}=1,510 R) and a sensitive strain *Kojiki* (LD_{50}=170 R) crossed to males of a marker strain, (*pe re*), and the reciprocal crosses, were irradiated with 1,000 R X-rays. The eggs were kept at 25°C, and irradiated at 10 min intervals during the period from 60 min (Meiosis I) to 230 min (Cleavage II) after oviposition. The results obtained are shown in Fig. 1-a and -b. As can be seen from Fig. 1-a sixfold difference in radiosensitivity was observed between *Kojiki* and *Kansen* at dividing phase, while no difference was observed at interphase. However, in the reciprocal crosses, (*pe re*×*Kojiki*)

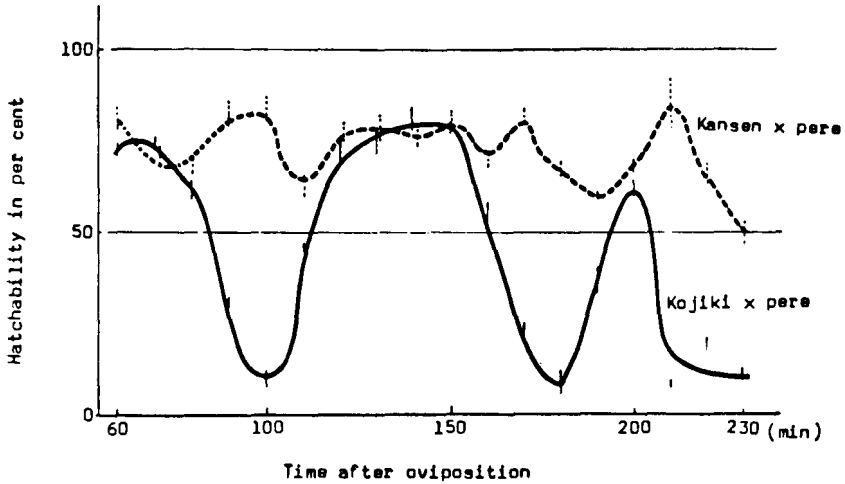


Fig. 1-a. Effect of X-rays on the hatchability during meiosis and early cleavage in oöcytes and/or in eggs of different sensitive strains of silkworm.

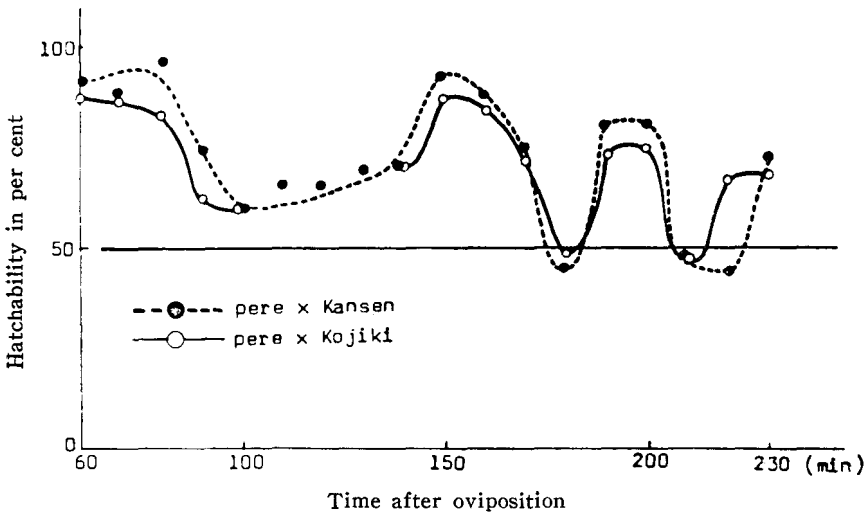


Fig. 1-b.

and (*pe re*×*Kansen*), the difference in sensitivity was almost nil between the two crosses regardless of different radiosensitivity between the strains used as male parents (Fig. 1-b). These findings suggest that the cytoplasmic factor is, at least in part, responsible for the differential

radiosensitivity among different silkworm strains.

Expt. 2. Eggs from inbred strains of *Kansen* and *Kojiki* and from hybrids of, in both directions, reciprocal crosses between the two strains were used as material. The eggs were kept at 25°C after deposition. 100 min eggs and 270 min eggs, corresponding to the stage of cleavage II and to cleavage III, were irradiated with 1,000 R of X-rays at 10 min intervals. The results obtained are presented in Figs. 2-a and -b for inbred- and hybrid-eggs, respectively. As shown in Fig. 2-a *Kansen* was 7–8

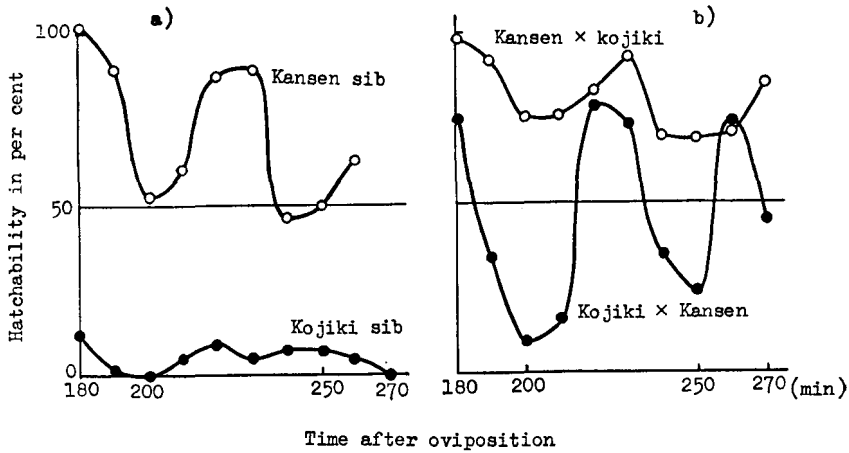


Fig. 2. Effect of X-rays on hatchability during early cell cleavage of inbred-eggs of two different strains in sensitivity and of hybrid-eggs obtained from the reciprocal crosses.

times as resistant as *Kojiki* and the difference between the two inbred strains was more pronounced at the resistant phase (or interphase). It is also seen from the figure that the resistant strain, *Kansen*, shows clearly a cyclic change in radiosensitivity, while no such change is observed in the sensitive strain *Kojiki*. (Fig. 2-b) Perhaps the latter strain is too sentitive to radiation. At any rate it was concluded from these experiments that an intrinsic difference is actually involved in the observed difference in radiosensitivity at early embryonic stage, although it may be modified to a great extent by the stage of embryonic development.

Studies on strain differences in radiosensitivity of the silkworm
IV. Variation in radiosensitivity to different X-ray doses during an early embryonic stage

Akio MURAKAMI

It was reported in my previous note that a marked cyclic change was observed in radiosentitivity in accordance with the division cycle of cleavage nuclei at an early developmental stage of the silkworm. These findings indicated that cells in the dividing phase were markedly sensitive to killing action of radiation, while those in interphase were not. These conclusions were obtained only after irradiation with 1,000 R X-rays. In order to obtain a better understanding of sensitivity changes with respect to the cell cycle, the present work was carried out with X-ray doses of 0, 500, 1,000, 1,500 and 2,000 R. Eggs were kept at 25°C from 210 min through 300 min after oviposition (during this time the eggs proceeded from cleavage I to cleavage III) and were irradiated with X-rays (180 kVp,

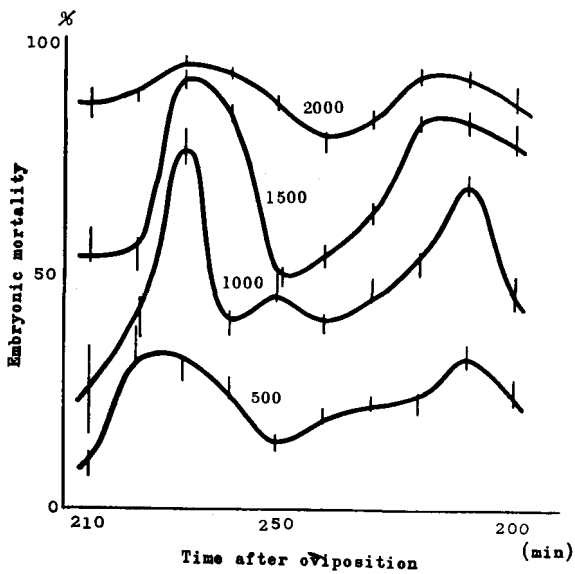


Fig. 1. Cyclic change in radiation sensitivity observed in synchronized early cleavage cells of silkworm for four different dose levels. The two highest peaks and the two lowest troughs for embryonic mortality correspond to cells at metaphase/anaphase and at interphase/prophase. The egg-age of 210 to 230, 240 to 290 and 300 minutes corresponds to the stage of 2, 4 and 8 nuclei respectively. The standard error of the mean of each point is given.

25 mA, 1.0 mm Al filter and dose-rate at 1,000 R/min) at 10 min intervals. The eggs used as material for wild type were F_1 eggs between females of C108 strain and males of a marker strain *pe re* which permitted simultaneously the examination of radiation-induced mutation frequencies. The eggs were collected every 10 min at 25°C. The radiosensitivity was measured in terms of embryonic mortality.

The relation between egg age and mortality for four different doses is shown in Fig. 1. Mortality increased with the does for the whole dose range and a cyclic change in radiosensitivity was observed within approximately 60 min cycle. With medium doses (1,000 and 1,500 R) a marked difference appeared with respect to sensitivity, while no clear-cut difference was observed with doses lower than 500 R and higher than 2,000 R. Such difference in sensitivity pattern expressed by the different doses applied may be explained by a differential reparability of the cells. Namely when cells are treated with higher doses, even those in resistant phase cannot repair radiation lesions, while after irradiation with lower doses, the cells in sensitive phase may be capable to repair them to in the same degree as when they were in the resistant phase. The dose-killing curved for 230 and 290 min-old eggs (both corresponding to sensitive phase) exhibited narrower shoulders than those for 210 and 260 min old eggs (both corresponding to resistant phase). The wide shoulders observed for cells at resistant phase seem to pepresent the existence of an active repair system which becomes incapacitated at higher X-ray doses.

Studies on strain differences in radiosensitivity of the silkworm
V. Relation between X-ray and UV-sensitivity in an early embryonic stage

Akio MURAKAMI

In a previous note marked differences in X-ray sensitivity were reported among strains with respect to the killing of the embryo. In the most resistant strain LD_{50} was approximately 7-8 times as high as that of the most sensitive strain. Recently a repair mechanism of UV-induced damage has been reported for microorganism. This posed the question whether or not a repair system for UV damage may be common phenomenon or at least may function also in some damages caused by ionizing radiation. Hence, this experiment was carried out. Materials used were some silkworm strains of different X-ray sensitivity: *Kansen* (1,510 R) and *Aojuku* (1,480 R) and *Kojiki* (170 R). LD_{50} values obtained for embryonic killing are given in parentheses.

UV-irradiation was applied to the eggs at an early cleavage stage with

a commercial germicidal lamp (*National Electric Co.*). Care was taken to avoid direct photoreactivation by visible light and all irradiation experiments were done in a dark room. Doses were adjusted by changing the duration of irradiation but keeping the distance to the lamp constant. The dose-rate used was 143 ergs $mm^{-2} sec^{-1}$. Irradiation was applied to 200, 210 and 220 min-old eggs (which corresponded to the 2nd cleavage) obtained from females mated with *pe re* males. Eggs were collected at 10 min intervals at 25°C. The UV sensitivity was measured in terms of hatchability.

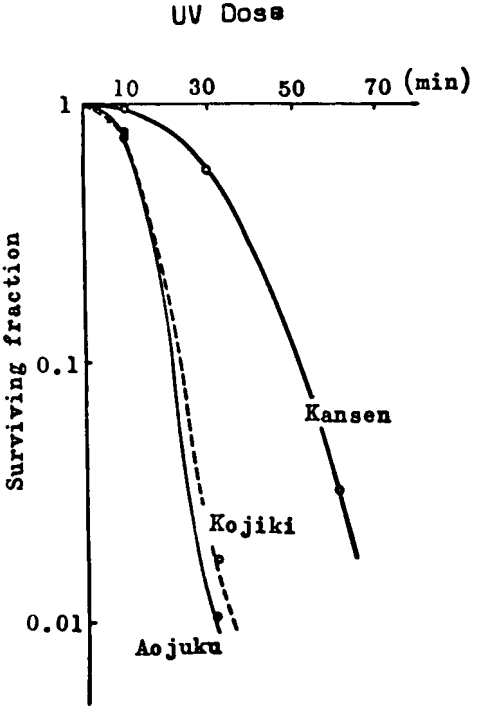


Fig. 1. Dose-hatchability curves for silkworm eggs (220 min-280 min after oviposition) irradiated with ultraviolet (dose-rate: 143 ergs $mm^{-2} sec^{-1}$).

The dose-response curve at 220 min old-eggs is presented in Fig. 1. As can be seen in the figure, the radioresistance of *Kansen* (LD_{50}: 10.3×10^4 ergs mm^{-2}) to UV-killing is approximately threefold higher than for both strains, *Kojiki* (LD_{50}: 27.5×10^4 ergs mm^{-2}) and *Aojuku* (LD_{50}: 27.5×10^4 ergs mm^{-2}). It is very interesting to note that the radiosensitivity of

strain *Aojuku* to UV-light has quite the same value as that of *Kojiki*, however, strain *Aojuku* has approximately 7 times higher radioresistance to X-ray killing in comparison with strain *Kojiki* as described earlier. There was the same situation observed for 200 and 210 min old-eggs even if the shape of dose-response relation was considerably different from the treated egg-age (or the time at the start of UV irradiation). This evidence suggests that the nature of UV-sensitivity to embryonic killing depends on the cell phase at the time of irradiation.

It is clear from the present experiment that no phenomenon of cross-resistancy between X-ray and UV light sensitivity to killing effects of silkworm embryos was observed. This suggests that the mechanism for X-ray killing and repair of silkworm embryos may be different from those for UV-light.

Relative biological effectiveness of fast neutrons for the induction of dominant lethals at various stages of male germ cells in the silkworm

Akio MURAKAMI

In the present study, the effect of fast neutrons and γ-rays on the induction of dominant lethal mutations was compared for various stages of male germ cells.

The sources of fast neutrons used were, 14 MeV neutrons derived from *T(d, n)He* reaction and 1.5 MeV neutrons from ^{235}U fission reaction. γ-rays were obtained from ^{137}Cs.

Irradiation experiments were performed at three germ cell stages: primordial germ cells in the embryos, early and late spermatogonia in early larval stages and mature sperm in the oldest pupae.

The incidence of dominant lethals was expressed by the percentage of non-hatched eggs of the total number of fertilized eggs. After exposure, the males of wild type strain C108 were mated to females having the marker genes *pe* and *re*.

