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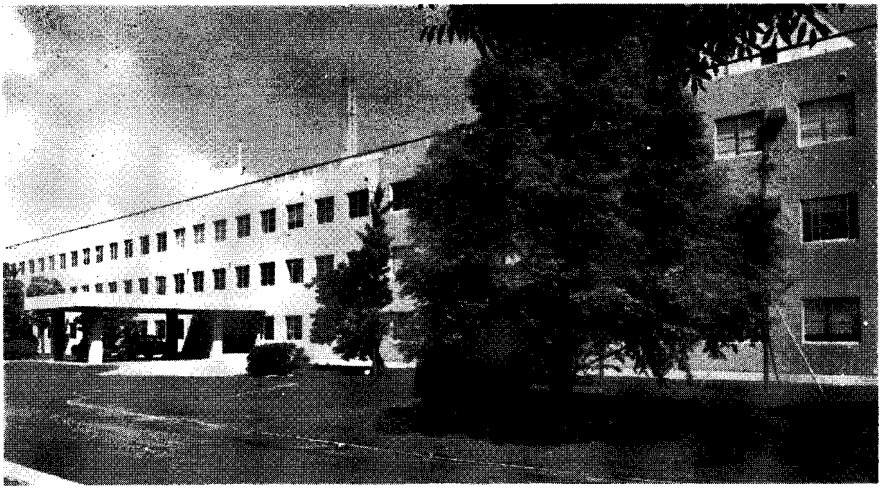

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CONTENTS

GENERAL STATEMENT

It is customary that this statement is written by the director. Therefore this will be my last statement for the annual report of the National Institute of Genetics, as my wish for resignation from the director's post was accepted by the Council and I shall retire by the end of the fiscal year (March 1969).

It was in the fall of 1955 when I came to Misima from Kyoto, where I served 35 years as assistant, assistant professor as well as professor at Kyoto University. Since then almost 14 years passed away.

Thanks to the collaboration of our staff members and through the good offices of the government officials, we could complete the new concrete main building and we succeeded in establishing 10 departments including molecular genetics, whose budget will be allocated from the next fiscal year. Thus our goal set at the start had been attained. Though the performance of our plans often lagged behind the set standards, we should be at least content with our present scale of work and our endevours to complete the departments. Our thanks are due to foreign organizations, especially the Rockefeller Foundation who provided us with many grants for genetic studies and funds for building a rice laboratory and seed storage room. We are also grateful to the National Institutes of Health for various grants for researches in special fields.

The year 1968 confronted the geneticists of Japan with a busy and difficult task, namely the organization of the XII International Congress of Genetics. We could welcome some 1,500 colleagues from abroad and the meetings were held in an atmosphere of true friendship and understanding thanks to our foreign colleagues, who cooperated with us to make the Congress a success.

In our institute, Dr. M. Kimura, head of the Department of Population Genetics was given an award from Japan Academy in May 1968. In November Dr. F. A. Lilienfeld was decorated with the Fourth Order of Sacred Treasure by His Majesty the Emperor of Japan for her services of many years.

Dr. Lilienfeld's first stay in Japan (Kyoto) was from 1929-1936 and the second one from 1950 up to the present. She worked with me first on wheat and is now interested in the study of chimeras induced by alien cytoplasms. She has read and corrected almost all of English manuscripts written by us, for which I want to thank her heartily on behalf of the institute.

H. Kihara

RESEARCH MEMBER

Director

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

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The 2nd Laboratory

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The 2nd Laboratory

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WADA, Bungo, Manager, Emeritus Professor of University of Tokyo

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PROJECTS OF RESEARCH FOR 1968

Department of Morphological Genetics

Genetics of the silkworm (TAZIMA and ONIMARU)
Repair processes in radiation mutagenesis (TAZIMA and ONIMARU)
Genetic studies of radiosensitivity in the silkworm (MURAKAMI)
Chemical mutagenesis in the silkworm (TAZIMA and ONIMARU)
Genetic studies on insect cells in tissue culture (KURODA and MINATO)
Developmental genetic studies on carcinogenesis in tissue culture (KURODA)
Effects of radiation on cells in tissue culture (KURODA)

Department of Cytogenetics

Cytogenetical and biochemical studies on tumor cells (MORIWAKI, IMAI, SEKIYA and YOSIDA)
Cytogenetical and biochemical studies of rodents in Southeast Asia and Oceania (YOSIDA, MORIWAKI, IMAI and TSUCHIYA)
Studies on chromosomal and biochemical polymorphism of black rats (*Rattus rattus*) (YOSIDA, MORIWAKI and TSUCHIYA)
Study on incorporation of isolated chromosomes into cultured mammalian cells (YOSIDA)
Experimental breeding and genetics of mice and rats (YOSIDA, MORIWAKI, TSUCHIYA, SAKAKIBARA and SONODA)
Morphological and genetical studies on some plant tumors (YONEDA and CHU)
Cytogenetical and biochemical studies on morning glory (YONEDA)

Department of Physiological Genetics

Population genetics of deleterious genes in natural populations of *Drosophila melanogaster* (OSHIMA and WATANABE)
Mechanisms of persistence of some lethal genes (OSHIMA and WATANABE)
Physiological and population genetics of sterile genes in natural populations of *Drosophila melanogaster* (OSHIMA and WATANABE)
Analysis of fitness in a fluctuating environment (OSHIMA and WATANABE)
Analysis of inversion chromosome in natural populations of *Drosophlia melanogaster* (OSHIMA and WATANABE)
Nucleus substitution in wheat and related species (KIHARA, SAKAMOTO and OHTA)

Basic studies on hybrid wheat breeding (KIHARA)
Cytogenetic studies in the tribe Triticeae (SAKAMOTO)
Collection and preservation of *Oryza* species (KIHARA)
Studies on cytoplasmic inheritance in higher plants (OHTA)

Department of Biochemical Genetics

Studies on transformation in higher organisms (NAWA, YAMADA and TSUJITA)
Genetical and biochemical studies of pteridine metabolisms in insects (NAWA and TSUJITA)
Studies on a gene for retarded moulting (*rm*) in the silkworm (TSUJITA)
Studies on the pteridine granule formation in larval hypodermal cells of the silkworm (TSUJITA and SAKURAI)
Analysis of gene action on cell differentiation in higher organisms (TSUJITA and NAWA)
Biochemical studies on the differentiation of muscle proteins in animals (OGAWA)
Genetical and biochemical studies of human serum proteins (OGAWA)
Genetical and biochemical studies on Japanese middle size dog (OGAWA)
Genetical and biochemical studies of the membrane protein of pteridine granules in the silkworm (SAKURAI and TSUJITA)
Genetics on isozymes in plants (ENDO)
Enzyme regulation in cultured organ of morning glory (ENDO)

Department of Applied Genetics

Quantitative genetic studies in poultry (SAKAI, KAWAHARA and FUJISHIMA)
Genetic studies in wild populations of Japanese quails (KAWAHARA)
Theoretical studies on breeding techniques (SAKAI and IYAMA)
Studies on competition in plants (SAKAI, IYAMA and MIYAZAKI)
Developmental genetics of quantitative characters in plants (SAKAI and HIGUCHI)
Studies on the effects of X-ray irradiation on quantitative characters of rice (IYAMA)
Genetic studies in natural stands of forest tree species (SAKAI and MIYAZAKI)
Zymographic studies in forest trees (SAKAI and MIYAZAKI)
Simulation studies on artificial selection (IYAMA)
Genetic studies of isolating barriers in *Oryza* (OKA aud CHU)
Numerical taxonomic studies in *Oryza perennis* (MORISHIMA and OKA)
Experiments on natural selection in wild and cultivated rice forms

(MORISHIMA and OKA)
Analysis of sterility genes in *Oryza* (OKA and MORISHIMA)
Analysis of genetic plant types in rice (MORISHIMA and OKA)

Department of Induced Mutation

Radiation genetics in mice (TUTIKAWA)
RBE and dose rate effects in higher plants (FUJII and AMANO)
Radiation genetics in *Arabidopsis* (FUJII)
Fine structure analysis in maize (AMANO)
Biological effects of ultraviolet radiation (KADA, FUJII, AMANO and HAYASHI)
Radiation-induced and chemical mutagenesis in microorganisms (KADA and SADAIE)

Department of Human Genetics

Genetic consequences of population trends (MATSUNAGA)
Dermatoglyphics (MATSUNAGA and MATSUDA)
Down's syndrome in Japan (MATSUNAGA, OISHI and KIKUCHI)
Cytogenetics in man (OISHI, KIKUCHI, SHIBATA and KUMAGAI)
DNA replication in human chromosomes (KIKUCHI and OISHI)
Biochemical studies on plasma proteins and enzymes (SHINODA)
Chemical modification of ribonucleic acids and their constituents (SHINODA)

Department of Microbial Genetics

Genetic fine structure analysis on microorganisms (IINO, ISHIDSU and YAMAGUCHI)
Genetics of cellular regulatory mechanisms (SUZUKI and ISHIDSU)
Genetics of bacterial flagella (IINO, ENOMOTO and SUZUKI)
Genetics of motility in bacteria (ENOMOTO)
Genetics of host range in bacteriophages (IINO, ENOMOTO and YAMAGUCHI)

Department of Population Genetics

Theoretical studies of population genetics (KIMURA)
Uses of computers in the theoretical studies of population genetics (KIMURA and MARUYAMA)
Studies on the genetic structure of human populations (YASUDA)

RESEARCHES CARRIED OUT IN 1968

I. CYTOGENETICS

Scientific Expedition for the Study of Rodents to South East Asia and Oceania. I. Members, Aims and Schedule of the Scientific Expedition

Tosihide H. YOSIDA

Supported by a grant-in-aid for scientific research from the Ministry of Education, we had organized a scientific expedition for the study of rodents to South East Asia and Oceania from September 20 to November 8, 1968. The members of the expedition were as follows:

Tosihide H. Yosida (Head)
Kazuo Moriwaki
Hirotami, T. Imai
Kimiyuki Tsuchiya
Tatsuo Udagawa

Department of Cytogenetics, National Institute of Genetics

——Forest Experimental Station, Ministry of Agriculture, Tokyo

Many species belonging to Muridae inhabit South East Asia and Oceania, but informations on these animals are not sufficient. Therefore, their study was undertaken with the following aims.

a) Research of polymorphism of the black rat, *Rattus rattus*: Presence of chromosomal polymorphism and transferrin of blood protein in the black rat was first detected in the Japanese population by Yosida *et al.* (this report, No. 15-18, 1965-1968) and Moriwaki *et al.* (this report, No. 18, 1968). The main purpose of our expedition was to examine whether such polymorphism should be found in the rats collected from several localities of South East Asia and Oceania.

b) Karyotaxonomical studies of rodent species: Karyological studies of species inhabiting South East Asia are very few. The second aim of the expedition was to survey the karyotypes of these animals and to determine the taxonomical relationships in this group.

c) Detection of suitable laboratory animals among the wild ones collected in South East Asia and Oceania: As to the rodents living in South East Asia, they were not yet tested for their usefulness as laboratory animals. In order to find this out, we had sent to Misima living animals of several species of rodents and we are breeding them in our laboratory.

d) Research on the damage to cultivated plants and forests by rodents: The rodents cause tremendous damage to cultivated plants and forests in several countries of South East Asia. The research was carried out mainly by Dr. Udagawa, one of our team, to devise appropriate methods of protection from these pests.

Schedule of the expedition was as follows:

Tokyo (left Sept. 20)→Tainan (Formosa)→Luzon (Philippines)→Bangkok (Thailand)→Kuala Lumpur (Malaya)→Singapore→Kotakinabalu (Borneo) →Bogor (Java)→Makassal (Celebes)→Brisbane and Cairns→(Australia)→ Wellington (New Zealand)→Pt. Moresby (New Guinea)→Mindanao (Philippines)→Hong Kong→Okinawa→Tokyo (arrived Nov. 8).

Scientific Expedition for the Study of Rodents to South East Asia and Oceania. II. Species and Chromosome Numbers of Rodents Collected from South East Asia and Oceania

Tosihide H. YOSIDA, Kimiyuki TSUCHIYA,
Hirotami T. IMAI and Tatsuo UDAGAWA[1)]

Species of rodents, their chromosome numbers and localities of collection in South East Asia and Oceania are listed in the following Table (Table 1).