The dose-response curves for the mature sperm were linear for both radiations, hence, the RBE was obtained very easily. However, for other germ cell stages, the relation was not linear and such straight direct comparison was impossible. Consequently, RBE values are represented arbitrarily as an inverse ratio of the respective doses required for the survival of 80% and 50%. They are given in Table 1.

The values for the relative efficiency of neutrons are larger for mature sperm than for pre-meiotic cells, *i.e.*, primordial germ cells and spermatogonia. In spermatogonia the incidence of dominant lethals was clear-

Table 1. Relative efficiency of ^{137}Cs γ-rays, 14 MeV fast neutrons and 1.5 MeV fission neutrons on the induction of dominant lethals in silkworm.

Germ cells	Radiations	R. B. E. Survival level 50%	80%
Primordial germ cells (egg stage)	(14 MeV neutrons / X-ray)	1.6	1.3
Spermatagonia (larval stage)	(14 MeV neutrons / X-ray)	4.4	3.6
	(1.5 MeV neutrons / X-rays)	11.2	9.5
Mature sperm (adult stage)	(14 MeV nentrons / X-rays)	8.2*	

* Calculated from the slope of the regression lines.

ly dependent on LET, as was previously observed for recessive visible mutations. However, the LET dependence of dominant lethals is more pronounced than that of recessive mutations, suggesting that the target size is larger for dominant lethals than for recessive mutations.

As to the interpretation of the stage difference in RBE, it may not be unreasonable to assume that germinal selection operates far more severely for dominant lethals produced in primordial germ cells than in sperm.

In spermatogonia, the RBE of 1.5 MeV fission neutrons was approximately 2 times as high as that of 14 MeV neutrons.

Effects of X-irradiation on aggregate-forming activity of embryonic chick and quail liver and heart cells

Yukiaki KURODA

Dissociated cells from embryonic tissues of organ rudiments, when cultured in gyrating flasks, formed aggregates having morphologically and biochemically tissue-specific characteristics. The patterns of the aggregates depend on the initial cell concentration, speed and temperature at which the rotation cultures are carried out. Under a constant condition, the cells show a consistent aggregation pattern; the effects on it of various external factors can be quantitatively estimated.

Livers and hearts were obtained from embryonic chicks and quails at various stages and were dissociated into single cells by treatment with trypsin. Cell suspensions each containing 10^6 cells per ml were introduced into Petri dishes and treated with 0R, 200R, 400R, 600R, 800R, 1,000R, and 1,200R of X-rays (175 KVp, 25 mA, distance 40 cm, filter 1.0 mm Al, dose rate 300 R/min). After irradiation 3 ml of each cell suspension treated with various X-ray doses were transferred into 25-ml Erlenmeyer flasks, which were rotated on a gyratory shaker at 70 rpm at 38°C.

The effects of X-irradiation on aggregation of dissociated 10-day embryonic chick liver and heart cells are shown in Table 1.

Table 1. Effects of X-irradiation on aggregation of dissociated embryonic chick liver and heart cells *in vitro*. (3×10^6 cells were rotated for 48 hours at 38°C at 70 rpm).

X-ray dose (R)	Liver cell aggregate		Heart cell aggregate	
	Average diameter (μ)	%	Average diameter (μ)	%
0	190.0	100	325.0	100
200	220.0	116	416.8	128
400	227.5	120	450.0	138
600	254.3	134	500.0	154
800	248.0	131	425.0	131
1,000	238.0	125	418.0	129
1,200	218.0	115	410.8	126

As shown in Table 1, aggregates obtained from rotation cultures of dissociated chick liver and heart cells were gradually increasing in the average diameter with the amount of X-rays until 600R, then decreasing it with still higher X-ray doses.

When liver and heart cells dissociated from 7-day and 10-day old embryonic quails were irradiated with various doses of X-rays, then tested for their aggregability in rotation culture, the average diameter of the resultant aggregates was increasing gradually with the X-ray dose until 400R, then decreasing with still higher doses.

It has previously been found that aggregate-forming substances which were produced from dissociated cells act as intermediates in the formation of aggregates and that such substances may be produced through RNA and protein syntheses (Moscona, 1961, 1963).

The findings in the present experiments suggest that the low doses of X-ray may stimulate the activation of aggregate-forming substances already present at the cell surface and the higher doses of X-ray may in-

hibit the new production of these substances through suppression of RNA or protein synthesis.

Skeletal abnormalities in the F_1 offspring of neutron-irradiated male mice

Kiyosi TUTIKAWA

Ehling has recently reported that part of overall genetic damage from irradiation can be detected by studies of skeletal abnormalities in the first generation of mice. He has also emphasized that the most sensitive indication of mutations in skeletal studies is probably a statistically significant increase of Class-1 multiple abnormalities and Class-1 abnormalities of the bilateral type of the appendicular skelton.

The present experiment was designed primarily to obtain an estimate of the frequency of neutron induced presumably dominant mutations affecting the skeleton. The following different preparation methods were used; the part of skeleton involving skull, cervical vertebrae and the first three thoracic vertebrae were macerated by means of the proteolytic enzyme papain, and the remaining parts were prepared by a modification of Dawson's technique.

The irradiation with 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. Male CBA strain mice were irradiated with doses of 242.5 and 485 rad neutrons, and subsequently the males were mated to PW strain females which were established as an inbred strain in our laboratory and are homozygous for six recessive genes (*a*, *b*, c^{ch}, *d*, *p*, and *se*). The F_1 offspring were sacrificed when about six weeks old, and the animals were examined by means of prepared skeletal specimens.

The examination of skeleton is still in progress. So far, only a small number of the papain-digested skeletons among all prepared specimens have been examined in detail. Animals with Class-1 single or multiple abnormalities of the axial skeleton which probably correspond to Ehling's classification, are briefly described as follows (single animals are identified by Arabic numerals);

1) *Control group*: (#188)Malformation of the skull similar to hydrocephalus. 2) *Offspring derived from irradiated males with 242.5 rad neutrons in postspermatogonial stages*: (#73) Malformation of the skull, shape anormalies of the interfrontal bone and foramen ovale (both), presphenoid-basisphenoid fusion, small body size; (#117) abnormal frontals, presphenoid-basisphenoid fusion, dyssymphysis of the C7 and Th1, small

body size; (#230) foramina transversaria imperfecta in the C3, C4, C5 on both sides and C6 on the right side, small body size. 3) *Offspring derived from irradiated males with 485 rad neutrons in postspermatogonial stages*: (#17) Malformation of the skull, shape anomalies of the interfrontal bone and foramen ovale (both), abnormal elongation of the incisor, presphenoid-basisphenoid fusion, basisphenoid-occipital fusion, dyssymphysis of the C4, foramina transversaria imperfecta in the C3, C5 on both sides and C4 on the right side, small body size; (#23) malformation of the skull, absence of the right tubercula anteria in the C6, small body size.

In the examination of these limited parts of the axial skeleton, the abnormality ratio per gamete for the 242.5 rad group was 0.0099 (3 out of 302 offspring), 0.0196 in the 485 rad group (2 out of 102 offspring), and 0.0033 in the control group (1 out of 303 offspring). The excess of abnormalities per rad was 2.7×10^{-5} for the 242.5 rad group and 3.3×10^{-5} for the 485 rad group with respect to irradiation in postspermatogonial stages. A more precise estimate of the frequency of neutron induced presumably dominant mutations affecting the skeleton will be possible when the experiment is complete.

VIII. RADIATION GENETICS IN MICROORGANISMS AND PLANTS

Selective Growth of Radiation Sensitive Mutants in *Escherichia coli* K12

Tsuneo KADA

Among many auxotrophic strains regarding threonine obtained from *Escherichia coli* K12 HfrH after ultraviolet irradiation, a strain named Hfr 1014 had some particularities. When cells grown in broth were washed and plated densely on threonine-free synthetic medium, some fast-growing colonies appeared very frequently (more than 10^{-3}). It was found that bacteria purified from these colonies had stronger sensitivities to either ultraviolet light or γ-rays than the parent strain Hfr 1014. Cells formed long filaments after very mild doses of radiations. Survival curves were very similar to those of fil^+ or lon^- strains.

The following experiments suggest that the strain 1014 has a deficiency at the usual threonine locus and an inactive suppressor gene for the strain which is linked to the leucine locus. This is indicated by thr^-_{1014} (Su^+). First, Hfr H (prototroph except B_1^-, Sm^s) was crossed to PA 309 (thr^- leu^- try^- his^- arg^- B_1^- lac^- gal^- man^- Sm^r) for 10 minutes and a threonine-independent strain witdout modification in other characters was isolated (PA 309 thr^+). Threonineless strains were then obtained by direct selection from a 5 minute conjugational culture between Hfr 1014 and PA 309 thr^+. These thr^-_{1014} strains were not leaky and their spontaneous reversion frequencies were between 10^{-6} and 10^{-7}. In the next experiment, Hfr 1014 was crossed to one of thr^-_{1014} recombinants named PA 309 thr^-_{1014} (thr^-_{1014} leu^- try^- his^- arg^- B_1^- lac^- gal^- man^- Sm^r) for 20 minutes and leu^+ Sm^r recombinants were selected. It was found that about 80% of them became concomitantly prototrophs as to threonine. Production of threonine prototrophs is not expected if both donors and recipients were complete threonine auxotrophs. It is reasonable to suppose that a leucine-linked suppressor gene exists in the Hfr 1014 and that it might have been transmitted into PA 309 thr^-_{1014} and suppressed this time actively thr^-_{1014}.

Because suppressed threonineless bacteria (thr^-_{1014} Su^+) grow scarcely in the absence of threonine, fast growing colonies were easily selected with high frequencies by plating these bacteria on threonine-free plates. The possibility that they reverted to thr^+ state involving true mutation in the threonine locus may be improbable because we know that frequencies of spontaneous true reversion in thr^-_{014} Su^- strains are low (between 10^{-6}

and 10^{-7}). On the other hand it seems more reasonable to suppose that the frequent production of fast-growing colonies must be related to some division abnormalities and that the genetic determinant(s) responsible for filamentation may promote specifically the growth of Su^+ cells. Present studies on mapping our fg (fast-growing) gene(s) indicated that they are located in the gal region of the chromosome.

Radiosensitization by Potassium Iodate and Related Compounds

Teuneo KADA

Potassium idoate and certain related compounds were found to be very efficient radiosensiltizers; their presence at non-toxic levels during irradiation increased radiation-induced cellular lethality. The test-strain used in these experiments was a threonineless and thiamineless derivative of *Escherichia coli* K12 Hfr H. The two auxotrophic characters of this strain were prepared for other purposes and are of no significance for the results described; the wild K12 strain behaves quite similarly.

Bacteria were grown exponentially in liquid broth, washed twice with M/15 phosphate buffer and resuspended in the same solution. Potassium iodate and other related compounds were dissolved in distilled water at diverse concentrations and sterilized by filtration. Usually one volume of reagent solution was combined with nine volumes of air-saturated bacterial suspension just before irradiation in order to obtain the desired drug concentrations. The bacterial suspension contained approximately 1×10^6 cells per ml at the time of irradiation. Air-saturated suspensions held at the melting temperature of ice were irradiated with γ-rays of ^{137}Cs at a dose rate of 667 rads/min. Survivors were estimated by the surface count technique of living bacteria on nutrient broth agar.

The effects of several chemicals on radiation inactivation of test bacteria were indicated in Table 1. Among the compounds tested, iodate, periodate anh paraperiodates possessed marked radio-sensitizing capacities at concentrations at which they were not toxic to non-irradiated bacteria. When the iodine atom was replaced in iodate by other halogen atoms, no sensitizing capacity was observed as was found in cases with chlorates or bromates. This also indicates that alikali ions are not responsible for the sensitization. Detalied studies with potassium iodate showed that the sensitizing capacity was pH-dependent and the maximum effect was found between pH 5 and pH 6. Because periodates are known to be mild oxidizing agents, the possibility is under examination if their reaction with cellular sulfhydryl key molecules might be involved under

Table 1. Sensitizing effects of potassium iodate and related compounds on γ-ray inactivation of colony-forming capacity in *Escherichia celi* K12

Reagnt present during irradiation (concentration)	pH of bacterial suspension	Radiation dose (R)	No. of viable cells per ml
None	7.0	0	1.0×10^{6}
	7.0	1290	8.6×10^{5}
	7.0	1460	7.7×10^{5}
	6.0	1290	8.1×10^{5}
	5.0	1290	6.4×10^{5}
Potassium iodate (2 mM)	7.0	0	1.1×10^{6}
	7.0	1290	3.8×10^{4}
	7.0	1460	5.9×10^{3}
	6.0	1290	1.0×10^{3}
	5.0	1290	0.9×10^{3}
Potassium periodate (0.2 mM)	7.0	0	1.0×10^{6}
	7.0	2000	5.2×10^{4}
Trisodium paraiodate (0.2 mM)	7.0	0	9.3×10^{5}
	7.0	2000	7.1×10^{4}
Potassium chlorate (2 mM)	7.0	0	1.1×10^{6}
	7.0	1460	7.4×10^{5}
Potassium bromate (2 mM)	7.0	0	9.6×10^{5}
	7.0	1460	9.1×10^{5}

irradiation.

Photoreactivation of UV-Induced Mutations in Maize

Tarô FUJII

Photoreactivation of UV-induced endosperm mutations (*Bz*-gene) was already reported by the present author (cf. Ann. Rep. 17 : 117-118). Besides the *Bz*-gene the materials used in that experiment had the dominant and the recessive Yg_2 and yg_2 gene (yellow green character) in male and female stocks, respectively. Mutation from *Yg* to *yg* and its photorecovery was examined in F_1 seedlings. The results are given in Table 1. Very narrow stripes of yellowish-green color were observed in several seedlings but partial mutations which appeared as sectors on a half or

one-fourth of the leaf area were very seldom. Most of these narrow sectors might be caused without *Yg*→*yg* mutation because their frequency is much lower than that of whole mutation (in *Bz*-gene, the proportion

Table 1. Frequency of *yg*-seedlings

Treatment			No. of seeds	No. of *yg*-seedlings (%)	No. of seedlings with narrow stripes (%)	Others* (%)
Control		Dark	3479	6 (0.17)	1 (0.03)	2 (0.06)
		PR	2516	10 (0.40)	0	0
γ-ray	250 R	Dark	4971	15 (0.30)	6 (0.12)	4 (0.08)
		PR	3944	28 (0.71)	1 (0.03)	3 (0.08)
	500 R	Dark	6134	41 (0.67)	9 (0.17)	8 (0.13)
		PR	6437	42 (0.65)	3 (0.05)	3 (0.05)
	750 R	Dark	3596	46 (1.28)	1 (0.03)	1 (0.03)
		PR	6569	71 (1.08)	4 (0.06)	16 (0.24)
UV	15 sec	Dark	2420	6 (0.25)	1 (0.04)	3 (0.12)
		PR	6182	11 (0.18)	1 (0.02)	4 (0.06)
	30 sec	Dark	4106	29 (0.71)	0	0
		PR	675	1 (0.15)	0	0
	60 sec	Dark	549	5 (0.91)	1 (0.18)	1 (0.18)
		PR	5904	12 (0.20)	0	3 (0.05)
	90 sec	Dark	4557	12 (0.26)	2 (0.04)	2 (0.04)
		PR	4490	20 (0.45)	6 (0.13)	1 (0.02)

* No. of seedlings with white stripes or other kinds of chlorophyll deficiency.

of partial mutation to whole mutation was comparable). Further, white stripes or other kinds of chrolophyll deficiency were also observed, but the occurrence of these mutant seedlings is left out of consideration.