Table 1. Names of species, collection localities and diploid chromosome numbers of rodents collected from South East Asia and Oceania

Species	Localities of collection	Chromosome number ($2n$)
Rattus annandalei	Kuala Lumpur, Malaysia	42
Rattus conatus	Cairns, Australia	32
Rattus canus	Kuala Lumpur	46
Rattus exulans	Luzon, Philippines	42 (44)
〃 〃	Makassar, Celebes	42
〃 〃	Bangkok, Thailand	42
Rattus fuscipes	Brisbane, Australia	38 (39)
Rattus hung	Hong Kong	42
Rattus muelleri	Kuala Lumpur, Malaysia	42
Rattus norvegicus	Taiwan, Philippines, Thailand, Australia, New Zealand	42
Rattus rajah	Bangkok, Thailand	52
Rattus rattus	Okinawa, Japan	42* (44)
〃 〃	Tainan, Taiwan (Formosa)	42*

[1)] Forest Experimental Station, Tokyo.

Table 1 (continued)

Species	Localities of collection	Chromosome number ($2n$)
Rattus rattus	Khao Yai, Thailand	42*
〃 〃	Luzon, Philippines	42**
〃 〃	Mindanao, Philippines	42**
〃 〃	Bogor, Java	42**
〃 〃	Makassar, Celebes	42**
R. rattus rattus	Brisbane and Cairns, Australia	38
〃 〃	Wellington, New Zealand	38
〃 〃	Port Moresby, New Guinea	38
R. rattus argentiventer	Kuala Lumpur, Malaysia	42**
R. rattus diardi	Kuala Lumpur, Malaysia	42* (43, 44)
R. rattus flavipectus	Hong Kong	42*
R. rattus jalorensis	Kuala Lumpur, Malaysia	42**
Rattus sp.	Hong Kong	42
Rattus sp.	Port Moresby, New Guinea	34
Rattus surifer	Kuala Lumpur, Malaysia	52
Rattus sabanus	Kuala Lumpur, Malaysia	42
Bandicota nemorivaga	Tainan, Formosa	44
Melomys cervinipes	Brisbane, Australia	54
Melomys littoralis	Cairns, Australia	52
Mus thai	Bangkok, Thailand	40
Mus caroli	Bangkok, Thailand	40
Uromys caudimaculatus	Port Moresby, New Guinea	54

* Polymorphism was found in No. 1 chromosomes (telocentrics and subtelocentrics).

** No polymorphism was found in No. 1 chromosomes (subtelocentrics). Seldom occorring chromosome number is given in parenthesis.

Scientific Expedition for the Study of Rodents to South East Asia and Oceania. III. Chromosomal Polymorphism and New Karyotypes of Black Rat, *Rattus rattus*, Collected in South East Asia and Oceania

Tosihide H. YOSIDA and Kimiyuki TSUCHIYA

It was already reported that black rats, *Rattus rattus*, collected in Japan and Korea were characterized by a polymorphism of the largest No. 1 chromosome pair, present either as a telocentric homomorphic

pair (T/T), or a telocentric and subtelocentric heteromorphic pair (T/S) or a subtelocentric homomorphic pair (S/S). (Yosida *et al.*, 1965, Chromosoma 16 : 70; 1968, this Ann. Rept. 18 : 51). Recently we found that No. 9 and No. 13 chromosomes showed also similar polymorphism by the presence of telocentric homomorphic (T/T), subtelocentric homomorphic (S/S) or subtelocentric and telocentric heteromorphic pairs (T/S).

A similar chromosomal polymorphism as in Japan was found in black rats collected in Okinawa, Hong Kong, Bangkok and Kuala Lumpur. On the other hand, all of *Rattus rattus* collected in paddy fields of Philippines, Java, and Celebes, showed that chromosomes No. 1, No. 9 and No. 13 were represented by S/S, S/S and T/T pairs. No chromosomal polymorphism was found in the rats collected in those countries.

Black rats collected in all localities of Far and South East Asia had the diploid chromosome number of 42, while all animals collected in Australia, New Zealand and New Guinea showed 38 diploid chromosomes. Two pairs of large metacentric chromosomes, one larger than the other, characterized the karyotypes of these animals. No. 1 and No. 9 chromosome pairs were usually of S/S type. The other chromosomes were similar to those of the Asian black rats. From the results of comparison of idiograms between Japanese and Australian black rats it is suggested that two arms of the large metacentric element in the latter might be homologous with No. 4 and No. 7 telocentric elements of the former, and two arms of the other smaller metacentrics might correspond to No. 11 and No. 12 telocentrics.

F_1 hybrids between Australian ($2n=38$) and Japanese rats ($2n=42$) showed 40 diploid chromosomes. It appears that among them 19 chromosomes came from the Australian rat and 21 chromosomes from the Japanese rat. Test crosses of F_1 animals and segregation of chromosomes are under investigation.

Scientific Expedition for the Study of Rodents to South East Asia and Oceania. IV. Comparative Idiogram Amalysis in Several Species Belonging to Genus *Rattus* Collected from South East Asia and Oceania

Tosihide H. YOSIDA and Kimiyuki TSUCHIYA

16 species belonging to genus *Rattus* were collected from South East Asia and Oceania. They showed various diploid chromosome numbers, such as 32, 34, 38, 42, 46 and 52. Among them, species with 42 chromosomes were found most frequently. From the above results it is

strongly suggested that the basic chromosome number in genus *Rattus* is $2n=42$, and the basic karyotype consists of 13 pairs of telocentrics, 7 pairs of metacentrics and telocentric X and Y elements, which were observed most frequently in *Rattus rattus* with chromosomal polymorphism.

The relationship between the karyotypes varying from the basic pattern and differentiation of species in genus *Rattus* was investigated.

(1) The following species had a basic chromosome number, but their karyotypes deviated slightly from the basic one. *Rattus norvegicus*, *Rattus jalorensis*, *Rattus argentiventer*, *Rattus anandalei*, *Rattus exulans*, *Rattus mulleri* and *Rattus* sp. Hong Kong.

(2) The following species had a smaller chromosome number than the basic one due to a change of Robertsonian type. *Rattus rattus rattus* ($2n=38$), *Rattus fuscipes* ($2n=38$), *Rattus* sp. New Guinea ($2n=34$), and *Rattus conatus* ($2n=32$).

(3) The following species had larger chromosome numbers than the basic one by non-disjunction. *Rattus rajah* ($2n=52$) and *Rattus surifer* ($2n=52$).

(4) Lastly, species with a markedly different karyotype from the basic one were *Rattus sabanus* ($2n=42$); 19 pairs of telocentrics and 2 pairs of metacentrics. *Rattus canus* ($2n=46$); 20 pairs of telocentrics and 3 pairs of metacentrics. *Rattus hung* ($2n=46$); 20 pairs of telocentrics and 3 pairs of metacentrics.

Scientific Expedition for the Study of Rodents to South East Asia and Oceania. V. Breeding of Wild Rodents Collected from South East Asia and Oceania

Kimiyuki TSUCHIYA and Tosihide H. YOSIDA

In order to find useful laboratory animals and to examine their behaviour, we had sent live specimens of several species of rodents collected from South East Asia and Oceania to our institute and we are breeding them in the laboratory. Names of species, number of propagated animals and breeding generations are given in the following table (Table 1).

All animals of Table 1 have been bred in the laboratory. Among them up to date in four species we were successful in breeding the animals to the first and second generations. We could also obtain hybrids between two subspeices of *Rattus rattus*. We found that *Rattus exulans* is a good experimental animal due to its small body and easy breeding. *Rattus conatus* is also good for cytogenetical study, because its chrosome number is smaller ($2n=32$) than that of other animals in the *Rattus* group.

Table 1. Species and number of animals breeding in laboratory

Collected locality and species name	No. of collected animals		No. of animals at 1st gener.		No. of animals at 2nd gener.		Total no. of animals under breed.	
	♀	♂	♀	♂	♀	♂	♀	♂
Okinawa (Naha city)								
Rattus rattus	1	1					1	1
Mus servicolor	1	1					1	1
Formosa (Tainan)								
Bandicota indica nemorivage	2	1					2	1
Rattus rattus	2	3					2	3
Thailand								
Rattus rattus	1	2					1	2
Malaysia (Kuala Lumpur)								
Rattus rattus diardii	2	2	13	15	0	2	15	19
R. r. Jalorensis	1	0					1	0
R. sabanus	2	1					2	1
R. rajah	1	1					1	1
R. mulleri	1	1					1	1
R. bowersi	1	1					1	1
Indonesia (Celebes)								
Rattus rattus	3	3					3	3
R. exulans (Java)	2	1	11	13			13	14
Rattus rattus	1	1					1	1
Australia (Brisbane)								
Rattus rattus rattus	2	2					2	2
R. fuscipes	2	2	5	8			7	10
Melomys cervinipes (Cairns)	3	1					3	1
R. r. rattus	2	2	11	10			13	12
R. conatus	2	1	10	11	3 Newly born		12	12(3)
New Guinea (Port Moresby)								
R. r. rattus	1	1	32	32			33	24
Mus musclus	2	1					2	1
F_1 hybrid between								
R. r. tanezumi (Japan)			3 litters					
× *R. r. rattus* (Aust.)			7	5			7	5
R. r. rattus (Aust.)			1 litter					
× *r. r. tanezumi* (Japan)			5	2			5	2

Rat Metaphase Chromosomes Incorporated into Mouse Metaphase Cells

Tosihide H. YOSIDA and Toyozo SEKIGUCHI[1]

The present paper deals with the observation of rat metaphase chromosomes incorporoted into mouse metaphase cells. As recipient cells a primary culture of mouse embryonic cells obtained from C57BL strain was used. Most of the cells within one week after cultivation showed normal diploid chromosomes ($2n=40$), characterized by telocentrics or acrocentrics. Donor chromosomes were isolated from the cells of rat ascites hepatoma AH-130. The rat cells had hypotetraploid chromosome number ($s=75$), including chromosomes of diverse shapes, such as meta-, submeta-, subtelo- and acrocentrics.

One, two and four days after addition of rat chromosomes to the culture, they were observed to be incorporated into the metaphase of the mouse cells at the rate of 0.3, 1.2 and 0.2 per cent, respectively. The incorporated rat chromosome could be distinguished from the mouse chromosomes by their characteristic shape, or by the occurrence of one or two extraordinary elements. The incorporated chromosomes sometimes showed signs of degeneration or were unusually slender, but in other cells they appeared quite normal.

The percentage of mitotic cells in proportion to interphase cells, four hours after treatment with colchicine, was one, two and four days after the addition of chromosomes, 7.2, 0.7 and 5.0, respectively. From the above results, it seems that some of mouse cells that had received rat chromosomes may have degenerated and therefore the frequency of chromosome integration into mouse cells was just opposite to the mitotic rates of the recipient cells.

Autoradiographic Analysis of the Rate of Protein Synthesis in Diploid and Tetraploid MSPC-1 Myeloma Cells[2]

Kazuo MORIWAKI and Hirotami T. IMAI

As previously reported (K. Moriwaki *et al.* 1968, this Annual Report 18 : 18-20), MSPC-1 mouse myeloma cells exhibited an obvious tendency to increase the rate of total protein synthesis per cell in parallel with the increase of tetraploid cells until 40 per cent in metaphase count,

[1] National Cancer Center Research Institute, Tokyo.

[2] This study was supported by Public Health Service research grant CA 07798-05 from the National Cancer Institute, U. S. A. (T. H. Yosida).

beyond which it reached a plateau. In that case, however, the rate of protein synthesis was determined from a mass of tumor cells of the order of 10^6. In the present study a further attempt was made to observe the variation in the rate of protein synthesis in individual tumor cells following tetraploidization.

The tumor cells used in this experiment were suspended in MEM medium with 5 percent calf serum and consisted of a mixture of diploid and tetraploid cells at the density of about 10^6 per ml. Tritiated leucine was added to the suspension at the concentration of 50 μC per ml. After two hour incubation at 25°C, the tumor cells were spread on microscopic slides by air drying method. After dipping in NR-M2 autoradiographic emulsion, the microscopic specimens were stored in a refrigerator for a three week exposure. The average number of deposited grains in a tetraploid cell was approximately two times larger than in a diploid cell.

This finding suggests a gene-dose dependent increase of the rate of protein synthesis in tumor cells. The discrepancy between our previous and present results might be explained by the following assumptions: During the transient stage from diploidy to tetraploidy the actual percentage of tetraploid cells is somewhat larger than that estimated from metaphase count, because in this stage a conciderable amount of binucleate cells appears in the tumor cell population and most of them are secored as diploid from metaphase figures. This could bring about an underestimation of tetraploid cell percentage. Furthermore, the part composed of binucleate cells can stay at interphase for a length of time several generation cycles of the mononucleate cells. Such a retardation of mitosis in the binucleate cells might also contribute to an increase of the actual percentage of tetraploid cells.