In the gamma-ray lots, mutation frequency after dark-treatment showed some fluctuations according to the dosage but it was possible to judge from the results that the post-treatments had no effect on gamma-ray exposures. In the UV-exposures, on the other hand, the saturation effect of mutation frequency at the highest dosage lot after dark-treatment and a clear decrease in mutation frequency after PR-treatment were again observed. Namely, a photorecovery phenomenon was observed only in UV lot as was observed in endosperm mutations.

Endosperm Mutations in Maize Induced by Fast Neutrons

Tarô FUJII

For the promotion of RBE study, 14 MeV neutron generator was

installed last year. Irradiated by 14 MeV neutrons and gamma-rays maize pollen with *Bz*-gene was crossed on the recessive stock. Mutation from *Bz* to *bz* could be observed in the F_1 seeds with bronzy aleuron color and the results are given in Table 1. Neutron flux was calculated with S, P reaction, and the neutron doses applied to pollen were decided

Table 1. Endosperm mutations after pollen irradiations

Treatment	No. of seeds	Average No. of seeds	No. of mutant seeds (per cent)	
			Whole	Partial
Control	2927	146.4	1 (0.034)	2 (0.068)
Gamma-rays				
300 R	2821	111.8	1 (0.065)	3 (0.106)
600 R	8642	96.0*	17 (0.197)	14 (0.162)
900 R	5230	118.9*	46 (0.880)	36 (0.688)
1500 R	14231	151.4	256 (1.799)	219 (1.539)
Neutrons				
154 rad	4631	178.1	31 (0.669)	24 (0.518)
284 rad	6973	120.2*	104 (1.491)	79 (1.133)
295 rad	5346	130.4*	65 (1.216)	92 (1.721)
545 rad	7470	138.6	170 (2.406)	234 (3.312)
2034 rad	3058	68.0	163 (5.330)	268 (8.704)
3700 rad	1157	19.3	85 (7.347)	99 (8.557)
10735 rad	72	3.6	1 (1.389)	9 (12.500)

* Some of the seeds were obtained from potted plants, therefore their seed set was more reduced than in plants grown in the experimental field.

by the first collision dose calculated on the basis of a chemical pollen analysis. Average seed set was not reduced in gamma-ray lots at the dosages used, but it clearly decreased in the neutron lots of more than 2000 rad. Two types, whole and partial mutation, were induced by both radiations. Frequency of the latter was slightly higher than of the former in gamma-ray lots, and the proportion of whole to partial mutations did not depend on the dosage. In the neutron lot, the proportion was the same as for gamma-rays in lower dosage lots, however the reverse relation was observed at dosages higher than 295 rad. Moreover, the increase in frequency of whole mutations became flattened in higher dosage lots in spite of the clear increase in partial mutations in these lots. Whole or partial mutations may arise from radiation damage of chromosome or chromatid units, respecitvely. If this assumption is correct, whole mutations should be associated with semilethal lesions or

feeble viability. Therefore, pollen grains which suffered radiation damage leading to whole mutations may have less survivals than those with partial mutations.

It is obvious that the mutation frequency after neutron irradiation is very much higher than that after gamma-ray irradiation; by gamma-ray irradiation 1.8% of whole mutation was induced at 1500 R, while it could be induced at less than 545 rad by neutron irradiation. As to partial mutation frequency, almost the same frequency was observed at 1500 R of gamma-rays and 295 rad of neutrons. The material employed in the present experiment had also the yg_2-gene and mutation from dominant to recessive could be observed in the F_1 seedlings. Examination is now underway and RBE value in the mutation frequency by pollen irradiation will be assessed after the experiments.

Sectorial Mutations Induced by Gamma-Ray Treated Fertilized Maize Endosperm

Tarô FUJII

Mutation frequency of whole and partial mutations was investigated after pollen irradiation. In this experiment, no partial mutations of yg_2-gene (plant color) in the F_1 seedlings was observed while both whole and partial mutations of *Bz*-gene (aleuron color) were found in the F_1 seeds (cf. pp 102 in this issue and Ann. Rep. 17 : 117–118). It is assumed that the differentiation into root and leaf occurred in the fertilized egg immediately after fertilization, and therefore, no partial mutations which are induced by the damage of chromatid units, could be observed. Pollen having *Bz* and *Yg*-genes was applied to a recessive stock, and the female plants were 24, 48, and 72 hours after pollination exposed to 1 kR of gamma-rays. Frequency of whole and partial mutations and relation between the frequency and exposure time were investigated in F_1 seeds and seedlings; the results are given in Table 1. Apparently, there were no appreciable radiation effects on their maturing and fertility.

In endosperm mutations, frequeny of whole mutations (*Bz*-gene) was clearly lower than after pollen irradiation even in the 24 hr lot, and it further decreased to about one half in 48 hr lot. The same frequency was observed in the 72 hr lot. On the contrary, frequency of partial mutation markedly increased in the 24 hr lot and about one half or more of the examined seeds had partial mutations in 48 and 72 hr lots. Most of these seeds had two or more mutated sectors per seed. The decrease of whole mutations was related to the increase of the partial kind.

In the F_1 seedlings, frequency of wholly mutated seedlings (*yg*-gene)

Table 1. Relation between the time from pollination to irradiation and frequency of whole and partial mutations

	Time between pollination and irradiation	No. of seeds or seedlings	Number of mutants (%)	
			Whole	Partial
Endosperm (*bz*-gene)	Pollen*	8858	72 (0.81)	51 (0.58)
	24 hr	5898	2 (0.03)	1744 (29.57)
	48 hr	6079	1 (0.02)	3986 (65.57)
	72 hr	6888	1 (0.02)	4100 (59.52)
Seedling (*yg*-gene)	Pollen*	7689	89 (1.16)	3** (0.04)
	24 hr	4110	65 (1.58)	28 (0.68)
	48 hr	4539	42 (0.93)	74 (1.63)
	72 nr	5614	25 (0.45)	389 (6.93)

* Results from the gamma-ray 900 R irradiation to pollen grains.

** These seedlings had very narrow yellowish-green stripes; see text.

in the 24 hr lot is the same as in pollen irradiations (the difference in dosage between two lots seems to be out of the question: it is merely 100 R), and it decreased to about one half and more according to the lapse of time after the pollination, i. e. 48 and 72 hours. But mutation frequency of the partial kind increased with passing time and that of the 24 hr lot was ten times higher than that of pollen irradiations. (Yellowish green stripes on seedlings after pollen irradiation could not be determined as partial mutation because of very narrow sectors; partial mutation could be expected to have sectors occupying a half or one-fourth of the leaf area). The frequency in the 48 hr lot was about twice as that in the 24 hr lot; it was inversely proportional to the whole type, and that in the 72 hr lot was about four times higher in spite of the decrease to one half of whole mutations. Thus decrease of whole and increase of partial mutations both in *Bz*- and *Yg*-genes with increasing time between pollination and irradiation showed a reciprocal relation in 24 and 48 hr lots, but the relation was complicated in 72 hr lot; the phenomenon observed in that lot could be the result of a large number of target cells according to repeated cell divisions.

In the present procedure, partial mutation could be observed in F_1 seedlings, but it could not be considered to be on effect of the damage suffered by chromatid units as was observed in pollen irradiations.

Modification of Killing Efficiency in Neutron Irradiations of *Arabidopsis* Seeds

Tarô FUJII

Dry and 24 hour steeped seeds of *Arabidopsis thaliana* were used. Thermal neutron exposures were carried out by the pneumatic tube Kyoto University Reactor at the output of 50 kW, the total neutron flux being 3.9×10^{12} at the minimum and 7.1×10^{13} at the maximum. The neutrons consisted of about 90% thermal and 10% fast neutrons, and the neutron field was strongly contaminated by gamma-rays. The same materials were also exposed to monoenergetic 14 MeV neutrons from T(d, n) reaction neutron generator in our institute. Total neutron flux was $3.8\times10^{11}\sim1.8\times10^{13}$. Decrease of survival rates after irradiation is shown in Table 1 together with the results from gamma-ray experiment for comparison with three kinds of radiation. In the experiment with thermal neutrons, severe killing effect was again observed like in our previous experiment: almost all plants died in the 71.5×10^{12} neutron lot of dry seeds and a similar survival rate was observed in the 35.6×10^{12} lot of wet seeds, while 37.5 kR of gamma-rays alone had no effect in the former case and 16.5 kR might have had a only slight effect in the latter case

Table 1. Killing effects of gamma-rays and neutrons for dry and wet seeds of *Arabidopsis*

Gamma-rays			Thermal neutrons			Fast neutrons		
Dosage (kR)	Dry (%)	Wet (%)	Dosage ($\times10^{12}$)*	Dry (%)	Wet (%)	Dosage ($\times10^{11}$)	Dry (%)	Wet (%)
10	91.6	92.7	3.9 (2.5)	80.7	76.7	3.8	85.8	83.9
30	90.8	75.6	15.4 (7.5)	85.2	39.7	4.6	80.6	78.6
50	92.8	21.7	26.6 (12.5)	72.5	6.0	15.5	73.9	51.0
70	93.3	0.0	35.6 (16.5)	72.5	2.1	19.0	66.2	32.4
100	82.4	0.0	43.0 (22.5)	56.3	0.0	35.0	64.2	34.6
			53.1 (27.5)	40.9	0.0	176.0	1.1	0.0
			60.8 (32.5)	14.1	0.0			
			71.5 (37.5)	1.9	0.0			

* Figures in parentheses show the contaminating gamma-rays in kR.

when compared with the results with gamma-rays. Thus the killing effect of thermal neutrons was very high, and it seems to have been the effect of high LET radiations modified by the water content of the material. But the assessment of thermal neutrons' RBE is very complicated because of its multienergetic condition and abundance of contaminating gamma-rays.

Fast neutron irradiations to dry and wet seeds also showed the effect of water content. Survival rates in wet seeds at 19 or 35×10^{11} neutron lots were about half of those obtained from dry seeds. Fast neutron doses given to seeds were calculated from the first collision dose converted from the given tissue dose by using chemical seed analysis, and dosage in rad unit was reckoned from the multiplid total neutron flux by 5.98×10^{-9}. From the calculations 3.8 and 35.0×10^{10} neutrons are equivalent to 2.27 and 20.93 krad, respectively. When the results were compared with that of gamma-rays, fast neutrons were nearly 10 times more effective in dry seeds and about 5 times more effective in wet seeds at LD_{50} level. Namely, modification of radiation damage due to water content was also observed in 14 MeV neutron exposures. Nevertheless, the modification factor is too large in the present experiment, though the environmental modification of radiation effect is, as a general rule, very small in high LET radiation. The problem is now to examine somatic mutations with specific locus method in order to throw a light on the modification of high LET radiation's damage due to water content.

Storage and Water Content Effects on Radiation Damage in Wheat

Tomoo MABUCHI and Seiji MATSUMURA

Dry seeds of diploid wheat containing 6.0, 12.5 and 15.0% water were sealed in ampules filled with oxygen, nitrogen or air and exposed to acute and chronic gamma-rays under room temperature (20°C) or dry ice temperature (ca. −80°C). In general, chronic irradiations were found to cause more inhibition than the acute ones, especially at higher irradiation doses (15 kR). There was no clear difference in the results between air and oxygen treatments after either acute or chronic irradiation. On the other hand, nitrogen treatments clearly showed a protective effect against the inhibition of seedling growth, especially under acute irradiation. Unexpectedly, post-irradiation storage had no effect on growth inhibition under acute irradiations. As to seed fertility, there was no marked difference between acute and chronic irradiations, and also between air and oxygen treatments. In respect to mutation, chronic

irradiations were found to be more effective than acute ones. On the other hand, in acute irradiations, a clear intensification of mutation induction due to storage was found only in nitrogen treatments. Comparison of air, oxygen and nitrogen clearly showed that nitrogen had a protectiv effect on mutation frequencies.

Seeds with 12.5% water content were somewhat inhibited by a 15 kR dose in air-filled ampules. In dry seeds (6.0%), a clear difference due to radiation dose and dose rate as well as post-irradiation storage and nitrogen treatment was observed. Seedling growth was markedly inhibited by acute gamma-irradiation at room temperature. However, there was a slight inhibition by exposure at dry ice temperature as compared with the non-irradiated control. A protective effect against gamma-ray damage was also observed both at room and dry-ice temperature. It should be noted that the storage effect could not be completely eliminated by combined treatments of low temperature and nitrogen under our experimental conditions. From experiments arranged with the intention to show the effects of varying temperature during treatment and storage, it was found that temperature during irradiations is the primary factor and storage temperature is of secondary importance as far as seeding growth is concerned.