Consideraing the above assumptions, the percentage of tetraploid cells seems to show an almost linear relationship between gene dosage and the rate of total protein synthesis in MSPC-1 tumor cells as a criterion of gene action.

Chromosome Alterations *in vitro* and *in vivo* of Cultured Hamster Cells Ttansformed by Chemical Carcinogen[1)]

Kunio SEKIYA

Neoplastic transformation of golden hamster embryonic cells was induced *in vitro* by treatment of the cultures for 10 days with 4HAQO

1) This study was supported by Public Health Service research grant CA 07798-05 from the National Cancer Institute, U. S. A. (T. H. Yosida).

(4-hydroxy-aminoquinoline-1-oxide) at a concentration of 10^{-5} M. The present report describes the neoplastic development of 4HAQO-treated cells and chromosomal alterations which had taken place due to the treatment.

Morphological features of transformation first appeared in cultures approximately 20 days after carcinogen administration, as indicated by a criss-cross arrangement of cells and loss of contact inhibition resulting in a "piling up" of cells. These cells, however, failed to give rise to tumor when transplanted either to the subcutis or the cheek pouch of adult hamsters. To acquire transplantability, the carcinogen-treated cells seemed to require further cultivation *in vitro*. About 150 days after treatment, the cultures started to show 100% transplantability to recipient animals; an inoculum of 10^6 cells was capable to produce tumors in all animals examined.

Chromosomes of these cells were studied at various stages of cultivation after treatment with the chemical. The modal chromosome number of the transformed cells was found to shift gradually from the normal diploid number, 44, to a hypodiploid 43. It seemed to be of fundamental importance to find out if this karyotypical change had anything to do with the cause of the neoplastic transformation of the culture or was merely brought about by environmental factors operating during the cultivation. To obtain critical information regarding this alternative, cells were transferred to various environmental conditions, and their karyotypes were carefully examined at certain periods after the transfer. The environments employed were as follows: 1) cultivation *in vitro*, 2) cultivation in the subcutis of the flank of adult hamsters and 3) cultivation in adult hamsters that had received whole-body irradiation with γ-rays. Cells grown under tissue culture conditions showed the mode at 43 chromosomes as expected, while those transplanted to hamsters, both irradiated and unirradiated, were found to possess a modal number of 45. The percentage of cells showing 43 chromosomes was higher in cells populations proliferating in irradiated animals than that in unirradiated ones. This fact may indicate that the irradiated hamsters offer conditions resembling more those *in vitro* than do the unirradiated animals, at least in terms of the maintenance of cells with a given karyotype. The alterations in karyotype was mostly characterized by a missing or an additional chromosome in comparison with the normal karyotype. Lack or addition of one chromosome seemed to occur randomly for any member of the chromosome complement. Therefore, these changes may be explained as resulting of non-disjunction of chromosomes in the mitotic processes.

It was found that cells transplanted to unirradiated hamsters showed a longer time lag before the production of a tumor as compared with those transferred to irradiated animals. It was also found that viability of transplanted cells was higher in the irradiated animals than in the unirradiated ones, and this tendency was emphasized when a smaller inoculum was used.

These results seem to be in favor of the idea that environmental factors play a key role in the establishment of a new karyotype. Some immunological mechanism might be involved in the *in vivo* alteration of the cromosome complement of the transformed cells.

Aggregate-Forming Activity of Rat Hepatoma Cells Induced by DAB

Yukiaki KURODA

In the process of malignant transformation of normal cells, changes in aggregate-forming activity which is a useful indicator of cell function determined quantitatively under strictly standarized conditions were examined. With the cooperation of Dr. Sato, Okayama University Medical School, liver cells from Donryu Rats, fed with 4-dimethylaminoazobenzene (DAB) for various days, were tested for their malignancy (ability to produce tumors in newborn rats), chromosome number and their aggregate-forming activity (aggregation pattern of the cells in rotation culture).

When strain dRLa-74 cells, which were derived from a male rat fed with DAB for 191 days were retransplanted into newborn rats intracerebrally, intraperitoneally or subcutaneously with an inoculum containing 10^5 to 5×10^5 cells per rat, the rats died with tumors at the rates of 4/9, 1/3 or 0/3, respectively. The number of chromosomes in strain dRLa-74 cells had a main diploid mode and another small triploid mode. The aggregate-forming activity of dRLa-74 cells was compared under the standard conditions of rotation culture with that of strain C251 cells which were derived from normal newborn rat livers. dRLa-74 cells formed after 24 hours of rotation culture aggregates which were significantly larger in diameter than those obtained from C251 cells.

C83T cells, which were derived from the liver of a female rat fed with DAB for 264 days, showed a moderate malignancy when they were retransplanted into newborn rats. C84AT cells, which were derived from the liver of a female rat fed with DAB for 312 days, showed a high malignancy. C84AT cells formed after 24 hours of rotation culture significantly larger aggregates than those of C83T cells.

In some other rat liver cells tested a close correlation was found between the ability of cells to produce tumors and their aggregate-forming activity. These facts coincide with the results which had been previously obtained with RSV-infected chick cells, mouse mammary gland tumor cells (Kuroda, Y. 1968. GANN 59 : 281), and mouse plasma tumor cells (Kuroda, Y. 1968. Ann. Rep. Natl. Inst. Genet. Japan 18 : 10). The increase in aggregate-forming activity of cells in carcinogenesis may provide an experimental clue to an explanation of the loss of contact inhibition (growth inhibition due to cell contact) and loss of histoformative structure of tumor cells.

Pachytene Pairing in a Translocation Heterozygote of Maize Having a Huge Knob Close to the Breakpoint

Yasuo OHTA

Chromosome knobs of maize in pachynema are a hereditary trait. Their position, size and shape are very constant features of the karyotype and can serve as cytological markers. Chromosome knobs consist mostly of heterochromatin, and are considered to be genetically inert (except for the abnormal chromosome 10).

An exceptionally huge knob ($3L^{111}$) was found on the long arm of chromosome 3 (position .58) in an inbred line, N. C. No. 12, derived from the variety Jarvis Golden Prolific (Ohta 1965). To examine the effect of this huge mass of heterochromatin on the synapsis during meiotic prophase, this inbred line was crossed to a translocation homozygote, T3-8h (furnished by Dr. E. B. Patterson), having the breakpoint at .53 on the long arm of chromosome 3.

Pachytene pairing in a translocation heterozygote, $3L^{111}$/T3-8h, was analysed. Three major types were found: 1) complete homologous pairing throughout the cross-shaped configuration, 2) complete asynapsis or asynapsis in each arm of the cross-shaped configuration in the segments adjacent to the breakpoint, and 3) partly homologous pairing and partly asynapsis. The observed frequency of each of the three types was about 1 : 1 : 1.

Preferential Pairing of Chromosomes in a Tetraploid Hybrid between *Oryza glaberrima* and *O. sativa*

Hiko-Ichi OKA

A tetraploid hybrid was obtained from a cross between induced tetra-

ploid strains of *Oryza sativa* L. and *O. glaberrima* Steud., which differed in glutinous (waxy) *vs.* non-glutinous starch (*gl*: +) and other genes. In the hybrid, as compared with the parental strains, there was a reduced number of quadrivalents and a relatively high degree of fertility. Segregation ratios for the glutinous gene observed in the back-cross and F_2 generations appeared to be modified not only by preferential homogenetic pairing but also by gametic selection. Taking into account the effect of gametic selection estimated from simplex heterozygotes, it was suggested that preferential pairing could take place to a certain extent. It seems to be difficult to measure quantitatively the degree of preferential pairing in tetraploid species hybrids, unless the effect of various segregation-distorting factors can be accounted for. (Published in Canadian J. Genet. & Cytol. 10: 527-535)

II. PHYSIOLOGICAL AND DEVELOPMENTAL GENETICS

Differentiation of Ommatidium-Forming Cells of *Drosophila melanogaster* in Culture

Yukiaki KURODA

In holometabolic insects such as Hymenoptera, Lepidoptera and Diptera a drastic change, called "metamorphosis," takes place from the larval into the adult stage through the pupal stage during which certain larval tissues and organs are destructed (histolysis) and adult structures are reorganized from primitive cell complexes, the imaginal discs. The eye-antennal discs are those from which the adult ommatidia, antenna, head hypodermis and perhaps the outer eye ganglion are differentiated during the pupal stage.

Eye-antennal discs which were dissected from mature third-instar larvae of *Drosophila melanogaster* grown under sterile conditions were cultured in chemically defined medium (Kuroda, Y. and Tamura, S. 1956. Med. J. Osaka Univ. 7: 137) and examined for the mechanism of differentiation of ommatidium-forming cells. It has previously been found that a marked differentiation of ommatidium-forming cells was observed when ring glands with brains and ventral ganglions or ecdysone analogues were added to the medium in which the eye-antennal discs were cultured (Kuroda, Y. and Minato, K. 1968. Ann. Rep. Natl. Inst. Genet. Japan 17: 28). In the presence of ecdysone and its analogues, ommatidium-forming cells in eye discs formed many clusters of several cells each after 24 hours of cultivation. The number of these cell clusters seemed to correspond to that of adult ommatidia.

When 5-bromodeoxyuridine, actinomycin D or puromycin was added to the medium at the concentrations of 10^{-5} M, 1 μg/ml or 10 μg/ml, respectively, the hormone-dependent differentiation of ommatidium-forming cells in eye-antennal discs was not affected after 24 hours of cultivation. This indicates that ecdysone and its analogues may induce the migration and orientation of ommatidium-forming cells by which morphologically distinct cell clusters are organized and that no new synthesis either of RNA or protein may be needed for the induction of cell orientation by the hormones.

Eye-antennal discs were irradiated with 0R, 500R, 1,000R, 1,500R, and 2,000R of X-rays (175 KVp, 25 mA, distance 40 cm, filter 1.0 mm Al, dose rate 300 R/min), and examined for the effects of X-rays on the differentiation of ommatidium-forming cells in the presence of ecdysone analogue.

In eye-antennal discs irradiated with less than 1,000 R of X-rays no marked change was observed in the differentiation of ommatidium-forming cells after 24 hours of cultivation. With 2,000R of X-ray their differentiation was almost completely inhibited when eye-antennal discs were irradiated at the start of the culture. 2,000 R of X-ray, however, had no effect on eye-antennal discs when they were irradiated after 4 hours of cultivation.

These results suggest that some substance(s) necessary for the differentiation of ommatidium-forming cells had preexisted in the eye discs in an unstable state at the start of the culture when the substance(s) was sensitive to 2,000R of X-ray and became stabilized after 4 hours of cultivation although the ommatidium-forming cells were morphologically not yet differentiated.

Effects of Ecdysone Analogues on Differentiation of Eye-Antennal Discs of *Drosophila melanogaster* in Culture

Yukiaki KURODA

It has previously been found that various steroids having ecdysone activity which have been isolated from plants were highly active in promoting the differentiation of eye-antennal discs cultured in chemically defined medium (Kuroda, Y. and Minato, K. 1968. Ann. Rep. Natl. Inst. Genet. Japan 18: 28). In a medium containing these steroids eye-antennal discs dissociated from mature third-instar larvae of *Drosophila melanogaster* showed a morphologically pronounced organization of ommatidium-forming cells which formed many cell clusters corresponding in number to adult ommatidia.

Table 1 shows the effects of ecdysone, inokosterone, ponasterone C and rubrosterone at various concentrations on the differentiation of ommatidium-forming cells in eye-antennal discs cultured *in vitro*.

Among ecdysone analogues tested, ecdysterone, inokosterone, and ponasterone C showed rather lower activity than rubrosterone, which had the highest activity on promoting the differentiation of ommatidium-forming cells in eye-antennal discs. This fact suggests a possibility that in the intact insect ecdysone may metabolite through several ecdysone analogues into rubrosterone or a related substance which may act on the target organs.

Table 1. Effects of ecdysone analogues on the differentiation of eye-antennal discs in culture

Substance	Concentration (μg/μl)	No. of explants tested	No. of explants in which ommatidia were differentiated	Per cent of differentiation
Control	0	16	0	0
Ecdysterone	10.0	16	15	94
	1.0	8	7	88
	0.1	6	2	33
Inokosterone	1.0	3	3	100
	0.1	9	4	44
	0.01	8	3	38
Ponasterone C	1.0	8	5	63
	0.1	8	2	25
Rubrosterone	10.0	8	6	75
	1.0	20	14	70
	0.01	11	10	91
	0.0001	13	12	92
	0.000001	12	6	50
	0.00000001	9	2	22

The Relationship between Cell Growth Cycle and Molting Cycle in Insect Epidermis

Kiyoshi MINATO

To elucidate further the mechanism by which the molting of the larval epidermis takes place through the action of a molting hormone, an analysis was carried out on the cell kinetics during the molting process of *Philosamia cynthia ricini* larvae.