Wave Length Dependence of UV Damage in Maize Pollen

Etsuo AMANO

As a preliminary experiment for future detailed investigation of photoreactivation in maize pollen (cf. Ikenaga and Mabuchi 1966, Rad. Botany *6*: 165–169), monochromatic ultraviolet (UV) lights were tested. Linked endosperm marker genes on chromosome 9 were used as the genetic system. Pollen of a multiple dominant stock, *C^I Sh Bz Wx, Y*, was irradiated in a single layer by monochromatic output of UV monochrometer (Bausch and Lomb, 500 mm, 1200 grooves/mm, F: 4.4). A 100 W short arc super high pressure mercury lamp (Ushio USH-100 D) was used as light source. The exit lens was replaced by a quartz plate and the output light was reflected vertically downward by a surface mirror, thus about 27×27 mm^2 field of irradiation was obtained for stainless steel sample pans of 25 mm diameter. Single layer of pollen grains was made by careful sieving the pollen into the pans. Both entrance and exit slits were set at 10 mm for high intensity irradiation. Because of the characteristic spectrum of the light source, UV intensity as measured by a thermopile (Eppley) and a microvoltmeter (Ohkura) varied 11.8 ergs/mm^2/sec at 250 mμ to 125 ergs/mm^2/sec at 365 mμ, but a constant dose of 4.15±

0.02×10^3 ergs/mm^2 was delivered at each wave length tested. The irradiated multiple dominant pollen was used to pollinate a multiple recessive tester stock in darkroom. All the procedures were done under yellow light of sodium lamp and pollinated ears were wrapped in aluminum foils to prevent photoreactivation. Results are shown in Fig. 1 in which

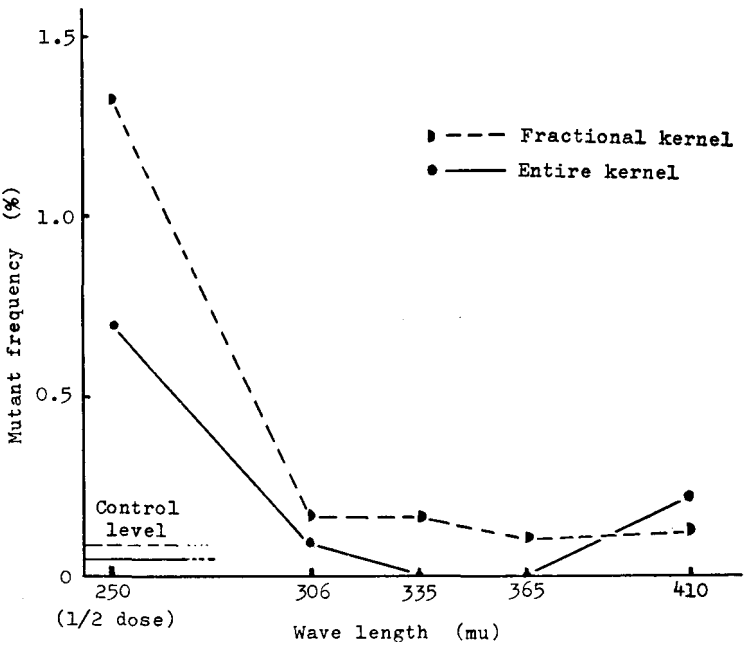


Fig. 1. Wave length dependenc of UV induced mutation. UV doses were 4000 erg/mm^2 constant except at 250 mu where the dose was 1/2 (see text).

dose of UV irradiations at 250 mμ were that of 1/2 at other wave lengths, since very poor seed set introduced by the full dose at this wave length made the mutation detection unreliable. The mutations scored were the total of single- and multiple-loci mutations and mosaics, and, in case of the fractional ones, mutations which occupied more than 1/8 of the endosperm surface were scored. The results indicated that among the tested wave lengths the most, and probably the only, effective wave length was 250 mμ. Since the wave lengths tested were not many and rather apart from each other, comparison with optical absorption characteristics of DNA may not be appropriate, but the experimental procedure toward this problem and study of photoreactivation will be improved in future.

Comparison of Photoreactivativating Activity on Different Chromosomes of Maize

Etsuo AMANO

Existence of photoreactivation of ultraviolet light (UV) irradiated maize pollen has been demonstrated by Ikenaga and Mabuchi (1966, Rad. Botany *6*: 165–169), who used *su* mutation as an index of UV damage. Amano and Mabuchi (Ann. Rep. No. 17: 114–115) tested it further using other linked endosperm marker genes, C^I *Sh Bz Wx*, and found that even damages due to chromosome aberration could be reduced by post-treatment with visible light. However, in the results of these two experiments, there seemed to be a difference in the spectrum of the photoreactivation. Ikenaga and Mabuchi reported more photoreactivation in whole kernel mutation than in partial mutation, but in Amano and Mabuchi's experiment with linked marker genes, more photoreactivation was observed in fractional (partial) mutation (more than 1/8 of endosperm surface). The present experiments were arranged to find out the cause of the discrepancy.

Pollen used was of the same stock as used in Amano and Mabuchi's experiment and only female testers were compared. The female stocks were selfed progenies of the previous experiments. A little modification was made in UV intensity (4×4 W germididal lamps, 200 ergs/mm^2/sec), and pollinations in *su* experiments were done in the field after dark with a flashlight. Other experimental procedures were basically the same as previously. Because of technical limitations, each dosage group was treated on a different day and this might be the reason for the fluctuating data. However, the results were basically the same for the two systems, indicating photoreactivation on both entire and fractional mutations. In the experiments with multiple recessive markers, most of the aberrant kernels showed loss of all marker genes or B-F-B mosaic type, in both entire and fractional kernel mutations. In case of loss of all marker genes in entire kernel mutants, fertilization by the treated pollen could be confirmed by the *Y* gene present in the pollen in contrast to the female. Such simultaneous losses of dominant genes or initiations of B-F-B cycles might involve chromosome breakage in some form. Some B-F-B type mosaics were also found in the *su* system. Photoreactivation were observed in both genetic systems, both mutation kinds, and in the chromosome breakage type UV damages. As for single locus mutations, the number of the mutants was not large enough for a definite conclusion, but some decrease of UV damages was observed in the groups treated by visible light.

Dosimetry Systems for the 14.1 MeV Neutron Generator

Masaru HAYASHI and Etsuo AMANO

In 1967, a Cockcroft-Walton accelerator type neutron generator (Toshiba NT-200-2) was installed in a newly built well shielded room in the Radioisotope Laboratory, National Institute of Genetics. This machine is to produce 14.1 MeV monochromatic fast neutrons by $^{3}H(d, n)^{4}He$ reaction for radiation biology experiments. Specifications of the machine are; maximum acceleration voltage: 200 kV, maximum ion current: 1 mA, and neutron output: more than 1×10^{10} n/sec.

Several dosimetrical methods were tested.

1) Proton measurement by plastic scintillator: A $2''\times2''\phi$ plastic scintillation detector of known efficiency set at 3.2 m from the target was used for both calibration and monitoring purposes. The signal pulses were analysed by a single channel pulse height analyzer (RCL) to obtain proton signals generated by 14.1 MeV fast neutrons. Since some problems were found in respect to the resolving time of the system, improvements are in progress.

2) Activation of sulfur: A few grams of sulfure were placed around the target and ^{32}P generated in sulfur by $^{32}S(n, p)^{32}P$ reaction was measured after processing, by a GM counting equipment of known efficiency for ^{32}P. This method can be used as simultaneous monitoring of actual radiation dosage delivered to the samples.

3) Activation of aluminum: $^{27}Al(n, \alpha)^{24}Na$ reaction was tested since aluminum can be used as metal and ^{24}Na, a gamma ray emitter, can be measured without processing. Accompanying product, ^{27}Mg, decays faster (1/2 life: 9.45 min.) than ^{24}Na. This will be a very convenient and fast means when simultaneous monitoring of delivered dose is needed as in the cases of radiation biology experiments.

4) Other methods tried or in progress: Silver activated glass rods in various casings have been tested with success to separate radiation components (contaminating gamma rays etc.) in radiation meaurement. Designing of an α particles counting apparatus to measure nuclear reactions $^{3}H(d, n)^{4}He$ is in progress. Though this is a promissing and orthodox method in radiation physics, good fit with the sample holder and other radiobiological set-ups must be taken into consideration in mounting the α particle detector.

IX. MICROBIAL GENETICS

Fingerprinting Analysis of Flagellin of Antigen Type *Salmonella* Recombinants

Shigeru YAMAGUCHI

The isolation of eleven types of antigen type recombinants obtained probably through intra-*H1* recombination from transductions between different g-complex antigenic *Salmonella* strains has been previously reported (Yamaguchi & Iino, 1967). In the present study, tryptic peptide maps of flagellins of each of these eleven types of recombinants were prepared and were compared with those of their parental type strains.

Flagella detached from cells by shaking (about 750 strokes/min, 25 mm amplitude) were purified by repeating low and high speed centrifugation, and were disintegrated into flagellin monomers by treatment with 0.01 N HCl for 30 min at room temperature. A sample of freeze-dried flagellin dissolved in 0.05 M phosphate buffer (pH 8.0) was digested with trypsin (2 % by weight) at 25°C for 24 hr. An aliquot of the digest was applied to a sheet (40×30 cm) of Tōyō No. 50 filter paper and subjected to electrophoresis in pyridine/acetic acid/water at pH 6.65 for 3.5 hr at 15 V/cm and twice to ascending chromatography with *n*-butanol/acetic acid/water (3 : 1 : 1). Peptide spots were developed with ninhydrin dissolved in acetone (0.25 % w/v).

The approximate number of spots was 35 to 36 for every specimen and almost all the spots except a few seem to be common to all parental and recombinant type strains. Five spots in total were distinguishable among the maps of the parental type strains: two spots (A and B) were specific for strain Tr6 (g_1, g_2, g_3, t : (e, n, x)), two (C and D) for Tr16 (g_3, g_4, f : (e, n, x)), and one (E) for Tr17 (g_1, g_2, g_4, g_5, m : (e, n, x)). These differing spots were found in various combinations on the maps of the antigen type recombinants. For example, three spots were found besides the common spots on the map of SJ1744, a recombinant between Tr6 and Tr16: one was the same as B specific for Tr6 and the other two were the same as C and D specific for Tr16. No other spot than those found on the maps of the parental type strains was detected.

On the basis of the assumption that the antigen type recombinants had resulted from a single crossover within *H1*, a structural gene for flagellin, in transduction, we have mapped out the location of the antigenic specificity-determining sections within *H1* as follows: $g_2-g_1-g_4-t-(g_3, g_5, m)$ (Yamaguchi & Iino, 1967). The differing peptides

disclosed here were now mapped out as additional markers. As the result, the following locations were assigned to sections *A* to *E* corresponding to peptides A to E: somewhere between g_4 and (g_3, g_5, *m*) for *A*, *C* and *D*, somewhere between g_1 and g_4 for *B*, and between g_1 and (g_3, g_5, *m*) for *E*.

Mapping of Some *fla* and *mot* Cistrons Closely Linked to *H1* in *Salmonella*

Shigeru YAMAGUCHI and Tetsuo IINO

By the use of deletion mutants and three-factor reciprocal crosses, the order of *fla* and *mot* cistrons closely linked to *H1* was determined.

Nine *H1*-linked stable *fla*$^-$ mutants were used: one of them derived from SJ241 (a : (enx)), a phase-1 stable strain of *Salmonella abortus-equi*, and the other eight from the derivatives (g : (enx)) of SJ241, given *H1*-g-alleles from g-complex antigenic strains.

Following experiments were carried out: 1) measurement of co-transduction frequency of their *fla*$^-$ sites with *H1*, 2) recombination and complementation tests by transduction in all their pairwise combinations, 3) recombination and complementation tests between them and the mutants of the already known *fla* and *mot* cistrons derived from *S. typhimurium* TM2 (Iino & Enomoto, 1966; Enomoto, 1966), and 4) three-factor cross tests, in which reciprocal transductions between *fla-306*, the *fla*$^-$ mutant of SJ241, and each of the other mutants were carried out and *H1* was used as the unselected marker.

The order of the co-transduction frequencies were *fla-204*, *-263*, *-351*, *-373*, *-227*, *-306*, *-251* and *-211*. Mutational sites of *fla-220*, *-227* and *-251* were found to be in *flaA*, and that of *fla-211* was in *flaD*. *Fla-373* was found to be a deletion mutant covering *flaA*, *D*, *B* and *motC*, and *fla-306* was also a deletion mutant covering a part of both *flaA* and *motC*. Three mutants, *fla-204*, *-263* and *-351*, constituted a new complementation group designated as *flaL*. Three-factor cross tests showed that the relative order of *flaA*, *D*, *L* and *H1* is *flaD-flaA-H1-flaL*. From these results and those reported previously (Iino & Enomoto, 1966; Enomoto, 1966), the following order can be inferred: *flaB-flaD-flaA-motC-H1-flaL*. Further, three-factor cross tests between a mutant in *flaL* and those in *flaC*, *E*, *K* and *J* originated from TM2 were also conducted, and it was shown that these cistrons are all on the same side of *H1* and *flaL* being the nearest to it.

Behavior of Conjugational Hemizygotes of *Salmonella typhimurium* in Special Reference to Motility Characters

Tetsuo IINO

Conjugation experiment was carried out between an *HfrB2·metA22·mot* strain, SJ1788, and an F^-·*athA·pro·flaA* strain, SL481, of *S. typhimurium*. When the mixture of *Hfr* and F^- bacteria (2×10^8 cells/ml) was inoculated on semi-solid plates and cultivated at 37°C, branched trails and swarms appeared on the plates. The number of branched trails was ca. 1 to 5 % of the inoculated bacteria, while the number of swarms was 1 % of branched trails at 24 hours' incubation. On further cultivation, swarms developped from minute colonies of branched trails, their number increasing up to 20 %. The clones which have grown as swarms were all fla^+·mot^+, and 91 % of them were met^- and the rest met^-·ath^-·pro^-. The bacterial clones grown from the minute colonies were all fla^-·ath^-·pro^-; that is the same as the F^- strain.

Those results are summarized as follows. After conjugation of SJ1788 and SL481, hemizygotes were produced. The hemizygotes currently segregate parental type cells at a considerably high frequency as compared with the combination of SJ457 and SJ152 (Iino and Suzuki, Annual Report No. **15**, 1964), producing branched trails on the indicator plates. The stable motile recombinants appear, forming swarms, throughout the period of persistence of hemizygotes.

By counting the number of minute colony clusters of each branched trail, it was shown that the number of branched trails with different cluster number distribute in such a way that $P=2^{-c}$, where P is the frequency of the branched trails with cluster number c. This equation indicates that 50 % of the hemizygotes segregate non-motile parental type cells in each cell division cycle.

Flagellar Characters of an *F-his* Strain of *Salmonella typhimurium*

Tetsuo IINO

SJ1795 (*F-his*$^+$/*his-712·ser·arg*) is a *Salmonella* strain carrying an episomal *F* factor which attaches the *his*-operon of *Salmonella* chromosome. The strain was originally derived from *S. typhimurium* LT2 (i : 1.2) by G. Fink, Department of Genetics, Cornell University, Ithaca, N. Y., U. S. A. It was found that SJ1795 is non-flagellate in phase-1 and produces curly flagella in phase-2. Therefore the bacteria cannot spread

on semi-solid plates in either phase. Transduction was carried out from the phase-2 culture of a diphasic *S. abony* (b : enx) strain, SW803. Motile transductional recombinants were selected for on semi-solid plates. The normal type transductional clones obtained were either normal-b in phase-1 and curly-1.2 in phase-2 or non-flagellate in phase-1 and normal-enx in phase-2.

Possibility that the *F* factor of SJ1795 carries the *H1-fla* gene cluster together with the *his*-operon was examined by conjungation analysis. SJ1795 was mated with a *fla*$^-$ mutant of strain SJ1780 (*his-486*, b : 1.2) and *his*$^+$ recombinants were selected for on minimal plates. The *his*$^+$ recombinants appeared at the frequency of 2 to 0.2 % of the inoculated bacteria. Twenty five such recombinants examined were all *fla*$^-$. When the recombinants were selected for from the same bacterial mixture on semi-solid plates, the motile recombinants, appearing at the frequency of 10^{-2} to 10^{-3} % of the inoculated bacteria, were all b : 1.2 type, and 56 % of them were *his*$^+$ and the rest *his*$^-$. Further conjugation analysis, in which a multiple auxotrophic mutant of *S. abony* was used as a female, indicated that *ser*, *leu* and *gal* markers were transferred together with *fla* and *H2* from SJ1795 to the female upon conjugation.

From these results it is concluded that the *F* factor of SJ1795 does not carry the *H1-fla* gene cluster but the strain can transfer its chromosomal markers, including the *H1-fla* cluster, with the same efficiency as in ordinary male and female conjugation.

Newly Isolated Arginine Sensitive Mutants of *Salmonella typhimurium*

Jun-ichi ISHIDSU

Mutation to arginine sensitivity was induced by N-methyl-N′-nitro-N-nitrosoguanidine in a wild type LT-2 strain of *S. typhimurium* and seven mutants were isolated after penicillin screening (*arg-s-2*～*8*). Among them, *arg-s-5* and *7* were sensitive only to arginine as previously isolated *arg-s-1* (Ishidsu, Ann. Rep. No. 14, 1963). The other five mutants (*arg-s-2*, *3*, *4*, *6* and *8*) were found to be sensitive also to uracil, though the response to uracil was weaker than that to arginine and as much as 1 millimole uracil per liter could not completely prevent their growth. They grew slowly in the minimal medium where *arg-s-1*, *5* or *7* can normally grow. In either case, however, they showed quite normal growth when the two substances, arginine and uracil, were added together to the minimal medium.

When *arg-s-3* was cultivated in a medium containing arginine and

uracil combined in various concentrations, growth of the mutant was completely inhibited immediately after either of the supplements was exhausted while the other still remained in a considerable amount. This indicates that these two substances repress the biosynthesis of each other in this mutant.

The precursors included in the biosynthetic pathway of arginine or uracil were tested separately or in various combinations to find out whether they have the ability to inhibit the growth or to remove the inhibition caused by the chemicals of the opposite pathway. The results are summarized in Table 1. It is noticeable from the table that chemicals which can inhibit the growth of *arg-s-3* when given alone have also the ability of neutralizing the toxic effect of inhibitors belonging to the other pathway, and that, in either pathway, the precursors before transcarbamylation (ornithine or aspartic acid) have neither the ability described above. It is also seen from the table that ureidosuccinic acid and dihydroorotic acid, though they enter the uracil synthesizing pathway after the transcarbamylation step, behave somewhat exceptionally and accelerate the growth when given together with ornithine, which never happens in the case of other precursors of uracil. Those observations seem

Table 1. Growth responses of some strains to various arginine and uracil precursors or related substances

Strain	*arg-s-1*				*arg-s-3*				*pyr-150*			
	none	ORN	$\xrightarrow{*}$CIT	→ARG	none	ORN	$\xrightarrow{*}$CIT	→ARG	none	ORN	$\xrightarrow{*}$CIT	→ARG
none	##	##	#	−	+	+	−	−	−	−	−	−
ASP	##	##	#	−	+	+	−	−	−	−	−	−
↓* URE	##	##	#	−	+	#	−	−	−	−	−	−
↓ DHO	##	##	##	±	+	#	±	−	−	−	−	−
↓ ORO	+	+	##	##	−	−	##	##	−	−	#	#
↓ URA	#	#	##	##	−	−	##	##	−	−	##	##
CYT	#	#	##	##	−	−	##	##	−	−	##	##
THY	##	##	#	−	+	+	−	−	−	−	−	−

ORN: ornithine, CIT: citrulline, ARG: arginine, ASP: aspartic acid, URE: ureidosuccinic acid, DHO: dihydroorotic acid, ORO: orotic acid, URA: uracil, CYT: cytosine, THY: thymine.

pyr-150: a mutant requiring both arginine and uracil for growth.

* steps of transcarbamylation.

to confirm the assumption that growth inhibition by arginine or by uracil is a result of inhibition of carbamyl phosphate formation which acts as a common substrate of ornithine- and aspartate-transcarbamylase (Ishidsu, Ann. Rep. No. **16**, 1965; No. **17**, 1966).

It is interesting to note that the spontaneous reversibility to nonsensitivity to arginine is quite different from that to uracil (very much higher in the latter case) in those mutants which are sensitive to both arginine and uracil, although they are considered to have acquired the character most likely by a single point mutation.

Conversion to Streptomycin-Sensitivity Induced by λ-Lysogenization Accompanying Increase in Reversion Frequency to Prototrophy in *Escherichia coli* K 12

Tsuneo KADA

The author has been studying the effects of episomal infections on the mutability at specific loci in bacteria. This report describes a pleiotropic modification observed by lysogenization with λ-phage of certain poly-auxotrophic streptomycin-resistant F^- strains of *Escherichia coli* K12. They were isolated as thr^-_{1014} recombinants by a 30 minutes conjugation between *E. coli* K12 *Hfr* 1014 (thr^-_{1014} B_1^- Sm^s) derived from *HfrH* after ultraviolet irradiation and *PA*309 thr^+ (F^- thr^+ leu^- try^- his^- arg^- B_1^- Sm^r) obtained by crossing *HfrH* with the original PA309. The typical strain utilized in the following experiments was named F^- *S*-1014 (thr^-_{1014} leu^- try^- his^- arg^- B_1^- Sm^r), possessing some UV-modified genes originated from *Hfr* 1014.

Many lysogenic strains were isolated by infecting free phages from the culture of *E. coli* K12 (λ) on F^- S-1014. They were purified by single-colony isolations and their genetic characters were studied. Among 19 lysogenic independent strains thus obtained, no observable change was found in 5 (type I), whreas the remaining 14 (type II) changed drastically their genetic behavior. While the original non-lysogenic strain F^- S-1014 and lysogenic strains of type I were able to grow in broth containing 2,000 μg/ml of streptomycin, type II strains were no more able to proliferate in the presence of more than 2 μg/ml of the antibiotic. Other modifications were concerned with reversion frequencies of auxotrophic characters. In Table 1, frequencies of spontaneous reversions as to several auxotrophic loci in three typical strains (F^- S-1014$(\lambda)^-$, F^- S-1014$(\lambda)^+$ type I and F^- S-1014$(\lambda)^+$ type II are shown. Though no marked modification exists between F^- S-1014$(\lambda)^-$ and F^- S-1014$(\lambda)^+$ type I, reversion frequencies were increased in F^- S-1014$(\lambda)^+$

Table 1. Frequencies of spontaneous reversions in strains of *Escherichia coli* K12. Lysogenic strains were derived from F^- S-1014. Bacteria were grown exponentially in broth and washed cell suspensions were appropriately diluted and plated on appropriate synthetic media to count revertants and on broth agar to determine the total cell number

Reversions studied	Frequency of spontaneous reversions		
	F^- S-1014(λ)$^-$ (Streptomycin-resistant)	F^- S-1014(λ)$^+$ type I (Streptomycin-resistant)	F^- S-1014(λ)$^+$ type II (Streptomycin-sensitive)
thr$^-$→*thr*$^+$	2.5×10^{-6}	2.0×10^{-6}	$>10^{-1}$
leu$^-$→*leu*$^+$	$<1\times10^{-9}$	$<1\times10^{-9}$	5.0×10^{-5}
gal$^-$→*gal*$^+$	4.0×10^{-8}	6×10^{-9}	3.8×10^{-7}
try$^-$→*try*$^+$	1.2×10^{-7}	1.8×10^{-7}	$>10^{-1}$
his$^-$→*his*$^+$	$<5\times10^{-8}$	$<5\times10^{-8}$	$>10^{-1}$
arg$^-$→*arg*$^+$	3.3×10^{-8}	7×10^{-8}	2.6×10^{-6}

type II. When many lysogenic strains were isolated from PA309, no similar observation was possible.

Introduction of extragenetic elements into the cell by episomes or their insertion into the chromosomes may cause modifications in the genetic character of the host. It is primarily supposed that, according to Gorini's finding of a suppression mechanism involving ribosomal change, auxotrophic characters might have been suppressed by a λ-introduced or λ-induced gene, thus producing complete or leaky prototrophic character. From the strain F^- S-1014, kanamycin-resistant or neomycin-resistant strains were isolated and lysogenized with λ. Lysogenic strains thus obtained became also sensitive to these antibiotics whose action sites are considered to reside in ribosomes. On the other hand, revertants regarding many loci should be again mutated to original auxotrophic state by means of a single event at the suppressor locus. Trials to demonstrate this possibility by P1-transduction experiments rather favour the assumption of a mutator acting non-specifically on auxotrophic loci. The actual relationship between the possible ribosomal change and the increased mutability regarding auxotrophic characters is not known. Further examinations are in progress to gain an insight into the mechanisms involved. The initial and important parts of these observations were done at the Institute of Physical and Chemical Research (Laboratory of Microbiology), Komagome, Tokyo.

X. HUMAN GENETICS

Sexual Variation in Finger Pattern Types and Ridge Counts

Ei MATSUNAGA and Ei MATSUDA

The dermal ridge patterns on finger tips can be studied by two different approaches, 1. by classification of the patterns into some types such as arches, loops (radial and ulnar) and whorls, and 2. using the pattern size as measured by ridge count. Arches are defined to have no ridge count, while loops have usually lower counts than whorls. Thus, pattern type and pattern size are not independent from each other. These two aspects, however, have hitherto been investigated quite separately, leaving some gaps to be clarified.

It is known that there is a sexual variation in the distribution of pattern types as well as of total ridge counts, *i.e.*, the sum of the ridge counts for each of ten fingers of a given individual. Table 1 illustrates the result of our study based on 200 males and 200 females, both healthy and unrelated subjects. As is shown in the table, males have, on the average, significantly larger total ridge counts than females, and whorls are significantly more common among males than among females, whereas the reverse is true with respect to the frequency of ulnar loops.

The larger total ridge count of the males than that of the females may be attributed mainly to two causes; one is the lower frequency of loops and higher frequency of whorls among the males than among the females, and the other is possibly a larger ridge count within the same pattern type on the same digit among the males than among the females. In order to check the latter point, mean ridge counts of a whorl and a loop were compared between males and females. Table 2 clearly shows

Table 1. Mean total ridge counts and percentages of different pattern types in 200 males and 200 females

Sex	Total ridge count		Pattern types (%)			
				Loop		Whorl
	Mean	S.D.	Arch	Radial	Ulnar	
Males	150.8	45.69	1.7	3.3	45.6	49.5
Females	139.2	42.04	1.9	2.6	53.2	42.4
Diff. (M−F)	+11.6*		−0.2	+0.7	−7.6**	+7.1**

* $P<0.01$, ** $P<0.001$.

Table 2. Mean ridge count of a whorl and a loop

Pattern type	Sex	Digits (left and right combined)					All digits
		I	II	III	IV	V	
Whorl	Male	21.0	16.8	18.5	19.0	16.5	18.6
	Female	19.3	15.8	16.9	18.2	15.4	17.4
	Diff. (M−F)	1.7	1.0	1.6	0.8	1.1	1.2*
Ulnar loop	Male	15.4	10.3	11.1	11.8	12.1	12.1
	Female	14.4	10.4	11.1	12.6	11.4	11.9
	Diff. (M−F)	1.0	−0.1	0	−0.8	0.7	0.2
Radial loop	Male	—	9.8	—	—	—	9.8
	Female	—	10.2	—	—	—	9.5
	Diff. (M−F)		−0.4				0.3

* $P<0.001$.

that whorls on every digit of the males have on the average larger ridge counts than those of the females, the difference in the overall mean being highly significant. On the other hand, no difference is found between the sexes in the mean ridge counts of loops, although there is more or less variation according to digits which may be due to chance fluctuation.

The above finding seems to be rather unexpected because the larger total ridge count among males than among females has generally been taken to imply that males had larger pattern size than females irrespective of the type, whorl or loop. Thus the mechanism for sexual variation in total ridge count appears to be more complicated than has been thought. The mean difference in the total ridge counts between males and females was found to be 11.6 ± 4.4 in our Japanese sample. Using the figures of Tables 1 and 2, it may be shown that about half of this difference is accounted for by the different ridge counts of whorls between the sexes, while the remaining half is due to the larger ridge counts of whorls than loops seen in both sexes multiplied by the different frequencies of these two types between the sexes.

Increased Finger Ridge Counts in Turner's Syndrome

Ei MATSUNAGA, Ei MATSUDA and Hidetsune OISHI

Recent advances in human cytogenetics have renewed the interest for

dermal ridge patterns that my be affected by chromosome aberrations. Of particular interest is the negative correlation of the finger pattern size in terms of total ridge count with the number of sex chromosomes present in various types of aneuploidy. Since there is an evidence that the total ridge count is controlled by autosomal genes, the above correlation has been interpreted to be due to non-specific dosage effect of whole sex chromosomes (Penrose, 1967). However, whether or not the variation between normal males and females is also caused by the same mechanism, had to be clarified.

In this connection, we carried out ridge counting of the finger tips in five cases of Turner's syndrome with reference to pattern types. The sex chromosome complements and the total ridge counts of these cases are given in Table 1. The mean total ridge count was 189.6, which is very high in agreement with the reports by previous workers. The percentage frequencies of arches, radial loops, ulnar loops and whorls were 2.0, 2.0, 44.0 and 52.0, respectively. When compared with the control series used in our previous report, these frequencies are similar to those found in normal males rather than to normal females, but the difference between our Turner cases and normal females was not significant.

Table 2 compares mean ridge counts per digit of our Turner cases

Table 1. Sex chromosome complements and total ridge counts in five cases with Turner's syndrome

	Sex chromosome complements	Total ridge count
Case 1	XO	151
Case 2	XO	200
Case 3	XO/XXX	150
Case 4	XO	264
Case 5	XO/XXr	184
	Combined	Mean: 189.6

Table 2. Mean ridge count of different pattern types

Pattern types	Turner cases	Normal males	Normal females
Whorl	20.4±1.4*	18.6±0.2	17.4±0.2
Ulnar loop	18.1±1.2**	12.1±0.2	11.9±0.2
Radial loop	21.0	9.8±0.8	9.5±0.8

* Significantly larger than normal females ($P<0.05$).

** Significantly larger than normal males and females ($P<0.001$).

with those of the control series according to different pattern types. It is to be noted that in Turner's syndrome the pattern size is substantially increased irrespective of the pattern type. The mean ridge count of a whorl was significantly larger than in normal females, and that of an ulnar loop was significantly larger than in normal males and females. As to radial loops, there was only one such pattern, which had 21 ridges.