It has previously been found that larval epidermal cells of this insect grew very synchronously for a relatively short period before molting. A detailed examination unexpectedly revealed that the cell division of the epidermis takes place not after but before DNA synthesis during the fourth larval instar. In the present investigation it was found that the occurrence of cell division prior to DNA synthesis also was taking place in the epidermis of the third instar larvae. These facts suggest that epidermal cells may not divide after DNA synthesis but continue in tetraploid state through the molting period and divide immediately before DNA synthesis in the next larval instar.

To ascertain this possibility the epidermal cells were labeled with a pulse of ^{3}H-thymidine at various periods in the third instar. Incorpo-

ration of ^{3}H-thymidine was found in mitotic figures of epidermal cells in the fourth instar larvae. When epidermal cells were continuously labeled with ^{3}H-thymidine throughout the fourth instar larval period, no grain was found in mitotic cells of the fourth instar larvae.

These results indicate that epidermal cells have a peculiar growth cycle in which they do not divide after their DNA synthesis in the same larval instar but divide after a long G_2 period (about 48 hours) in which they pass through molting to the next larval instar.

Cytoplasmic Male Sterility and Virus Inoculation in *Capsicum*

Yasuo OHTA

Phenotypical similarity between cytoplasmic and virus induced male sterility in certain plants is well known.

Cytoplasmic male sterility in *Capsicum annuum* L. is controlled by a sterile cytoplasm (*S*) and a male sterile gene (*rf*, original symbol, *ms*). (*S*) *rfrf* plants are pollen sterile, (*S*) *RfRf* or (*S*) *Rfrf* plants are pollen fertile, as are plants having normal cytoplasm (*N*).

In the first experiment, (*S*) *Rfrf* plants obtained from a cross (*S*) *rfrf* Fresno Chile×(*N*) *RfRf* Fushimiamanaga and (*N*) *RfRf* plants (Fushimiamanaga) were inoculated with an ordinary strain of cucumber mosaic virus (CMVo), ordinary and yellow strains of tobacco mosaic virus (TMVo and TMVy), and broad bean wilt virus (BWV) (designated as tobacco ringspot virus (TRSV) in previous reports).

(*S*) *Rfrf* plants inoculated with CMVo or BWV showed reduced pollen fertility which was 64.2 to 68.0 per cent on the average. On the other hand, (*N*) *RfRf* plants showed little or practically no change in pollen fertility. In the second experiment, both (*S*) *Rfrf* and (*N*) *RfRf* plants were inoculated with three strains of cucumber mosaic virus (ordinary (CMVo), necrotic spot (CMVn), and leguminous (CMV1)), BWV, alfalfa mosaic virus (AMV), and a mottle strain of potato virus X (PVXm). Again, CMVo- and BWV inoculation produced a reduced fertility in (*S*) *Rfrf* plants which was 72.5 to 75.3 per cent on the average. A similar result was obtained also by CMV1 inoculation in which pollen fertility was reduced to 77.6 per cent in (*S*) *Rfrf* plants.

Selfing of those inoculated plants in the first experiment was successful, and the progenies were examined for pollen fertility. Since the inoculated plants were heterozygous for the fertility gene, a 3 fertile: 1 sterile ratio was expected. Two lines from a plant inoculated with CMVo having lowest pollen fertility (42.8%) and a line from a TMVy-

infected plant having normal pollen fertility (95.1%) produced many more sterile plants than expected. The observed ratio for the above three lines is more likely to be the 1:1 ratio expected from a backcross to (*N*) *rfrf* plants. But such a possibility was refuted by the segregation ratio for two characters of the fruit, erect *vs.* pendent, and oval *vs.* round. In addition to steriles, some plants showed reduced pollen fertility (semi-fertiles and semi-steriles). Thus in the progeny of a (*S*) *Rfrf* plant inoculated with CMVo and a (*S*) *Rfrf* plant inoculated with TMVy, pollen fertility tended to be lower.

It may be said, therefore, that a particular cytoplasm (*S*) or at least cytoplasm (*S*) together with *Rfrf* genotype is responsible in some way for the production of reduced pollen fertility by specific viruses.

Organ Differentiation of Isolated *Crepis* Leaves *in vitro*

Yoshiaki YONEDA

In many plant tissues cultured *in vitro*, externally supplied plant hormones have been found to induce callus or organs differentiation such as rooting and bud formation. This phenomenon could present a suitable means for analysing the hormonal mechanism of differentiation. In the present study, it is described how isolated leaf blades of *Crepis capillaris* were responding to different hormones or hormone combinations.

As a basal medium, Linsmaier & Skoog (1950)'s mineral salts containing organic constituents without hormones were used. Agar was used at 0.7% concentration. *Crepis* plants were cultured on the basal medium containing 0.5 ppm kinetin (KT medium), and leaf blades (leaf terminal, 1 cm long) were severed for inocula which were transplanted to various hormones containing media. For differentiation experiments, indoleacetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin (KT) and yeast extract (YE) were added singly or in combinations. The leaf blades were incubated for one month at 27°C in the dark and examined for differentiation.

On the basal medium, leaf blades showed no morphological changes. On the medium containing 0.1 to 10 ppm of IAA (IAA medium), the leaf blades became transparent and soon many roots appeared. Over the veinlets of these leaf blades occurred many protrusions consisting of small seemingly meristematic cells, some of which seemed to produce roots. In contrast, KT (0.05–0.5 ppm) did not produce any morphological change in the leaves. On IAA-KT combined media, rooting and bud initiation was observed depending on the concentration of both hormones. For

bud initiation, the most effective concentration of KT was 0.5 ppm when combined with IAA at 1.0 ppm. The general tendency observed was that KT increasingly suppressed root initiation with increasing concentration, and that increasing concentration of IAA reduced the inhibitory effect of kinetin.

When 2,4-D or NAA were added instead of IAA and combined with KT at 0.5 ppm, bud initiation was also observed at 0.1 ppm of 2,4-D and 0.1 to 1 ppm of NAA. Higher concentration of 2,4-D completely suppressed bud initiation. On media containing 2,4-D at 0.1 ppm and KT at 0.1 ppm, only undifferentiated growth took place, resulting in slowly growing firm, solid callus. Addition of YE (0.5%) to this medium (2,4-D-KT-YE medium) also produced callus, though rootings were observed in this case. The callus was soft and friable and grew very rapidly.

These results indicate that rooting, callus formation and bud initiation seem to be controlled by the kind and concentration of the hormone. Kinetin favors induction of bud initiation and IAA stimulates rooting, while undifferentiated callus growth is observed by 2,4-D.

III. BIOCHEMICAL GENETICS

Genetic Effects of DNA in *Ephestia*

Saburo NAWA and Masa-Aki YAMADA

A heritable change induced by DNA in *Ephestia* had been already reported (this Annual Report, No. 18). The method used in these experiments consists of the treatment of larvae or eggs, having the recessive genotype *a*, with DNA from wild type or DNA from *a*-genotype. Adults developed from treated larvae or eggs were mated to *a*/*a* animals to observe possible changes in eye color in their progeny (BF_1). The appearance of wild type eyes in BF_1 was repeatedly observed, confirming the previously reported results. In the overall experiments to date, 19 cases of black-eyed mutant out of 138,917 BF_1 were obtained in the case of +DNA treatment, while no black-eyed mutant out of 46,229 was observed in the treatment with homologous DNA extracted from *a*-strain. The difference between the effect of +DNA and *a*-DNA is significant ($x^2=5.5$, $P\simeq 0.02$). Black-eyed mutants obtained by +DNA treatment were successively backcrossed to *a*/*a* until segregation of black and red eyes occurred. Then, in successive sister-brother matings, black-eyed animals were bred to each other and have been kept as mutant lines.

Since cases of nonspecific mutations induced by DNA have been known in *Drosophila*, a possibility might be considered that the change of red to black in our experiment is due to an accidental mutational effect of DNA such as replacement of a nucleotide or nucleotides in the *a*-locus leading to something like an intra-cistronic suppressor mutation. If so, amino acid compositions of tryptophan pyrrolase (TP) of mutant lines would be altered, since the gene a^+ is considered to be the structural gene for TP. Therefore, properties of the enzyme from mutant lines were compared with that from wild type. It was found that all mutant lines had TP activities nearly the same as the wild type. On starch gel electrophoresis, TP activity was detected as a single, slightly diffuse band. It was observed that TP from all of the mutant lines had the same mobility as TP of wild type, suggesting identical amino acid composition. Although it became possible to purify the enzyme about 80 fold from a homogenate of adults by ammonium sulfate fractionation, treatment with calcium phosphate gel, followed by chromatography on DEAE-Sephadex, an attempt to get an antibody for the enzyme was unsuccessful.

Further Evidences of Uptake of DNA by *Ephestia* Eggs

Masa-Aki YAMADA and Saburo NAWA

Evidence of DNA uptake by *Ephestia* eggs was previously reported (this Annual Report, No. 18). The ^{3}H-labelled DNA ($1.5\times10^{6}-6.2\times10^{5}$ cpm/μg) was prepared from a thymineless mutant of *Salmonella* cultured on a medium containing ^{3}H-thymidine. Eggs ranging from 0 to 18 hours after they had been spontaneously laid in plastic boxes were dechorionated. After washing with saline, they were immersed in the ^{3}H-labelled DNA solution for a definite time and treated with DNase to remove the DNA attaching to the egg surface. After washing with saline, DNA was extracted from the eggs by SDS-phenol method. The radioactivity was measured by a low back gas flow counter. When eggs were immersed in the DNA solution (300 μg/ml) for 2, 4 and 6 hours, the radioactivities per 500 μg DNA extracted from the eggs were 99, 296 and 339 cpm respectively. When eggs were treated with 100, 200 and 300 μg/ml DNA solutions, the radioactivities per 500 μg DNA extracted from the treated eggs were 672, 1529 and 2171 cpm respectively. The repeated experiments gave similar results, showing that the uptake of DNA by eggs depends on the concentration of the DNA solution and the time of their immersion in DNA. DNA extracted from the eggs treated with ^{3}H-DNA for 2, 4 and 6 hours was submitted to fractionation by centrifuge chromatography on DEAE-celullose paper pulp. The elution pattern of the extracted radioactivities is given in Table 1. In the eggs treated for 2 hours, radioactivities were found in the fractions of high molecular weight. Radioactivities extracted from the eggs treated for 4 or 6 hours shifted to the fractions of lower molecular weight. These results indicate that the foreign DNA is incorporated into the eggs in high molecular state.

Table 1. Fractionation of the DNA extracted from the eggs treated with ^{3}H-DNA

Fraction No.	(Average Molecular weight)	2 hours		4 hours		6 hours	
		Radioactivity (%)	O.D.	Radioactivity (%)	O.D.	Radioactivity (%)	O.D.
1	(5×10^{2})	2.4	0.65	10.4	0.61	0	0.81
2	(2×10^{3})	5.4	0.92	1.2	1.03	0	0.80
3	(5×10^{4})	19.3	2.01	21.9	0.96	73.6	0.98
4	(5×10^{5})	46.8	3.15	67.1	1.29	17.9	1.20
5	(1×10^{6})	22.8	4.13	6.5	5.60	8.5	3.81
6	(5×10^{6})	3.6	1.80	3.7	1.75	0	1.31

Genetical and Biochemical Studies of Lethal Lemon Silkworm Larvae

M. TSUJITA and S. SAKURAI

It had been reported (Tsujita 1956) that normal ($+^{lem}$), lemon (*lem*) and lethal lemon (*lem*^{l}) are multiple alleles of the gene located at 22.3 units on chromosome 3.

In the lethal lemon larvae melanin pigment is scarcely produced in the cuticular layer of the hypodermis and also their mandibular cuticle does not sufficiently develop owing to incomplete hardening of cuticular scleroprotein, so that they cannot chew up mulberry leaves and starve to death after the first moulting. They exhibit a distinctly yellow body color and in their hypodermis a large amount of pteridine granules containing sepiapterin and uric acid is produced.