Since sexual variation in pattern size among normal subjects was found for whorls but not for loops, the above finding suggests that the genetic mechanism whereby changes in pattern size are produced in aneuploid cases of the sex chromosomes may differ from that responsible for the variation between XY and XX individuals.

The Complete Amino Acid Sequence of a λ Type Bence-Jones Protein

Tomotaka SHINODA

Attempts were made to test the hypothesis that all light chains of immunoglobulin, κ and λ, have a variable amino-terminal half and an invariant carboxyl-half and likewise to test the relationships between the genetic factor and the chemical structure of the protein. With regard to these points of view, the complete amino acid sequence of a λ type human Bence-Jones protein, designated Ha, has been determined, including the assignment of the amide groups.

The results are as follows:

(1) The examined protein has 217 amino acid residues, including three extra residues which are inserted between Asn-28 and Gly-31. (2) Many amino acid interchanges in the amino-terminal half (residue number from 1 to 112) are found to be random. (3) Approximately the half of the molecule with initiation of Gln-113 is found to be constant with the exception of lysine-arginine interchange, which is associated with a genetic factor. (4) The protein has the arginine residue at position 195, suggesting that it is of type Oz(−). (5) The protein has a blocked amino-terminal, presumably a pyrrolidonecarboxylic acid. (6) From sequence comparison, there are some evidences for conservation of certain areas in the primary structure of light chains, that is, the positions that are variable in λ-chains are generally variable in the counterpart positions of κ-chains, and *vice versa*.

The evidences seem to support the concept that the light chains of

Penrose, L. S.: Finger-print pattern and the sex chromosomes. Lancet **i**: 298–300, 1967.

immunoglobulins have a variable amino-terminal half and an invariant carboxyl-terminal half that carries the genetic factors. (The entire work was performed at Indiana University, Indiana, U.S.A.)

Cytogenetic Study on Familial Down's Syndrome

Yasumoto KIKUCHI, Hidetsune OISHI, Akira TONOMURA[1] and Takehiko KURITA[2]

It is now known that both in Europeans and Japanese more than 90% of patients with Down's syndrome are of 21-trisomy, while the remaining small portion consists of cases with translocation and mosaicism. In order to find the incidence of chromosome mutation leading to translocation Down's syndrome, 508 patients were examined by means of leucocyte culture. Of the 508 cases, 26 (5.12%) were found to be of translocation type; *i.e.*, 12 t(GqGq), 13 t(DqGq) and 1 was a mosaic of 45,XY,D−, G−, t(DqGq)+/46,XY,D−, t(DqGq)+. In cases with t(DqGq), 5 (including the mosaic) were inherited, 6 were sporadic, and the parents of the remaining 3 have not yet been examined. In cases with t(GqGq), 2 were inherited, 7 were sporadic, and the parents of the other 3 were not examined.

The family members of the patients with inherited translocation were investigated. In the first family, the patient had (DqGq) translocation; the father was a mosaic of 46,XY/47,XY, t(DqGq)+, and was phenotypically normal; the mother had apparently normal karyotype; a phenotypically normal sib of the patient had not yet been examined (Tonomura *et al.* 1962[3]). In the second family, the patient was a mosaic of 45,XY, D−,G−, t(DqGq)+/46,XY,D−, t(DqGq)+; one cell line was of a balanced translocation type and the other was of a translocation-trisomic type; the mother was a carrier of translocation in the balanced form, and has previously had 2 stillbirths; the father had apparently normal karyotype; a sib of the patient has not yet been examined but had normal phenotype. In the third family, the patient had t(DqGq); the mother and the third sib of the patient were carriers; the father, the first sib and the maternal grandparents had apparently normal karyotype; the second sib died at early infancy. In this family, the translocation occurred *de novo* in one of the parents of the carrier mother. In the remaining 4 families, the

[1] Department of Cytogenetics, Tokyo Medical & Dental University.

[2] Pediatric Clinic, Kohnodai National Hospital.

[3] Tonomura, A., T. Honda, and T. Kurita, 1962. Chromosome abnormalities in a child with Down's syndrome and in its father. Proc. Jap. Acad. **38**: 526-530.

translocation chromosomes of the patients were inherited from the carrier mothers.

The above data indicate that, excluding the unexamined cases, the approximate frequencies of inherited and sporadic translocation among Down's syndrome were 1.8% and 3.3%, respectively, and that the population incidences of these two translocation types are of the order of 3×10^{-5} and 5.5×10^{-5}, respectively.

Maternal age at the patient's birth has also been subjected to statistical analysis. Mean maternal ages for the patients with inherited and sporadic translocation were 30.5 ± 2.69 years and 27.9 ± 3.66 years, respectively. These mean ages are not different from that of the control population (28.5 ± 4.27 years).

An Incomplete No. 5 Chromosome Pair in Two Patients with the *Cri du Chat* Syndrome

Hidetsune OISHI, Yasumoto KIKUCHI and Ei MATSUDA

An incomplete chromosome pair in group B has been found in the *cri du chat* syndrome (Lejeune *et al.*,[1] 1963). Chromosome analyses revealed that the deficiency consisted in the deletion of the short arm of no. 5. However, it seems that the magnitude of the deletion varies somewhat from case to case, and this could be reflected in the clinical features of the patients with the syndrome. From this point of view, the present study deals with two cases of that deficient chromosome.

Case 1 was a 4 months old boy and his cry reminded of the *cri du chat*. The gestation lasted normally for 40 weeks and the birth weight was 2000 g. At the time of his birth, the age of the mother and father was 28 and 32 years, respectively. On both hands of the patient were observed transverse palmar creases and whorls on all fingers except the first with a lateral pocked loop, though the dermal ridges on the fingers were poorly developed. The patient died of pneumonia 7 months after birth. A 3 years old sister was phenotypically normal.

Case 2 was an 18 months old girl who was the first-born child of a 26 years old mother and 30 years old father. The gestation was 40 weeks and the birth weight was 2060 g. The patient exhibited an odd cat-like cry, and the parents said that the characteristics cry became less pronounced as the child grew older. The dermatoglyphics of the patient

[1] Lejeune, J., J. Lafourcade, R. Berger, J. Vialatte, M. Boeswillwald, P. Seringe, and R. Turpin, 1963. Trois cas de délétion partielle du bras court d'un chromosomes. C. R. Acad. Sci. Paris **257**: 3098-3102.

were as follows, and transverse palmar creases were also noted on her hands:

Right hand.—Palmar formula, $7\cdot5''\cdot5'\cdot4-t'-A^u/A^c\cdot U/0\cdot0\cdot0\cdot L$, *at'd angle*=64°; digits=Ucp, W, W, W, W, total ridge count=59.

Left hand.—Palmar formula, $7\cdot5''\cdot5'\cdot3-t'-A^u/A^c\cdot L/L\cdot0\cdot0\cdot L$, *at'd angle*=58°, digits=U, W, W, W, W, total ridge count=64.

The clinical features, such as mental and growth retardation, microcephaly, round face, hypertelorism, antimongoloid slant of palpebral fissures, low-set ears and short neck which are frequently associated with this syndrome, were found in both cases. However, there were also epicanthus, micrognathia and congenital heart disease in case 1, but not in case 2.

Chromosome analyses of these patients revealed that one of the chromosomes of group B was lacking a portion of the short arm. The chromosomes of group B of ten cells were magnified about 20,000 times from photomicrographs by a photographic enlarger. The figures were then measured by a planimeter. The lengths of the chromosomes were also recorded in the same cells. The deficient chromosome corresponded to chromosome no. 5 in the size of the long arm, and the deleted portion amounted to 64.7% of the short arm by area measurement and 49.4% by length measurement in case 1, and respectively 61.8% and 43.8% in case 2. The parents had in both cases apparently normal chromosome sets.

Dumar and Kitzmiller[2] (1964) assumed that the deletion comprised about the mass of a chromosome of group G, and McCracken and Gordon[3] (1965) reported similarly that almost all of the short arm was absent in their case. Ricci *et al.*[4] (1965) also observed a deletion of more than a half short arm in this syndrome. However, no exact measurements of the deletion, in comparison with a variable appearance of clinical features, were recorded so far.

Clinical Conditions of Patients with Apparently Normal Chromosomes, IV

Hidetsune OISHI, Yasumoto KIKUCHI and Kunihiko SHIBATA

Chromosome analyses performed on cultured leucocytes of patients

[2] Dumars, K., Jr., and N. Gaskill, 1964. Le cri du chat (crying cat) syndrome. Amer. J. Dasease Child. **108**: 533–537.

[3] McCracken, J., and R. Gordon, 1965. "Cri du chat" syndrome. Lancet **i**: 23–25.

[4] Ricci, N., B. Bentimiglia, B. Dallapiccola, F. Franceschini, and G. Preto, 1965. "Cri du chat" syndrome. Lancet **i**: 1278–1279.

having various pathological conditions showed that they had apparently normal chromosome assortments of 46 chromosomes. Cases of some interest are listed below.

	Name	*Age*	*Legal sex*	*Clinical condition*
a) Multiple deformations with mental retardation				
64	Y.M.	3 months	M	Prominent occiput; malformed and low-set ears; cleft palate; congenital heart disease; transverse palmar creases; overlapped fingers; hemangioma
65	I.S.		M	Cleft palate; hare-lip; malformed ears; hydrocephalus
66	H.E.	1 year	F	Flat occiput; hypertelorism; strabismus; high-arched palate; micrognathia; short neck; cubitus valgus
67	A.K.	5 months	F	Arnold-Chiari malformation; lacunal skull; hydrocephalus; myelomeningocele; bladder-rectal incontinency; paraplegia of the leg
68	H.K.	3 years	F	Oblique palpebral fissures; aplasia of the XII rib; spasmophilia; logopathy
69	H.K.	1 month	M	Microphthalmia; cataract; low-set and malformed ears; micrognathia; short neck
70	K.S.	2 months	M	Exophthalmos; low-set ears; hydrocephalus
71	M.Y.	1 year	F	Moebius's singn; strabismus; high-arched palate; short neck; cubitus valgus; syndactyly
72	S.H.	2 months	F	Flat nasal bridge; short upturned nose; low-set ears; high-arched palate; webbed neck
73	J.M.	6 years	M	Malformed ears; cleft palate; micrognathia; hypoplasia of the XII rib; transverse palmar creases
74	E.N.	2 years	F	Congenital heart disease; narrow palpebral fissures; transverse palmar creases; logopathy
75	S.N.	3 years	M	Transverse palmar creases; presence of the XIII rib; incurved fifth fingers; increased iliac index (94°); spasmophilia
76	J.Y. & T.Y. (twin)	4 months	M	Hirschsprung's disease
77	Y.K.	4 years	M	Oblique palpebral fissures; incurved and

				short fifth fingers with clinodactyly; aplasia of the XII rib
78	Y.O.	2 years	M	Hypertelorism; antimongoloid slant eyes; epicanthus; strabismus; malformed ears; micrognathia; short neck
b) Sex anomalies				
79	S.I.	2 months	M	Male pseudohermaphroditism
80	Y.I.	16 years	F	Male pseudohermaphroditism
81	O.T.	3 years	M	Slender body; short neck; shield chest; retentio testes; flat occiput; high-arched palate; logopathy; mental retardation (Klinefelter's syndrome?)
82	T.K.	21 years	M	Male pseudohermaphroditism
c) Hereditary disease				
83	H.A.	6 years	M	Congenital ichthyosis; spastic paraplegia; logopathy; mental retardation

XI. APPLIED GENETICS

General and Differential Relative Growth Rates of Mutant Lines of Rice

Hiko-Ichi OKA and Hiroko MORISHIMA

Last year (Ann. Rep. 17, p. 33-34), we reported an observation of variations in growth pattern among 23 mutant lines radiation-induced from a rice variety, Norin 8, though the results were incomplete. Further study of the data gave the following results. Between-line variations in the final size, the time at which half of the final size was attained ($t_{1/2}$), and the growth rate at $t_{1/2}$, were studied for the panicle and first to fourth (from the top) internode. For each of the above three values, principal components were extracted from correlations between the five organs, and the "genetic vectors" showing genetic contributions of the components to the respective organs were computed by "Hashiguchi's method (Hashiguchi and Morishima, in press). In the final size, two latent phases of genetic variation were distinguished, one showing the general size, and the other showing an allometric pattern that brought about variation between "upper-elongation" and "lower-elongation" types. This pattern is the same as reported last year. Variations in the growth rate of organs similarly comprised an isometric and an allometric phase. In the allometric phase, lines whose panicle and third internode elongated relatively slowly had a relatively high growth rate for the first and second internodes. Such lines tended to be the "upper-elongation" types at maturity. As the panicle and third internode elongate some days before the elongation of first and second internodes that results in heading, the allometric pattern of growth rate suggests that the lines vary in the rhythm of development. This was proved by an observation of the change of relative growth rate in the period from panicle elongation to heading. It was suggested that genes modify the rhythm of development and bring about the size variations observed at maturity. This work was supported by a grant of the Ministry of Education, Government of Japan.

Further Observations on the Effects of the Earliness Gene, *E*, in the Genetic Background of a Variety, Taichung 65

Kuo-Hei TSAI* and Hiko-Ichi OKA

Taichung 65, a representative Ponlai rice variety, and its early isogenic

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lines, A3 and B96, were compared regarding the growth pattern of organs, temperature response and yielding capacity in varying environment, in order to estimate the effects of the *E* gene-block carried by the isogenic lines. A comparison of lines derived from BC_7 and BC_{10} generations showed that three additional back-crosses with Taichung 65 slightly reduced the effect of the *E* gene-block on heading date. Perhaps *E* might be linked with genes exaggerating its effect. The primary effect of the *E* gene-block seemed to promote flower initiation and subsequent growth of certain flower organs. This resulted in increase or decrease of growth rate, growth duration and final size of various organs that develop after flower initiation. For instance, the *E* gene increased the growth rate of the panicle and reduced its growth duration, but decreased the growth rate of the first leaf (from the top) and extended its growth duration. Further, it increased sensitivity of the plants to temperatures in the flower-initiation period. But the *E* gene did not seem to affect the seasonal and regional adaptabilities of the original genotype. In yielding capacity, the early isogenic lines (about 10 days earlier than Taichung 65) were comparable to other early Ponlai varieties, and when grown in the northern region of Taiwan in the second crop, they gave better yield than Taichung 65. As to the pleiotropic effects of the *E* gene, as many as 32 were listed. They were considered to suggest the developmental paths through which "ripples provoked by a stone" were transmitted. The experiments on adaptability and yield were conducted in Taiwan, supported by a grant of the Science Development Council of the Republic of China.