Using normal, lemon and lethal lemon larvae the activity of tyrosinase in larvae immediately after the first moulting was examined by disc-electrophoresis. The activity of protyrosinase in lethal lemon larvae could not be discriminated from that of normal larvae. However, in lethal lemon larvae a weaker tyrosinase activity was detected than in normal larvae.

It has been known that the conversion step from sepiapterin to tetrahydropteridine is catalysed by pterine reductase. The activity of pterine reductase of normal, lemon and lethal lemon larvae was reported in our previous paper (Tsujita 1963). According to experimental results normal larvae show a strong activity of pterine reductase and lemon larvae also have some though weaker activity. However, lethal lemon larvae lack the activity of this enzyme so far as the method which we used is concerned. In spite of this fact, some amount of isoxanthopterin in addition to a large amount of sepiapterin could be detected in lethal lemon larvae.

It was proved that a much smaller amount of alkali isoluble matter and tyrosine metabolite incorporated into this insoluble matter was found in lethal lemon larvae than in normal larvae. And it seems that the principal components of this alkali insoluble substance is scleroprotein of the cuticular layer of larval hypodermis.

It has been known (Tsujita 1956) that embryos homozygous for the *lem*^{l} gene, usually develop normally to black young larvae and die immediatly after the 1st moulting. Namely, in the cross $+/lem^{l} \times +/lem^{l}$ or $+/lem^{l} \times lem/lem^{l}$, the yellow lethal larvae can be recognized in the 2nd instar. On the contrary, in the cross $lem/lem^{l} \times +/lem^{l}$ or $lem/lem^{l} \times lem/lem^{l}$, however, all homozygous lem^{l}/lem^{l} individuals die within the

eggs which show through the semi-translucent shells the yellowish-brown dying larvae.

In my previous paper (Tsujita 1963) the following relationship was found between the presence or absence of pterine reductase in eggs immediately after they were laid by their mother moth and the stage at which the homozygotes for *lem*^{l} die. When these homozygotes develop from the biginning in the presence of the enzyme, they become normal black young larvae and they are able to hatch out by chewing the egg chorion. However, when they develop in the absence of the enzyme, they become yellowish-brown young larvae and cannot hatch out owing to incomplete differentiation and insufficient hardening of their mandible cuticle. It may be safely said that enzyme activity is directly controlled by the genes $+^{lem}$, *lem* and *lem*^{l}.

According to Kaufman (1962) sepiapterin and its hydrated derivatives act as cofactor in the hydroxylation of phenylalanine to tyrosine. Therefore a hypothesis based on Kaufman's finding was considered for the explanation of the cause of lethality of the mutant larvae. Namely, in normal and lemon larvae tyrosine is sufficiently produced before and at the time of new formation of the cuticular layer because phenylalanine hydroxylase stimulated by the cofactor activity of sepiapterin can normally catalyse the conversion of phenylalanine to tyrosine. On the contrary, in the lethal lemon larvae sepiapterin loses its cofactor activity owing to the lack of pterine reductase, so that phenylalanine hydroxylase cannot sufficiently catalyse the conversion from phenylalanine to tyrosine, resulting in the production of a small amount of melanin pigment and incomplete differentiation, *i.e.* insufficient hardening of the cuticular layer of the larval hypodermis.

Genetical and Biochemical Studies of Lethal Albino Silkworm Larvae

M. TSUJITA and S. SAKURAI

The experimental results of linkage test (Tsujita 1956) showed that the lethal albino gene (*al*) is not located on chromosome 3, and is inherited independently of *lem*^{1} gene. However, the phenotype of lethal albino larvae resembles that of lethal lemon larvae. Namely, melanin pigment of both is scarcely produced in the cuticular layer of the hypodermis and their mandibular cuticle does not sufficiently develop due to incomplete hardening of cuticular scleroprotein, so that they cannot chew up mulberry leaves and starve to death after the first moulting. The lethal

albino larvae have partly light brown and partly white body color and their hypodermal cells contain a large amount of pteridine granules filled with pteridine metabolic products and uric acid. A weaker tyrosinase activity is detected in them than in normal ($+^{al}$) larvae, although the activity of protyrosinase is normal. Furthermore, a very low activity of phenylalanine hydroxylase was detected in the larvae of this mutant.

0.2 g of frozen albino larvae immersed in 1 ml of 30% alcohol was homogenized in a glass homogenizer and heated for 20 minutes in an water bath at a temperature of 95-100°C in order to extract pteridine compounds. After centrifugation at 3000 rpm for 10 minutes, 0.15 ml of supernatant was used for the separation of several pteridines by two-dimensional paper chromatography. According to experimental results a much smaller spot of isoxanthopterin was observed than in normal larvae ($+^{al}$). However, a very large spot showing light greenish-blue fluorescence which was not observed in normal larvae appeared in the place of isoxanthopterin.

The activity of pterine reductase and pterine dehydrogenase was normal. Also, according to determination of the amount of uric acid by uricase method, it is normally produced in the mutant larvae.

Based on these experimental results, it is assumed that the formation step of some precursor of sepiapterin is blocked in the mutant larvae and that this abnormal pteridine metabolism have an intimate relation to the weak activity of tyrosinase and phenylalanine hydroxylase.

Preliminary examination of the Cofactor Role of Sepiapterin for Phenylalanine Hydroxylase

M. TSUJITA and S. SAKURAI

Materials and methods: normal larvae ($+^{al}$, $+^{lem}$), lethal albino (*al*) larvae and lethal lemon larvae (lem^l) were used as materials.

A mixture of 1 ml of 0.01 mole phosphate buffer solution (pH 6.8) and 0.5 g of frozen larvae was homogenized under ice cooling and centrifuged at 10,000 rpm for 30 minutes. The supernatant was used as crude enzyme solution.

The following modified reaction solution obtained after the method used by Kaufman (1959) was prepared. Namely, a mixture of i) 0.5 ml of 0.5 m mole L-phenylalanine solution (L-phenylalanine was dissolved in 0.01 mole phosphate buffer at pH 6.8), ii) 0.2 ml of sepiapterin solution containing 100 μg pigment per ml, iii) 0.2 ml of NADPH* solution con-

* Abbreviations used: NADP, nicotinamide-adenine dinucleotide phosphate; NADPH, reduced form of NADP.

taining 100 μg reduced NADP* per ml, and iv) 0.2 ml of crude enzyme solution was incubated at 25°C for 30 minutes and then 2 ml of 12% TCA was added to the solution to stop the reaction. After centrifugation of the solution at 3000 rpm for 10 minutes, the supernatant was used for the determination of the amount of formed tyrosine.

0.05 ml of 0.1 mole iodine solution was added to a mixture of 1 ml of supernant and 0.4 ml of 2 mole tris buffer solution. After keeping the mixture at room temperature for 2 minutes, 0.2 ml of 0.1 mole sodium thiosulfate was added and then the solution was diluted to 3 ml of the final volume by addition of distilled water. The amount of formed tyrosine was determined by measuring with Beckman's spectrophotometer at 310 mμ wave length.

Results: tyrosine formation was recognized in the reaction solution containing the crude enzyme solution of normal larvae ($+^{lem}$, $+^{al}$). Further, almost the same results were obtained in both cases of reaction solutions with addition of sepiapterin or without it.

Next, in the reaction solution containing the crude enzyme solution prepared from lethal lemon larvae the formation was found of a much smaller amount of tyrosine than in the reaction solution of normal larvae.

In the reaction solution of albino lethal larvae formation of a much smaller amount of tyrosine was recognized than that of the normal strain ($+^{al}$). Besides, it is a remarkable fact that the production of tyrosine increased in the reaction solution by addition of sepiapterin.

Thus, so far as the present in vitro obtained results are concerned, lethal lemon larvae showed low activity of phenylalanine hydroxylase in comparison with the normal larvae ($+^{lem}$). However, it was difficult to confirm the cofactor role of sepiapterin for phenylalanine hydroxylase for these mutant larvae.

On the contrary, the lethal albino larvae exhibited not only weak tyrosinase activity but also low activity of phenylalanine hydroxylase. Thus, the fact that the amount of tyrosine in the reaction solution of the mutant larvae was increased by addition of sepiapterin seems to indicate the cofactor role of sepiapterin for phenylalanine hydroxylase.

Genetical and Biochemical Studies of Pteridine Granules in Hypodermal Cells of Silkworm Larvae: Significance of Polypeptides inside the Granules

S. SAKURAI and M. TSUJITA

Comparative studies of peptidase activity in the body fluid and hypodermal tissue of larvae during the period from 4th instar to mounting stage of the 5th instar were carried out using as materials C-124, a hybrid (Daizo x W-C) with normal hypodermis and w^{ox} oily mutant strain.

Body fluid was taken from the caudal horn by cutting off the apex of the horn.

1 g of fresh larvae hypodermis together with 3 ml of 0.02 mole veronal buffer (at pH 7.6) was homogenized in a glass homogenizer and centrifuged at 10,000 rpm for 30 minutes. The supernatant was used as hypodermis extract.

Substrates used for the detection of peptidase were leucyl-glycyl-glycine in case of aminopeptidase and glycyl-tyrosine, alanyl-histidine, glycyl-glycine, alanyl-alanine and alanyl-glycine in case of dipeptidase.

Mixture of 0.05 ml of body fluid or hypodermis extract and 0.3 ml of 0.04 mole substrate solution (substrate was dissolved in 0.02 mole veronal buffer at pH 7.6) was incubated at 37°C for 2 hours and the activity of enzymes was analysed by paper chromatography. Chromatograms were run in n-butanol-acetic acid-water (100 : 20 : 4 v/v) for 20 hours and developed with 0.2% ninhydrin-acetone solution.

In both, body fluid and hypodermis extract, the activity of amino- and dipeptidase was detected. So far as the present experimental results are concerned, the extract of hypodermal tissues showed a stronger activity of alanyl-glycine dipeptidase than the body fluid. Furthermore it was found that the activity of amino- and dipeptidase of hypodermal tissue and body fluid gradually increased with time during the period from the 5th day of the 5th instar to mounting stage.

It is well known that the silkprotein, fibroin, is a very specific protein composed of 4 main amino acids, *i.e.* glycine, alanine, serine and tyrosine, and that it is synthesized in gland cells of the posterior division of the silkgland, especially in a later larval stage, *i.e.* mounting stage.

Although amino acids required for fibroin formation are partly synthesized in silk gland cells, it is not sufficient to provide a large amount of amino acids which is necessary for rapid synthesis of fibroin. Consequently, there must be some other source of those amino acids.

Each of two materials used, normal (Daizo x W-C) and w^{ox} strain, has the following characteristics; in hypodermal cells of the former a large

amount of pteridine granules is produced, and in those of the latter such granules are scarcely formed. Using two strains, dry weight of the middle and posterior division of the silkgland was measured at a definite time during the period from the 5th day of the 5th instar to mounting stage in order to examine the activity of fibroin secretion in silkgland cells.

It was found from experimental results that secretion of fibroin in silkgland of normal larvae was in the mounting stage more rapidly increased than in the w^{oz} mutant larvae.

Our previous report showed that a large amount of granules containing pterine pigment-polypeptide complexes and a uric acid-polypeptide complex lose their function in larval mounting stage, and that polypeptides combined with pigment or uric acid are released from granules and excreted from hypodermal cells into the body fluid. It is considered that in hypodermal cells and body fluid these polypeptides are decomposed by peptidases and that free amino acids derived from polypeptides are absorbed into the gland cells of the posterior division of the silkgland and are an important source of the materials used for fibroin synthesis.

Genic Analysis for Peroxidase Isozymes in *Oryza sativa* and *O. perennis*

B. B. SHAHI, Toru ENDO and Hiko-ichi OKA

Eight strains belonging to the Asian form of *Oryza perennis* and *O. sativa* were inter-crossed, and peroxidase isozymes of the hybrid plants were observed by the starch-gel electrophoresis method. Isozyme bands C2 and C4 were found to be specified by dominant genes pe^{C2+} and pe^{C4+}, respectively. Bands A2 and A4 were allelic and controlled by co-dominant allels, pe^{A2} and pe^{A4}, respectively. Their heterozygotes showed zymogram A2-A3-A4, in which A3 was a hybrid dimer enzyme. From diallel crosses among ten plants of a population of *O. perenis*, a regulatory gene R^{C4} was found which suppressed band C4.