Analysis of variance in diallel crosses with parents in nested, hierarchal, classification

Tohru FUJISHIMA

The purpose of this study was to provide analytical procedures of diallel crosses in which the parental lines employed were further classified by breeds or races, nested or hierarchal classification. According to whether purebreds are included or not, there are three different analytical methods. The mathematical model common to those methods for X_{ijklm} which is the mth observation in crosses between jth maternal parent within ith breed and lth paternal parent within kth breed is as follows:

$X_{ijklm}=\mu+g_i+g_k+s_{ik}+g_{j(i)}+g_{l(k)}+s_{j(i)k}+s_{il(k)}+s_{j(i)l(k)}+m_i+m_{j(i)}+r_{ik}+r_{j(i)k}+r_{il(k)}+r_{j(i)l(k)}+e_{ijklm}$, $m=1, 2, \ldots, n$, where the g is the general combining ability, the s is the specific combining ability, the m is the maternal effect and the r is the reciprocal effect, and in Method 1 i, $k=1, 2, \ldots, a$, $j(i)$, $l(k)=1, 2, \ldots, k$, in Method 2 i, $k=1, 2, \ldots, a$ $(i\neq k)$, $j(i)$,

Table 1.

Source of variation		Method 1	
		df	Sums of squares
G.C.A. for breed	(a)	$a-1$	II−2I
G.C.A. for line within breed	(b)	$a(k-1)$	III−II
S.C.A. for breed	(c)	$1/2a(a-1)$	IV−II+I
S.C.A. for cross between line and breed	(d)	$a(a-1)(k-1)$	V−2IV−III+II
S.C.A. for line within breed	(e)	$1/2a(k-1)(ak-a+1)$	VI−V+IV
M.E. for breed	(f)	$a-1$	VII
M.E. for line within breed	(g)	$a(k-1)$	VIII−VII
R.E. for breed	(h)	$1/2(a-1)(a-2)$	IX−VII
R.E. for cross between line and breed	(i)	$a(a-1)(k-1)$	X−VIII−2IX+VII
R.E. for line within breed	(j)	$1/2a(k-1)(ak-a-1)$	XI−X+IX
Error		$a^2k^2(n-1)$	XIII−XII
Total		a^2k^2n-1	XIII−I

Method 2		Method 3	
df	Sums of squares	*df*	Sums of squares
$a-1$	II−2I′	$a-1$	II−2I′
$a(k-1)$	III−II′	$a(k-1)$	III−II
$1/2a(a-3)$	IV−II+I″	$1/2a(a-1)$	IV−II+I″
$a(a-2)(k-1)$	V−2IV−III−II′	$a(a-1)(k-1)$	V−2IV′−III+II
$1/2a(a-1)(k-1)^2$	VI−V+IV	$1/2a\{(k-1)(ak-a-1)-2\}$	VI−V+IV″
$a-1$	VII	$a-1$	VII
$a(k-1)$	VIII−VII′	$a(a-1)$	VIII−VII
$1/2(2-1)(a-2)$	IX−VII	$1/2(a-1)(a-2)$	IX−VII
$a(a-2)(k-1)$	X−2IX−VIII+VII′	$a(a-1)(k-1)$	X−2IX−VIII+VII
$1/2a(a-1)(k-1)^2$	XI−X+IX	$1/2a(k-1)(ak-a-1)$	XI−X+IX
$ak^2(a-1)(n-1)$	XIII−XII	$ak(ak-1)(n-1)$	XIII−XII
$ak^2n(a-1)-1$	XIII−I	$akn(ak-1)-1$	XIII−I

G.C.A. ... General combining ability
M.E. ... Meternal effect
S.C.A. ... Specific combining ability
R.E. ... Reciprocal effect

$l(k)=1, 2, \ldots, k$, and in Method 3 $i, k=1, 2, \ldots, a$, $j(i), l(k)=1, 2, \ldots, k$ ($j(i)\neq l(k)$ when $i=k$).

The tables of analysis of variance corresponding to those methods are summarized in Table 1, where for Method 1,

$$\mathrm{I}=(X.....)^2a^2k^2n,\ \mathrm{II}=\sum_i(X_{i}....+X_{..i}..)^2/2ak^2n,\ \mathrm{III}=\sum_{ij}(X_{ij}...+X_{..ij}.)^2/2akn,$$
$$\mathrm{IV}=\sum_{ik}(X_{i\cdot k}..+X_{k\cdot i}..)^2/4k^2n,\ \mathrm{V}=\sum_{ikl}(X_{i\cdot kl}.+X_{kli}..)^2/2kn,\ \mathrm{VI}=\sum_{ijkl}(X_{ijkl}.+$$
$$X_{klij}.)^2/4n,\ \mathrm{VII}=\sum_i(X_{i}....-X_{..i}..)^2/2ak^2n,\ \mathrm{VIII}=\sum_{ij}(X_{ij}...-X_{..ij}.)^2/2akn,$$
$$\mathrm{IX}=\sum_{ik}(X_{i\cdot k}..-X_{k\cdot i}..)^2/4k^2n,\ \mathrm{X}=\sum_{ikl}(X_{i\cdot kl}.-X_{kli}..)^2/2kn,\ \mathrm{XI}=\sum_{ijkl}(X_{ijkl}.-$$
$$X_{klij}.)^2/4n,\ \mathrm{XII}=\sum_{ijkl}X^2{}_{ijkl}./n,\ \mathrm{XIII}=\sum_{ijklm}X^2{}_{ijklm}$$

for Method 2,

$$\mathrm{I}=(X.....)^2/ak^2n(a-1),\ \mathrm{I}'=(X.....)^2/ak^2n(a-2),\ \mathrm{I}''=(X.....)^2/k^2n(a-1)(a-2),$$
$$\mathrm{II}=\sum_i)X_{i}....+X_{..i}..)^2/2k^2n(a-2),\ \mathrm{II}'=\sum_i(X_{i}....+X_{..i}..)^2/2k^2n(a-1),$$
$$\mathrm{III}=\sum_{ij}(X_{ij}...+X_{..ij}.)^2/2kn(a-1),\ \mathrm{IV}=\sum_{ik}(X_{i\cdot k}..+X_{k\cdot i}..)^2/4k^2n,\ \mathrm{V}=$$
$$\sum_{ikl}(X_{i\cdot kl}.+X_{kli}..)^2/2kn,\ \mathrm{VI}=\sum_{ijkl}(X_{ijkl}.+X_{klij}.)^2/4n,\ \mathrm{VII}=\sum_i(X_{i}....-X_{..i}..)^2/$$
$$2ak^2n,\ \mathrm{VII}'=\sum_i(X_{i}....-X_{..i}..)^2/2k^2n(a-1),\ \mathrm{VIII}=\sum_{ij}(X_{ij}...-X_{..ij}.)^2/$$
$$2kn(a-1),\ \mathrm{IX}=\sum_{ik}(X_{i\cdot k}..-X_{k\cdot i}..)^2/4k^2n,\ \mathrm{X}=\sum_{ikl}(_{i\cdot kl}.-X_{kli}..)^2/2kn,\ \mathrm{XI}=$$
$$\sum_{ijkl}(X_{ijkl}.-X_{klij}.)^2/4n,\ \mathrm{XII}=\sum_{ijkl}X^2{}_{ijkl}./n,\ \mathrm{XIII}=\sum_{ijklm}X^2{}_{ijklm}$$

for Method 3,

$$\mathrm{I}=(\mathrm{X}.....)^2/akn(ak-1),\ \mathrm{I}'=(\mathrm{X}.....)^2/akn(ak-2),\ \mathrm{I}''=(X.....)^2/n(ak-1)(ak-2),$$
$$\mathrm{II}=\sum_i(X_{i}....+X_{..i}..)^2/2kn(ak-2),\ \mathrm{III}=\sum_{ij}(X_{ij}...+\mathrm{X}_{..ij}.)^2/2n(ak-2),$$
$$\mathrm{IV}=\sum_i(\mathrm{X}_{i\cdot i}..)^2/kn(k-1)+\sum_{i\neq k'}(X_{i\cdot k'}..+\mathrm{X}_{k'\cdot i}..)^2/4k^2n,\ \mathrm{IV}'=\sum_i(X_{i\cdot i}..)^2/$$
$$kn(k-2)+\sum_{i\neq k'}(X_{i\cdot k'}..+X_{k'\cdot i}..)^2/4k^2n,\ \mathrm{IV}''=\sum_i(X_{i\cdot i}..)^2/n(k-1)(k-2)$$
$$+\sum_{i\neq k}(X_{i\cdot k'}..+X_{k'\cdot i}..)^2/4k^2n,\ \mathrm{V}=\sum_{il}(X_{i\cdot il}.+X_{ili}..)^2/2n(k-2)$$
$$+\sum_{i\neq kl}(X_{i\cdot k'l}.+X_{k'li}..)^2/2kn,\ \mathrm{VI}=\sum_{ijkl}(X_{ijkl}.+X_{klij}.)^2/4n,$$
$$\mathrm{VII}=\sum_i(X_{i}....-X_{..i}..)^2/2ak^2n,\ \mathrm{VIII}=\sum_{ij}(X_{ij}...-X_{..ij}.)^2/2akn,$$
$$\mathrm{IX}=\sum_{ik}(\mathrm{X}_{i\cdot k}..-X_{k\cdot i}..)^2/4k^2n,\ \mathrm{X}=\sum_{ikl}(_{i\cdot kl}.-X_{kli}..)^2/2kn,$$
$$\mathrm{XI}=\sum_{ijkl}(X_{ijkl}.-X_{klij}.)^2/4n,\ \mathrm{XII}=\sum_{ijkl}X^2{}_{ijkl}./n,\ \mathrm{XIII}=\sum_{ijklm}X^2{}_{ijklm}$$

Electrophoretic variation in enzymes of tobacco plants

Sumiko NARISE

Our local varieties of tobacco are apparently different from the flue-cured ones in many respects, either physiological or morphological. The purpose of this experiment was to find the differences, if any, between them on the basis of variation in enzymes, i.e. variation in isozyme constitution.

Three of Japanese varieties, Matsukawa, Kirigasaku and Ohdaruma, and the same number of flue-cured varieties, Bright Yellow, Hicks and Coker 139, were used. The buffer-extract from leaf-nerves, leaf-mesophylls, calyxes, corollas, stamens, pistils and ovaries from six strains were separately subjected to electrophoresis on acrylamide, and esterase and acid phosphatase come into view by using appropriate reagents.

The results obtained were: 1) Seven different bands of esterase were found in the Japanese strains, while nine appeared in the fluecured tobaccos. Among them, one was common to all organs of different strains, whereas two were common to different organs of flue-cured strains only. 2) There were five different bands of acid phosphatase, two of which were specific to the flue-cured strains, while one of the remaining three was specific to the Japanese strains.

Interaction between sire genotypes and photoperiodic treatment in Japanese quails

Takatada KAWAHARA

The results described in the present report were obtained from 31 sire families of Japanese quail reared in Auburn University. The experiment was carried out with three photoperiodic treatments. Photoperiods applied were: (1) 24 hour light with no dark period (24L:OD), (2) 18 hour light with 6 hour darkness (18L:6D) and (3) 12 hour light with 12 hour darkness (12L:12D) per day. Other environmental conditions including air temperature and humidity were kept as constant as possible. Observed traits were body weight at various stages of development, sexual maturity, testis weight, egg weight, hen-day egg production rate from first egg to 16 weeks of age. Egg production rate was adjusted according to sexual maturity and ordinary egg production rate from 12 to 16 weeks after hatching. Analysis of variance of the data obtained revealed that the interaction between sire genotypes and photoperiodic treatment was significant for sexual maturity and ordinary egg production rate (Table 1). These interactions are understood to have occurred through

Table 1. Analysis of variance for sexual maturity, ordinary and adjusted egg production rates

Source of variation	Degrees of freedom	Mean square		
		Sexual maturity	Ordinary egg production rate[1)]	Adjusted egg production rate[1)]
Sire genotype (G)	30	116.17*	258.30*	182.12
Photoperiod (P)	2	8587.36**	1209.39**	666.57**
24L:OD vs. 18L:6D and 12L:12D (P_1)	1	4148.53**	2352.43**	1259.32**
18L:6D vs. 12L:12D (P_2)	1	13026.18**	66.35	73.82
Interaction	60	118.23**	227.35*	164.44
$G \times P_1$	30	25.84	186.09	140.66
$G \times P_2$	30	210.61**	268.61*	188.22
Error	111	67.99	160.22	147.54

*, **: Exceed the 5% and 1% significance levels, respectively.
1): Arc sin $\sqrt{\text{Percentage}}$ transformation.

different responses of genotypes to light period of 18 and 12 hours per day. The interaction, however, was not significant in egg production rate adjusted according to sexual maturity. This means that the statistically significant interaction in ordinary egg production rate is the effect of differences in the pheses of egg production.

Experiment on bilateral asymmetry in wings of *Drosophila melanogaster*

Takashi NARISE and Kan-Ichi SAKAI

In the experimental study on bilateral asymmetry in wing length in *Drosophila melanogaster*, it was observed that some flies, though infrequently, of the natural population had asymmetrical wings. Therefore, it was expected that the genetic system producing asymmetrical wings persists in natural populations. The present paper describes the results of an inbreeding experiment with three wild strains of *D. melanogaster*, MS-1, Suyama and Katsunuma. The initial number of inbred lines examined was 100 per strain. The number (%) of lines yielding offspring having asymmetry in wing length is presented in Table 1.

It was found that inbreeding disclosed the presence of genes controlling asymmetrical development of wings, and the number (%) of pathological lines increased up to different generations. Conclusions drawn

Table 1. The number (%) of inbred lines yielding offspring having asymmetry in wing length

Inbreeding generation	1	2	3	4	5	6	7	8	9	10	11	12
MS-1	2.0	7.6	6.2	7.4	8.3	10.2	17.6	12.0	23.1	6.0	0.0	0.0
Suyama	1.0	2.0	3.0	5.3	13.0	12.5	5.6	0.0	0.0	0.0	0.0	0.0
Katsunuma	2.0	4.1	4.4	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

from this experiment are: 1) There is a concealed pool of genes responsible for asymmetrical development of wings in wild populations of *D. melanogaster* which is disclosed by the effect of inbreeding. 2) The rate of disclosure varies from population to population due either to difference in gene constitution or buffer system in organic development.

Multidirectional selection in Japanese quails

Teruo INOUYE and Kan-Ichi SAKAI

It is known that the body size and the shank length of Japanese quails are genetically positively and closely correlated. This report deals with the result of a multidirectional selection for body size in various combinations with shank length. The selection was conducted in four directions: for larger body size and longer shanks and for smaller body size and shorter shanks, on the one hand, and for larger body size and shorter

Table 1. Cumulative selection differential and genetic gain obtained after four generations of consecutive multidirectional selection in Japanese quail

Selection group		1 ++*)	2 +−*)	3 −+*)	4 −−*)
Cumulative selection differential	Body weight, *g.*	34.80	9.85	−3.70	−22.10
	Shank length, *mm.*	3.16	−2.31	2.48	−2.72
Genetic gain	Body weight, *g.*	17.95	0.95	2.50	−11.40
	Shank length, *mm.*	2.32	−1.47	2.05	−1.81
Selective efficiency (%)**	Body weight	51.58	9.64	−67.57	51.58
	Shank length	73.42	63.77	82.46	66.54

*) The first positive or minus sign indicates the direction of selection for larger or smaller body weight respectively, while the second is for shank length; +−, for example, means selection for heavier body weight in combination with shorter shank length.