Isozyme Variations to Organ Specificity in *Oryza perennis*

B. B. SHAHI, T. ENDO and H. I. OKA

Variations in peroxidase and acid phosphatase isozymes among the vegetative and reproductive organs of three *Oryza perennis* strains were

observed by the starch-gel electrophoresis method. Each organ showed its characteristic zymogram which was more or less modified with aging, while the variation pattern differed among strains. In the stigmas before pollination, both peroxidase and acid phosphatase isozymes were silent, but some of them became active after pollination. The acid phosphatase isozymes of the anthers containing pollen grains showed trailing nature. In plants which were heterozygous for a locus specifying a set of allelic isozymes of peroxidase (pe^{A2}: pe^{A4}), the isozyme bands were fully expressed only in a certain organ but were suppressed in other organs in different manners, and the pattern of suppression differed according to strains. Further, the lemma and palea and some other flower organs showed particular bands which were absent in the vegetative organs of the plant but were found in those of another distantly related strain. It was suggested that the synthesis of isozymes in an organ might be controlled by some inducer-producing genes which take part in organ differentiation.

Partial Purification and Characterization of Genetically Different Acid Phosphatase of Human Red Cells

Tomotaka SHINODA

Partial purification and comparison of general characteristics were made using three genetically different types of acid phosphatase [EC 3.1. 3.2, orthophosphoric monoester phosphohydrolase] isolated from normal human red cells. Heterogeneity was observed on chromatography as well as on starch gel electrophoresis made with CM-or DEAE-cellulose and NaCl gradient. The partially purified enzyme appeared to show essentially the same electrophoretic pattern on starch gel as the fresh hemolyzate, except that a faster cathodal component was absent in the purified material. Heat stability of acid phosphatase carried out at 50°C was found to be different according to the phenotypes of the enzyme, the order of their stability being B>AB>A. Average times at which a half amount $t_{1/2}$, of the original enzyme activity of types A, AB and B remained, were 4.1, 4.5 and 6.8 minutes, respectively. A change in electrophoretic pattern on starch gel was noticed either by heat treatment or by incubation of the sample with oxidized glutathione. A new zone with slow mobility and reduced activity appeared by these treatments. The effect of guanidine on the enzyme was tested as a function of its concentration, but no significant difference was observed among different types of enzyme. Upon incubation with guanidine a rapid fall

of activity took place at a relatively low concentration of guanidine and, with increasing of its concentration the activity gradually decreased to 10% of its original strength. The partially purified sample was slightly more susceptible to the treatment as compared with the enzyme of stroma free hemolyzate.

When stroma free hemolyzate was submitted to gel filtration on a Sephadex G-75 column, the enzyme, regardless of its type, was eluted as a single peak slightly behind hemoglobin. This suggests that multiple isozyme components of each type are similar in molecular size. By gel filtration, the specific activity was found to be increased several times with respect to enzyme units per gram of hemoblobin. By combination with several procedures, types AB and B were purified approximately 1,500 fold.

Further characterization of purified enzyme is now going on.

Amino Acid Sequences Around Cystine Residues and COOH-terminus of γM-globulin

Tomotaka SHINODA

Chemically pure κ-type human γM-globulin was submitted to structure analysis with special interest in the position of cystine residues in the molecule. Pepsin digest of intact γM-globulin was first treated with Sephadex G-10 in N acetic acid, and the fractions containing disulfide bridges were pooled and lyophilyzed.

Purification of the peptides was performed by column chromatography and high voltage electrophoresis in a volatile buffer system. Amino acid sequences of respective peptides were carried out by Edman's degradation method. At present four disulfide bridges were identified, and their sequences were as follows: 1) Val-Cys-Leu: Ala-Cys-Glu, 2) Ile-Thr-Cys-Leu : Tyr-Thr-Cys-Val-Ala-Val, 3) Tyr-Cys-Ala-Val-Val-Asn : Thr-Cys-Thr-Phe-Ser-Gly, 4) Thr-Cys-Leu : Thr-Cys-Thr (Asx, Thr_2, Ser, Pro_2, Val, Leu, His, Lys) Leu-Gln. The COOH-terminus of μ-chain was sequenced as Met-Ser-Asx-Thr-Ala (Gly, Thr)-Cys-Tyr-COOH. The cystine residue in this sequence was recovered in the S-carboxy-methylated form, indicating that it was involved in an interchain disulfide bond that was selectively cleaved and alkylated during chain separation. The fragment does not have the reported sequence adjacent to the disulfide bonding of the heavy and light chains of a γM-globulin; and it is not analogous to the proline rich sequences adjacent to the H-H interchain bonds of human and rabbit γ-chains. Hence, the unique cystine residue of μ-chins

is probably involved in the intermolecular disulfide bridge linking five 7 S γM monomer units to form polymeric 19 S macroglobulins. Indeed, when CNBr fragments were prepared from the whole γM-globulin, the COOH-terminal fragment was not eluted at the position of the S-carboxymethylated fragment, presumably because it was in the dimeric form. Fragment IO-3 isolated from peak 4 (sequence : Gln-Arg-Gly-Glx-Pro-Leu-Ser-Pro-Gln-Lys-Tyr-Val-Thr-Ser-Ala-Pro-Met) has a strong homology in sequence to residues 108 to 125 of the Fc region of rabbit γ-chain. Although human μ-chains appear to have an identical sequence for the COOH-terminal pentapeptide, there is no similarity to the corresponding portion of either human or rabbit γ-chains.

The entire work was carried out at Indiana University, U.S.A.

Peroxidase Isozymes in Four Strains of Morning glory

Yoshiaki YONEDA

Among the stocks of morning glory, *Pharbitis nil*, we have in the National Institute of Genetics, four geographical strains: TKS strain, a normal standard strain selected by Dr. Y. Takenaka from a Japanese cultivariety; Peking Tendan strain collected by Dr. H. Kihara in Peking in 1938; Nepalese strain collected by Dr. S. Nakao in Nepal in 1952; African strain collected by Dr. K. Furusato in Africa in 1959. This last strain could be easily crossed with the other three strains, but the morphology of its leaf showed a reduced type and its flowers opened in late September. In this report, the peroxidase isozyme patterns of leaves of these four strains were compared by the method of starch gel electrophoresis (Endo, T. 1968, Plant & Cell Physiol. 9:333). Peroxidase isozymes were detected by H_2O_2-benzidine staining.

As previously reported (Yoneda and Endo, 1967, this report 18:43), the TKS strain had 7 anodal peroxidase bands, designated as 1 A to 7 A from the original line, and one sharp cathodal band, designated as 1 C. The 1 C band appeared in leaves, stems and roots irrespective of age, with the exception of seeds and young cotyledons. One line was found which had no 1 C band (Endo, T. 1966, this report 17 : 50).

The isozpme pattern of Peking Tendan strain was very similar to that of TKS strain. It had a sharp cathodal 1 C band. The Nepalese and the African strains had also a similar isozyme pattern to that of TKS, except on the following points. The Nepalse strain had the same sharp cathodal isozyme, 1 C, but one more band appeared between the fastest moving anodal band (7A) and the second one (6A). In the African strain,

a few more bands were also recognized between 6A and 7A bands. Another difference was found in that a sharp cathodal moved at 22 mm from the start line, in contrast to the 1 C band at 18 mm in the other three strains in four hour electrophoresis under 10 V/cm voltage gradient. This band was designated as 2 C.

The African strain was crossed to TKS and Peking Tendan strains and the F_1 hydrids were analysed for the cathodal band. All hybrids had both 1 C and 2 C isozyme bands and no hybrid enzymes were observed. This suggests that the cathodal isozyme bands, 1 C and 2 C, are controlled by different genes.

A new finding was that the TKS, Peking and Nepalese strains had in common 1 C isozyme, while the African strain had 2 C isozyme.

IV. EVOLUTIONARY GENETICS

Intraspecific Differentiation of Cytoplasm in *Aegilops caudata*

Hitoshi KIHARA and Yasuo OHTA

Aegilops caudata L. includes two varieties, vars. *typica* and *polyathera* Boiss. The former is awnless and is found mainly in Greece and Crete, while the latter is awned and is distributed solely in Turkey.

The reciprocal hybrids between two varieties were easily obtained. The chromosome behavior was normal throughout meiosis in F_1, but the hybrids were almost completely pollen sterile.

As expected, with the advance of restoration backcrosses (RB) in the F_1 between *typica* (♀) and *polyathera* (♂), the pollen fertility was strikingly increased until it became complete in RB_3. On the contrary, the substitution backcross lines (SB_1–SB_3) were totally pollen sterile.

The reciprocal hybrids having the cytoplasm of *polyathera* gave quite unexpected results. Both restoration and substitution backcrosses gave rise to fertile offspring. A difference is only in the speed of recovering full fertility. Contrary to our expectation, the RB_1 and RB_2 were completely sterile and from RB_3 fertility was getting higher until in RB_4 one plant had completely dehiscent anthers with 94.4% fertile pollen and the remaining 14 had an average fertility of 55.7%.

From these investigations, it may be concluded that two varieties of *Ae. caudata* are slightly different in their genome and also in the cytoplasm.

Interspecific Hybrid Between the Two Species of the Genus *Taeniatherum* of the Tribe Triticeae

Sadao SAKAMOTO

The genus *Taeniatherum* comprises two diploid annual species (2n=14), *Tn. asperum* (Simonk.) Nevski and *Tn. crinitum* (Schreb.) Nevski, distributing widely from Central Europe to Central Asia. A strain of *Tn. asperum* (strain no. 7065) collected at Pul-i-Khumri, Afghanistan, was crossed reciprocally with a strain of *Tn. crinitum* (no. 7064) found at Karaji, Iran. Only three F_1 plants, one from *Tn. asperum*×*Tn. crinitum* and two from the reciprocal combination, were obtained.

F_1 plants showed vigorous growth and marked heterosis was observed in the number of tillers, plant height, and length of top internode, flag

leaf and spike. However, the number of spikelets per spike was intermediate between the parents.

Average chromosome pairing per cell of PMC's of the F_1 was $0.2_{IV}+0.1_{III}+5.8_{II}+1.9_{I}$. Pollen fertility of non-dehiscent anthers of the F_1 was very low (0.2-0.5%) and no seeds were obtained at maturity.

From these observations it is concluded that the two diploid species of *Taeniatherm* contain very similar but structurally differentiated genomes.

Scientific Expedition for the Study of Rodents to South East Asia and Oceania VI. Serum Transferrin Polymorphism in *Rattus rattus* Collected in Southeast Asia and Oceania

Kazuo MORIWAKI, Kimiyuki TSUCHIYA and Harumi SAKATA

Recently a genetic polymorphism of serum transferrin in *Rattus rattus* has been found in the natural population of Japan (Moriwaki, K. *et al.* 1969. Genetics, in press). Three types of transferrin pattern, TfR, TfRN and TfN, could be identified by starch gel electrophoresis. TfR was characterized by two fast moving bands, TfN by two slowly moving bands and in TfRN all four bands were evident. These bands migrated into the region between slow α_1-globulin and fast α_1-globulin. The overall frequencies of TfR, TfRN and TfN in the natural population of Japan were 0.74, 0.21 and 0.05, respectively.

A similar kind of electrophoretic survey has been extended to serum specimens of *Rattus rattus* collected in South East Asia and Oceania. The same type of *Rattus rattus* as that in Japan was obtained in the former, not in the latter. The localities and frequencies of TfR, TfRN and TfN were as follows: Okinawa (32 samples) 0.75, 0.22, 0.03; Luzon (44 samples) 0.62, 0.33, 0.05; Thailand (39 samples) 0.15, 0.26, 0.59. Apparently a higher occurrence of Tf^N gene in the Thailand population seems to be an important finding with regard to the origin of this gene in the species *Rattus rattus*.

In Java and Makassar *Battus rattus argentiventer* is widely distributed. All specimens collected in Java (16 samples) and Makassar (57 samples) showed an similar pattern of transferrin as TfR of *Rattus rattus* in Japan.

A serum transferrin type, tentatively designated as TfRo, which exhibited a smaller mobility on starch gel than TfN of Japanese *Rattus rattus* was observed in *Rattns rattus* of Oceania. All individuals collected from New Zealand (22 samples), Australia (28 samples) and New Guinea (13 samples) were of this TfRo type. In F_1 hybrids obtained in our laboratory between *Rattus rattus* of Japan and that of Australia, a mixture

of TfR and TfRo emerged, though they were somewhat faint.

Differentiation of Pathogenic Races of *Piricularia oryzae* into two Groups, "Indica" and "Japonica"

Hiroko MORISHIMA

To obtain a summarized picture of host-parasite relationships, the data reported by Kozaka and Matsumoto (1967) for 484 varieties of *Oryza sativa* from various countries of the world, each inoculated with 21 pathogenic races of *Piricularia oryzae* collected from eight countries, were analysed from the viewpoint of numerical taxonomy. The two methods used, pattern analysis and principal component analysis, gave consistent results. The pathogenic races examined were clearly divided into two groups, one mainly pathogenic to rice varieties of the Indica type and the other to the Japonica type. They were called the "Indica" and "Japonica" race groups. The rice varieties could also be divided into two major groups of differential reactions to the races, which were represented by the Indica and Japonica types. In addition, variations in general resistance of rice varieties and in general pathogenecity of blast races were observed. An about 40% fraction of the multi-dimensional variation was estimated to be due to general resistance, but the fraction due to general pathogenecity seemed to be of lower magnitude. The variation pattern in pathogenecity of the fungus seems to reflect that in resistance of the host, as if a shadow follows the shape of a body. (Published in SABRAO Newsletter 1: 81-94.

Genetic Basis of the Weakness of F_1 plants in *Oryza breviligulata* and *O. glaberrima*

Yaw-En CHU and Hiko-Ichi OKA

The weakness of F_1 plants was frequently found between strains of *O. glaberrima* Steud. (cultivated) and *O. breviligulata* Chev. et Roehr. (wild), which are endemic to West Africa. From the segregation pattern of normal and weak plants in three-way crosses, a set of complementary dominant lethals, W_1 and W_2, was found to control the F_1 weakness. About one half of *breviligulata* strains tested had either W_1 or W_2, while most of *glaberrima* and semi-wild strains had W_1 only or neither. They showed no particular trend of localization in geographical distribution. The F_1 weakness was found to be due to a disturbance in tissue differ-

entiation of the adventitious roots, which was directly controlled by the complementary lethals. The undifferentiated tissues of induced callus, derived from weak plants, grew as well as those from normal plants.

A Crossing Barrier Isolating *Oryza perennis* subsp. *barthii* from its Related Taxa

Yaw-En CHU and Hiko-Ichi OKA

The African subspecies of *Oryza perennis* Moench, called *barthii* does not easily produce viable F_1 seeds when crossed with its wild and cultivated relatives (other forms of *perennis*, *O. sativa* L., *O. breviligulata* Chev. et Roehr., and *O. glaberrima* Steud.). When *barthii* is used as the pollen parent, the F_1 embryos and endosperms begin to deteriorate about six days after fertilization, resulting in shrunken and inviable F_1 seeds. When *barthii* is used as the maternal parent, the F_1 zygotes deteriorate about three days after fertilization. In both directions of the cross, tissue differentiation in the zygotes was disturbed. The chance to obtain viable F_1 seeds was in both less than 5%. The results of crossing experiments using exceptionally obtained F_1 plants indicated that the crossing barrier was controlled by a set of complementary dominant lethals. The difference in the stage of zygote deterioration between reciprocal crosses was explained by the assumption that the genes would primarily interrupt tissue differentiation in the endosperm. As *barthii* is a cross-pollinated plant sympatrically growing with its wild and cultivated relatives, this crossing barrier may protect it from frequent hybridization.

Variations in Peroxidase, Acid Phosphatase and Esterase Isozymes of Wild and Cultivated *Oryza* Species

B. B. SHAHI, Hiroko MORISHIMA and Hiko-Ichi OKA

A total of 541 strains belonging to 21 *Oryza* species were observed for leaf-blade isozymes of peroxidase, acid phosphatase and esterase. The species markedly differed in zymogram variability. Widely distributed wild species were generally highly variable, while localized wild species as well as cultivated ones showed a limited variation. Though certain species had isozyme bands peculiar to them, identification by zymogram seemed to be difficult on account of wide variations within species. In view of the complicacy of the data, the technique of pattern analysis was used for obtaining an integrated picture. In the scatter diagram thus

obtained, closely related species were placed near each other, though strains of a species were distributed in a wide range. In the scatter diagram for acid phosphatase zymograms, species of the *Sativa* and *Officinalis Groups* were separated and strains of the latter were divided into two groups having the bands of odd and even numbers. In both peroxidase and acid phosphatase, the cultivated species, *sativa* and *glaberrima*, had no zymogram peculiar to them as all their zymograms were present among those of their respective wild progenitors, *perennis* and *breviligulata*. The zymograms of *sativa* varieties were only two which represented the Indica and Japonica types, respectively, and it appeared as if a part of *perennis* strains having certain isozymes could have been domesticated. However, *sativa* varieties showed 19 different zymograms of esterase, and the Indica and Japonica types differed in the frequencies of those peculiar to *sativa*.

V. MATHEMATICAL AND STATISTICAL STUDIES ON POPULATION GENETICS

The Evolutionary Rate at the Molecular Level

Motoo KIMURA

From the data of amino acid differences between homologous proteins and the total number of nucleotide pairs making up the genome, it was estimated that in the evolutionary history of mammals, nucleotide substitution has proceeded roughly at the rate of one substitution every two years.

On the other hand, based on the concept of the cost of natural selection (substitutional load), Haldane (1957) estimated that, in the standard rate evolution, a new allele may be substituted in the population roughly every 300 generations.

The large discrepancy between these two estimates may most easily be interpreted by assuming that a majority of the observed differences in amino acids between homologous proteins are the result of random fixation of selectively neutral mutations.

If this interpretation is correct, we may conclude that random genetic drift is playing a very important role in forming the genetic structure of biological populations.

For details, see Kimura (1968).

The Average Number of Generations until Fixation of a Mutant Gene in a Finite Population

Motoo KIMURA and Tomoko OHTA

Since gene substitution plays a key role in the evolution of the species, it is desirable to know the average number of generations until fixation of a mutant gene in a finite population (excluding the cases of eventual loss).

By using the diffusion models in population genetics, we have found the solution to this problem.

Let

$$T_1(p)=\int_0^\infty t\frac{\partial u(p,t)}{\partial t}dt\,, \qquad (1)$$

where $u(p, t)$ is the probability that the mutant allele becomes fixed by

the t-th generation, given that its frequency is p at $t=0$. Then, it can be shown that $T_1(p)$ satisfies the following differential equation,

$$\frac{d^2T_1(p)}{dp^2}+\frac{2M_{\delta p}}{V_{\delta p}}\frac{dT_1(p)}{dp}+\frac{2u(p)}{V_{\delta p}}=0\,, \tag{2}$$

where $M_{\delta p}$ and $V_{\delta p}$ are the mean and variance of the rate of change in the frequency of the mutant gene per generation and $u(p)=\lim_{t\to\infty}u(p,t)$ is the probability of ultimate fixation. The mean time until fixation, $\bar{t}_1(p)$, which is equal to $T_1(p)/u(p)$, can be obtained as the solution of equation (2), namely,

$$\bar{t}_1(p)=\int_p^1 \psi(\xi)u(\xi)\{1-u(\xi)\}d\xi+\frac{1-u(p)}{u(p)}\int_0^p \psi(\xi)u^2(\xi)d\xi\,. \tag{3}$$

In the above formula,

$$u(p)=\int_0^p G(x)dx\Big/\int_0^1 G(x)dx \quad\text{and}\quad \psi(x)=2\int_0^1 G(x)dx\Big/\{V_{\delta x}G(x)\}\,,$$

in which

$$G(x)=\exp\left\{-\int_0^x \frac{2M_{\delta\xi}}{V_{\delta\xi}}d\xi\right\}\,.$$

If the mutant gene is selectively neutral, formula (3) reduces to

$$\bar{t}_1(p)=-\frac{1}{p}\{4N_e(1-p)\log(1-p)\}\,. \tag{4}$$

For the cases of additive gene effect and overdominance, formula (3) was evaluated by numerical integration. Generally, the time until fixation becomes smaller with additive advantageous effect and larger with overdominance. The analytical results were verified by Monte Carlo experiments.

The details will be published in GENETICS.

Linkage Disequilibrium due to Random Genetic Drift

Tomoko OHTA and Motoo KIMURA

Linkage disequilibrium between 2 linked loci in finite populations may be caused by epistatic interaction in fitness and/or by random genetic drift. The latter effect on linkage disequilibrium was investigated by using the diffusion models. In the present investigation the variance of the linkage disequilibrium coefficient was worked out, assuming no selec-

tion.

Let N_e be the effective population number and c the recombination fraction between the 2 loci, with alleles A_1 and A_2 in the first locus, and B_1 and B_2 in the second. We will denote by g_1, g_2, g_3 and g_4 the initial frequencies of 4 gamete types, A_1B_1, A_1B_2, A_2B_1 and A_2B_2 respectively. Let $p=g_1+g_2$, $q=g_1+g_3$ and $D=g_1g_4-g_2g_3$. Then the expected value of the square of linkage disequilibrium at time t can be expressed as follows,

$$E\{D_t^2\}=\sum_{i=1}^{3} C_i\left[\frac{pq(1-p)(1-q)}{2(1+\lambda_i)}+\frac{1}{4}(3+4R+2\lambda_i)\right.$$
$$\left.\times D(1-2p)(1-2q)+D^2\right]e^{\lambda_i t/N_e} \qquad (1)$$

In the above formula, λ_i's are the 3 eigenvalues of the Kolmogorov forward equation involved. The coefficients C_i's are the functions of λ_i's as follows,

$$C_1=-\frac{1+\lambda_1}{(\lambda_2-\lambda_1)(\lambda_3-\lambda_1)}(\lambda_2+\lambda_3+2N_ec+2.5)$$

$$C_2=-\frac{1+\lambda_2}{(\lambda_1-\lambda_2)(\lambda_3-\lambda_2)}(\lambda_1+\lambda_3+2N_ec+2.5)$$

$$C_3=-\frac{1+\lambda_3}{(\lambda_1-\lambda_3)(\lambda_2-\lambda_3)}(\lambda_1+\lambda_2+2N_ec+2.5)$$

Some numerical values of λ_i's are listed in the following table.

Table 1. Values of λ_i's for various values of N_ec, ranging from $N_ec=0$ to $N_ec=5$.

N_ec	$-\lambda_1$	$-\lambda_2$	$-\lambda_3$
0.0	0.50	1.50	3.00
0.2	0.67	1.66	3.27
0.4	0.78	1.88	3.55
0.6	0.84	2.13	3.83
0.8	0.88	2.39	4.12
1.0	0.91	2.66	4.42
2.0	0.97	3.96	6.07
3.0	0.98	5.13	7.89
4.0	0.99	6.22	9.79
5.0	0.99	7.28	11.73

From direct evaluation of formula (1), it was found that the variance of the linkage disequilibrium coefficient is fairly large with small $N_e c$. Details will be published in GENETICAL RESEARCH.

Rate of Decrease of Genetic Variability in a Linearly Subdivided Population

Takeo MARUYAMA

The asymptotic rate of decline of heterozygosity in a linearly subdivided population, in which migration occurs only between adjacent colonies, was investigated. If we denote the number of colonies in the population by n, the number of diploid individuals in a single colony by N and the migration rate between adjacent colonies by $m/2$, the rate is approximately equal to

$$\frac{m\pi^2}{2n^2} \tag{1}$$

when

$$\frac{m\pi^2}{2n^2} < \frac{1}{2Nn} . \tag{2}$$

Thus for these cases the rate is proportional to migration rate but independent of colony size. When the inequality (2) is reversed, the rate is approximately equal to

$$\frac{1}{2Nn} . \tag{3}$$

Thus it is equal to the rate in a panmictic population of nN diploid individuals, and therefore the effect of subdivision of the population disappears as far as the rate of decrease in heterozygosity is concerned.

Approximation formulas for the eigenvectors associated with the dominant eigenvalues were also obtained. Let f_{ij} be the probability that two homologous genes taken one from the i-th colony and the other from the j-th colony are identical by descent, and let $h_{ij}=1-f_{ij}$. Then the asymptotic form of the matrix $[h_{ij}]$ is given by

$$[h_{ij}] \propto \left[\left|\sin\frac{i-j}{n+1}\sin\frac{i+j}{n+1}\right|\right]_{n\cdot n} \quad i, j=1, 2, \ldots n , \tag{4}$$

when the dominant eigenvalue is most closely approximated by (1), and the associated eigenvector of the eigenvalue approximated by (3) is

$$[h_{ij}] \propto \begin{bmatrix} 1 & 1 & \cdots\cdots & 1 \\ 1 & 1 & \cdots\cdots & 1 \\ \vdots & \vdots & & \\ 1 & 1 & \cdots\cdots & 1 \end{bmatrix}_{n \cdot n} . \qquad (5)$$

These results were checked by numerical calculations. A few examples are presented in Tables 1 and 2.