**) Genetic gain divided by cumulative selection differential ×100.

shanks and for smaller body size and longer shanks, on the other. The result of the consecutive selection is presented in the table. Data show that the selection in favor of or against the two correlated characters was quite effective whereas that in favor of one but against the other was less effective.

Fertility, hatchability, viability up to 8 weeks of age and number of days to first egg were investigated in connection with the effects of the selection. It was observed that the (++) selection and the (−−) selection were in general weaker than (+−) and (−+) selection in relation to the fitness of the bird.

Biochemical relationships among different organs of tobacco plants

Sumiko NARISE and Kan-Ichi SAKAI

It was demonstrated in the previous issue of this Annual Report (No. 17) that an electrophoretic method was useful for biochemical comparison of different organs in a plant. This paper describes a further technique improvement yielding higher distinctness and repeatability in the pattern of protein bands than before. The improvement consisted of a fractionating procedure of protein into five fractions by the aid of ammonium sulfate at pH 7.0 of different concentration: 0 to 30, 30 to 45, 45 to 60, 60 to 75 and 75 to 100% saturation. The five fractions thus separated were subjected to electrophoretic analysis. In order to measure the similarity among organs, an index was constructed by summing up the squares of differences between different organs, in respect of staining intensity of bands. It was called similarity index and had the following formula;

$$S_{Y(X)}=1-\frac{\sum_{i=1}^{n}(y_i-x_i)^2}{(v-1)^2 n}$$

where $S_{Y(X)}$ is the similarity index comparing Y-th with X-th organ. y_i and x_i stand for the rating of staining intensity of i-th band of Y-th and X-th organs. v is the number of ratings: six ratings from 0 to 5, and n is the number of bands found in either of the two organs compared. The S value becomes unity if two organs are quite identical with regard to protein band distribution but zero if no common band is detected between the two organs. The S values among different organs are given in Table 1.

The findings according to Table 1 are as follows: 1) All organs, ir-

Table 1. Similarity indices among different organs

		Flower parts			Calyx	Bract leaf	Leaf		
		Pistil	Stamen	Petal			Upper	Intermediate	
Flower parts	Pistil	0.773	0.667		0.697	0.745	0.710	0.707	
	Stamen		0.763	0.734	0.721	0.671	0.624	0.667	
	Petal				0.669	0.614	0.623	0.600	
						0.676	0.652		0.658
	Calyx					0.649	0.691	0.692	
	Bract leaf						0.723	0.655	
Leaf	Upper							0.691	

respective of whether they are floral or foliar, have more than fifty percent of protein constituents in common. 2) Organs constituting flower parts exhibit satisfactory high values among themselves, but lower values with calyx, bract leaf or leaf. 3) It is interesting to take note of the relationship between leaves, on the one hand, and calyx and bract leaf, on the other. The biochemical relationship between the calyx and either the upper or the intermediate leaf are not particularly close. The relationship between the bract leaf and the upper leaf is apparently high, despite of the low value between upper and intermediate leaf.

Competition in relation to tree age in Cryptomeria japonica

Shigesuke HAYASHI and Kan-Ichi SAKAI

Transverse disks of 196 trees were collected from an artificial plantation some fifty years old of Cryptomeria japonica at Kagoshima University. Position of each tree in the forest was recorded on a section paper. The disks were measured for yearly growth in eight directions and every five years the mean diameters of stem were used for the calculation of correlations between adjacently growing trees. Mean diameter and the correlation coefficients were as follows:

Age in years		5	10	15	20	25	30
Diameter (cm)		2.72	4.44	7.68	11.36	14.38	16.64
r_{ij}	vertical*	0.423	0.622	0.162	−0.063	−0.078	−0.157
	horizontal*	0.485	0.596	0.221	0.128	0.117	−0.004
	pooled	0.448	0.608	0.192	0.032	0.021	−0.080

Age in years		35	40	45	50
Diameter (cm)		18.26	19.44	20.18	20.92
r_{ij}	vertical*	−0.226	−0.245	−0.243	−0.246
	horizontal*	−0.082	−0.136	−0.159	−0.183
	pooled	−0.154	−0.190	−0.201	−0.214

* Vertical and horizontal stand for r's computed from pairs of trees growing in vertical and horizontal rows to the slope, respectively.

It has been published recently that $r_{i,\,i+1}$ of stem diameters between two adjacently growing Cryptomeria trees tells the status of intertree competition: positive and high values indicate little or no competition while zero or negative values show intense competition (Sakai, Mukaide and Tomita, 1968 in Silvae Genetica). It is found from the above table that for the first 10 years, intertree competition appeared to be nil, but was apparent in later years. It is thus concluded that in the forest investigated, trees younger than 10 years were free from intertree competition, but older trees 10 and 15 years of age started to compete with each other and the competitive effect was accumulative year after year.

Analysis of a clonal progeny in a natural forest of Cryptomeria

Kan-Ichi SAKAI, Shigesuke HAYASHI, Kihachiro OHBA and Koji TOMITA

It is known that Crytomeria japonica propagates either vegetatively or by seed in nature. This study aimed at distinguishing a clonal progeny from others in a natural forest of Cryptomeria by means of an identity index which estimates the degree of similarity between a given tree and other trees. It is computed on the basis of several needle characters:

$$I_{j,\,i}=1-\frac{\sum_{m=1}^{n} D^2{}_{m(j,\,i)}}{n} \quad \text{and} \quad +1\geq D\geq -1\,,$$

where $I_{j,\,i}$ is the identity index comparing j-th tree with a given i-th tree. $D_m(j,\,i)$ is the difference in m-th character between j-th and i-th tree, n being the number of characters to be compared.

In this study, 226 Cryptomeria trees in a natural forest of about 750 m^2 were measured for five needle characters: twistedness, angle of insertion, stiffness, density and intensity of color. They were classified into 39

groups including each more than 2 trees besides 13 single trees. The distribution of group size is presented in the following table.

	Number of trees per group										Total number of groups	Mean number of trees per group
	1	2-4	5-7	8-10	11-13	14-16	17-19	20-22	23-26	27-29		
Number of groups	13	19	11	2	3	0	3	0	0	1	52	5.12

At least, some of the group are regarded as clones, and the variance of some quantitative characters measured within them was apparently smaller than the total variance of the forest. It thus becomes possible to make a genetic study of quantitative characters in a natural forest of Cryptomeria japonica.

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ABSTRACTS OF DIARY FOR 1967

February	24	151st Meeting of Misima Geneticist' Club
March	27	76th Biological Symposium
April	3	77th Biological Symposium
April	21	78th Biological Symposium
May	12	152nd Meeting of Misima Geneticist' Club
May	22	79th Biological Symposium
June	9	153rd Meeting of Misima Geneticist' Club
June	17	27th Meeting of the Board of Councillors
July	14	154th Meeting of Misima Geneticist' Club
August	15, 16	The 6th Summer Seminar on Genetics for High School Teachers of Biology
September	22	155th Meeting of Misima Geneticist' Club
November	11	Public Lectures on Genetics (at the National Science Museum, Ministry of Education, Tokyo)
November	28	156th Meeting of Misima Geneticist' Club
December	9	Japanese Poultry Breeder's Association
December	14	157th Meeting of Misima Geneticist' Club

FOREIGN VISITORS IN 1967

Jan.	15	SCHEIN, M. W., National Science Foundation, Washington, D. C., U.S.A.
Mar.	23	TCHAN, Y. T., Department of Agricultural Microbiology, University of Sydney, Sydney, Australia
		QUISENBERRY, K. S., Agricultural Research Consultant, Sarasota, Florida, U.S.A.
Mar.	27	YAMAMOTO, N., Felis Institute, Temple University, Philadelphia, U.S.A.
Mar.	28	JESENA, C. C., Department of Agronomy, University of Philippines, The Philippines
Apr.	3	KANAGIR, D., Department of Biochemistry, Institute of Nuclear Sciences "Boris Kidrich", Yugoslavia
Apr.	5-6	GARCIA, A., World Seeds, Inc., California, U.S.A.
Apr.	20-22	GOTTSCHALK, W., Institute of Genetics, Bonn University, West Germany
Apr.	26	ROBERTS, W., Boyce Thompson Institute for Plant Research, N. Y., U.S.A.
Apr.	29	TOKUNAGA, C., Department of Zoology, University of California, Berkeley, California, U.S.A.
May	2-17	STICH, H. F., Department of Biology, McMaster University, Hamilton, Ontario, Canada
June	6	EVERSON, E. H., Department of Crop Science Wheat Breeding and Genetics, Michigan State University, East Lansing, Michigan, U.S.A.
June	11	SOEKARNA, D., Research Institute of Pest and Diseases of Crop Plants, Bogor, Indonesia
June	26	NAVARATNA, S. K., Agricultural Officer, Division of Botany, Central Agricultural Research Institute, Ceylon
June	30	SOEKARNA, D., Research Institute for Pest and Diseases of Crop Plants, Bogor, Indonesia
		MOSKOWITZ, M., Department of Biological Sciences, Purdue University, Lafayette, Indiana, U.S.A.
July	11	KOJIMA, K., Department of Genetics, Texas University, U.S.A.
July	17	LAMM, R. D., Colorado State Representative, Denver, Colorado, U.S.A.
July	19	HODGE, W. H., Tokyo Office, National Science Foundation, U.S.A.
Aug.	4	O'NELL, E., CSIRO, Division of Plant Industry, Canberra

City, A.C.T., Australia

Aug. 7–8 SUEOKA, N., Department of Biology, Princeton University, Princeton, N. J., U.S.A.

Aug. 19 GARBER, B., College of Medicine, Department of Biochemistry, State University of New York, U.S.A.

Aug 26 BULL, H. B., Department of Biochemistry, University of Iowa, U.S.A.

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Oct.	2	FINLAY, K. W., Waite Agricultural Research Institute, Glen Osmond, South Australia
		HSIEH, S. C., Taiwan Agricultural Research Institute, Taiwan
Oct.	9	PAPAZOV, N., Bulgarian Ambassador at Tokyo
Nov.	20	SCOSSIROLI, R. E., Instituto di Genetica, Università di Bologna, Italia
Dec.	18	COYNE, B. J., c/o Committee on Mathematical Biology, University of Chicago, U.S.A.
		KOMING, D. R., N. I. H. Pacific Office, National Institute of Health, U.S.A.
		ANGELONE, L., N. I. H. Pacific Office, National Institute of Health, U.S.A.

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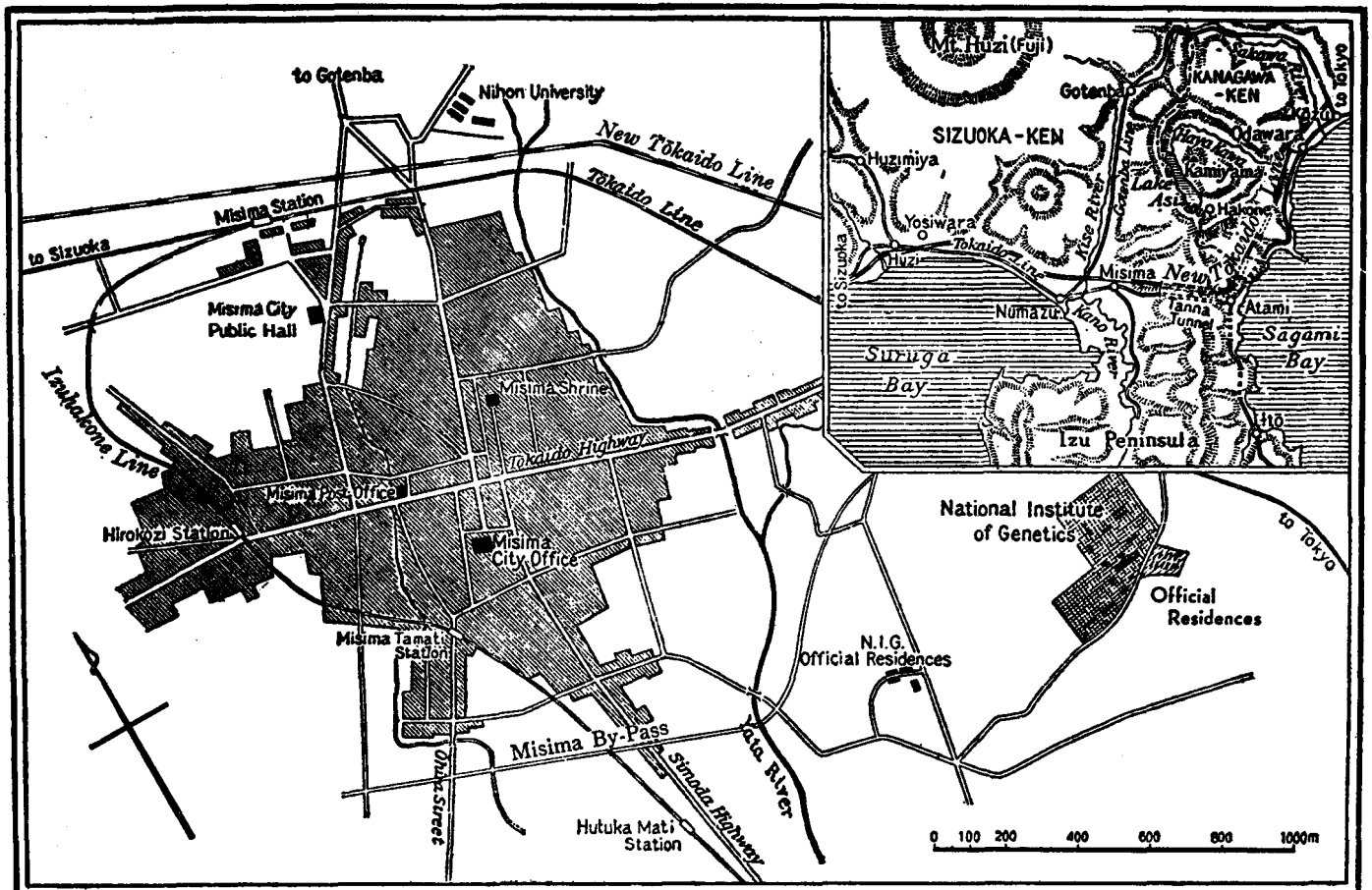
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