Table 1. Comparison of eigenvalues obtained by approximation formula (1), or (3), with exact values obtained numerically

Number of colonies (n)	Colony size (N)	Migration rate (m)	Dominant Eigenvalue Approximation by (1)	Exact value by the power method
30	2	0.4	0.00216	0.00213
20	2	0.4	0.00474	0.00436
20	5	0.1	0.00123	0.00110
10	5	0.05	0.00245	0.00214
10	5	0.05	0.00245	0.00241
10	5	0.03	0.00131	0.00148
10	5	0.01	0.000471	0.000494
10	5	0.005	0.000240	0.000247
10	50	0.005	0.000213	0.000247
10	50	0.003	0.000131	0.000147
10	50	0.001	0.0000472	0.0000490
			by (3)	
10	10	0.5	0.00464	0.00500
10	50	0.2	0.000924	0.001000
10	50	0.15	0.000901	0.001000
10	150	0.05	0.000300	0.000333
10	500	0.05	0.0000967	0.0001000
10	1500	0.05	0.0000329	0.0000333
10	5000	0.05	0.00000989	0.00001000
10	1000	0.005	0.0000428	0.0000500
10	5000	0.005	0.00000974	0.00001000

Table 2. Comparison of eigenvector (matrix) given by (4) with exact eigenvector obtained numerically by the power method. Because of identity $h_{ij}=h_{ji}=h_{n-i+1,\ n-j+1}=h_{n-j+1,\ n-i+1}$ of the elements in the vector, only one quarter of them are presented. $n=10,\ m=0.01,\ N=5$.

$i=$ \ $j=$		1	2	3	4	5
1	By formula (4)	0				
	By power method	0.0005				
2	By formula (4)	0.0143	0			
	By power method	0.0108	0.0014			
3	By formula (4)	0.0367	0.0223	0		
	By power method	0.0296	0.0201	0.0022		
4	By formula (4)	0.0649	0.0505	0.0282	0	
	By power method	0.0549	0.0454	0.0274	0.0027	
5	By formula (4)	0.0961	0.0817	0.0594	0.0312	0
	By power method	0.0843	0.0749	0.0568	0.0320	0.0030
6	By formula (4)	0.1273	0.1129	0.0901	0.0624	0.0312
	By power method	0.1149	0.1055	0.0876	0.0628	0.0336
7	By formula (4)	0.1554	0.1411	0.1188	0.0906	
	By power method	0.1438	0.1344	0.1166	0.0919	
8	By formula (4)	0.1778	0.1634	0.1411		
	By power method	0.1682	0.1589	0.1411		
9	By formula (4)	0.1921	0.1778			
	By power method	0.1859	0.1766			
10	By formula (4)	0.1971				
	By power method	0.1951				

One-dimensional Stepping Stone model of Finite Length

Takeo MARUYAMA

The so-called "stepping stone model" of population structure proposed by Kimura [1] was analysed in a one-dimensional case of finite length, with special reference to variance and correlation of gene frequencies between colonies.

The analysed model is as follows. A population consists of n colonies of finite size, each located at grid point of one-dimensional lattice of finite length. A non-terminal colony receives immigration from each of its adjacent colonies at the rate $m/2$ per generation, and a terminal colony receives immigration only from its corresponding subterminal colony at the rate m. We consider a locus segregating two alleles A_1 and A_2, and assume that each colony receives immigration, at the rate m_∞ per gene-

Table 1. Comparison of values by theoretical formulas (1) and (2), and by Monte Carlo method. The diagonal entries of the table are variances and the off-diagonal entries are genetic correlations between colonies specified. $n=10$, $m_\infty=0.01$, $m_1=0.1$, $2N=50$ and $\bar{q}=0.5$

Colony	1	2	3	4	5	6	7	8	9	10	
1	.0559	.7466	.5252	.3517	.2303	.1499	.0982	.0659	.0465	.0354	(Theoretical)
	.0585	.7964	.5676	.3645	.2514	.1507	.1158	.0932	.0295	.0491	(Monte Carlo)
2		.0545	.6904	.4627	.3030	.1927	.1292	.0867	.0611	.0465	(Th.)
		.0554	.6957	.4732	.3048	.1938	.1372	.1175	.0982	.0800	(M.C.)
3			.0499	.6556	.4297	.2797	.1833	.1230	.0867	.0659	(Th.)
			.0463	.6908	.4437	.2923	.1904	.1212	.0782	.0932	(M.C.)
4				.0478	.6399	.4169	.2732	.1833	.1292	.0982	(Th.)
				.0461	.6699	.4303	.2720	.1547	.1131	.1287	(M.C.)
5					.0469	.6355	.4169	.2797	.1972	.1499	(Th.)
					.0438	.6398	.4107	.2219	.1909	.1131	(M.C.)
6						.0469	.6399	.4297	.3030	.2303	(Th.)
						.0450	.6450	.3902	.3367	.2385	(M.C.)
7							.0478	.6556	.4627	.3517	(Th.)
							.0415	.6306	.4008	.3367	(M.C.)
8								.0499	.6904	.5252	(Th.)
								.0421	.6392	.5090	(M.C.)
9									.0545	.7466	(Th.)
									.0473	.7650	(M.C.)
10										.0559	(Th.)
										.0512	(M.C.)

ration, from an external colony in which the frequency of allele A_1 is constant with time and equal to $\bar{q}$.

An approximation of the covariance of the frequencies of allele A_1 between colonies at equilibrium is given by

$$c_{ij}=c\sum_{k=1}^{2n-2}\frac{\cos\frac{(i-1)(k-1)\pi}{n-1}\cos\frac{(j-1)(k-1)\pi}{n-1}}{1-\left[(1-m_\infty)\left\{1-m\left(1-\cos\frac{(k-1)\pi}{n-1}\right)\right\}\right]^2} \quad i, j=1, 2, \ldots n$$

in which c is a constant and the subscripts indicate colonies. Therefore the genetic correlation between colonies is

$$r_{ij}=\frac{c_{ij}}{\sqrt{c_{ii}c_{jj}}} \tag{1}$$

And the variance in the frequencies of allele A_1 in the i-th colony is given by

$$v_1=\frac{\bar{q}(1-\bar{q})}{1+2N\{1-2\beta^2-\alpha^2-4\alpha\beta r_{1,2}\}}$$

$$v_i=\frac{\bar{q}(1-\bar{q})}{1+2N\{1-2\beta^2-\alpha^2-2\alpha\beta(r_{i,\,i-1}+r_{i+1,\,i})-2\beta^2 r_{i+1,\,i-1}\}} \quad \text{for } i=2, 3, \ldots n-1 \tag{2}$$

and

$$v_n=v_1,$$

where $\alpha=(1-m_\infty)(1-m)$ and $\beta=(1-m_\infty)m/2$.

The validity of the analysis was checked by Monte Carlo experiments. One example is presented in Table 1.

[1] Kimura, M., 1953. "Stepping stone" model of population. Ann. Rept. Nat. Inst. Genetics, Japan 3: 62-63.

Distribution of Parent-offspring Distance in Mishima District

Norikazu YASUDA

The previous report (Ann. Rep. No. 18, pp. 68-70, 1967) was about a study on the distribution of matrimonial distance in Mishima district. The frequency of couples whose marital distance is r was derived from a square-root-exponential function; namely, $C\cdot r\cdot\exp(-k\sqrt{r})$, where $C=k^4/12$ and k is a constant. The present report is concerned with the dis-

tribution of parent-offspring distance; namely, distance between birthplaces of parent and their offspring. This type of distribution in man is important in studies on gene dispersion or migration.

Two histograms, each consisting of father-offspring and mother-offspring distances, were preliminarily obtained from the Nakazato area of Mishima district. Some characteristic features of the two distributions, in comparison with those of matrimonial distance, are summarized in Table 1.

Table 1. Some features of distributions of parent-offspring and marital distance in Mishima district

Type of distance	Frequency of zero distance	Mode of distribution (km.)	Type of migratory function*
Father-offspring	442/988	0	normal
Mother-offspring	74/988	2	normal
Matrimonial	1/944	0.5	square-root-exponential

* Tentative.

Although conclusions are still tentative, the results suggest that the form of distributions between marital and parent-offspring distances seems to be essentially different, particularly in the neighborhood of zero distance. Columns 2 and 3 of the table also suggest that migratory activity is much higher in females than in males even in a sedentary population like Nakazato.

The project will be continued to clarify the above points.

VI. EXPERIMENTAL STUDIES ON POPULATION GENETICS

Significance of Loci of the Persisting Lethal Genes

Chozo OSHIMA and Takao K. WATANABE

From the results of our experiments during the past five years, six kinds of clustering lethal genes were confirmed to have been maintained for more than three years in natural populations in Kofu and Katsunuma. The loci of these lethal genes on the second chromosome were determined by using dominant marker genes *Sp* (Sternopleural), *Bl* (Bristle) and *L* (Lobe). They were divided into three groups; the first located at the terminal region of the left arm, the second located at the mid-left arm and the third located near the centromere region. The results were compared with that obtained by Spiess *et al.* (1963 Genetics 48 : 1377—1388) and the similarity was found between the loci of persisting lethals in natural populations and those of lethals clustering in a cage population. Since 1965, a visible recessive mutant gene, named reduced bristle (*rbl*), has been extracted every year at a frequency as high as 3—4 per cent. The viability of homozygous flies for the gene was reduced to semilethality. The locus was determined to be 82.3 by using two dominant marker genes, *L* and bw^D (brown dominant).

About ten kinds of autosomal inversions were observed in natural populations in Kofu and Katsunuma. Six of them have been maintained at a relatively high frequency. The equilibrium frequencies of *In*(2*L*)*B*, identical to *In*(2*L*)*t* and *In*(2*R*)*C*, identical to *In*(2*R*)*NS* were about 35 per cent and 25 per cent respectively. *In*(2*L*)*B* suppresses completely the crossing over between loci 0 and 48.5 and *In*(2*R*)*C* acts in similar way between loci 70 and 100. The relationship between the mitotic chromosome and the genetic map has been established (A. Hannah 1951 Advances in Genetics 4 : 87—125, T. Hinton 1942 Genetics 27 : 119—127). The terminal regions of both arms and that near the centromere of the mitotic chromosome were assumed to be the regions of infrequent crossing over. On the contrary, two regions between 10 and 45 and between 60 and 100 loci of the genetic map were thought to be regions of frequent crossing over, because genes in these regions were concentrated in small parts of both distal portions of the mitotic chromosome. Loci 32 and 82 of the genetic map were about at the midpoints of the frequent crossing over regions, but these two points might be included in the relatively infrequent part due to interference with crossing over.

An adaptive gene complex might result from natural selection in such

crossing over infrequent regions. When a deleterious gene arises as mutation in such regions, its deleterious effect in the heterozygote might be covered by the epistatic actions of an adaptive gene complex of the background.

The hypothesis that the paracentric inversions *In*(2*L*)*B* and *In*(2*R*)*C* were adaptive gene complexes, may be supported by their persistence and wide distribution in natural populations. If a lethal gene arose by mutation in such inversions, it would be maintained naturally, and if it occurred between the centromere and the end of inversions, it would be also maintained. From the fact that *l* 201 gene (47.9) has been maintained for at least three years together with *In*(2*R*)*C*, an adaptive gene complex may have been formed in the region including the inversion and both sides of the centromere.

Allelic Relations between Lethal Genes Distributed in Natural Populations

Chozo OSHIMA and Takao K. WATANABE

In late October of 1967, a particular collection of flies was carried out

Table 1. Frequencies of intrapopulational allelisms and interpopulational allelisms.

Locality	Population	No. of lethal chromosome	Frequency of intrapopulational allelism	Population	Distance between populations	Frequency of interpopulational allelism
Katsunuma	A	34	5.35%	A-B	430 m	5.34%
	B	27	3.70	B-C	450	4.40
	C	16	2.50	C-D	450	5.00
	D	25	13.33	B-D	630	6.81
				A-C	880	5.70
				A-D	950	8.82
		Mean	6.22%	Mean	632 m	6.01%
Kofu	E	10	6.67	E-A	14000 m	6.47
				E-B	14000	7.04
				E-C	14000	3.75
				E-D	14000	9.60
				Mean	14000 m	6.72%

Overall allelic rate: 6.44%

simultaneously at four sites in Katsunuma and one site in Kofu. The distances between four sites; A, B, C and D were relatively short between 430 and 950 meters, and the distance between them and site E in Kofu was about 14 kilometers. The allelism test was performed by intercrossing 112 *Cy*/lethal balanced strains extracted from 5 populations located at A, B, C, D and E sites.

From the results of a total of 6216 crosses, allelic rates within the five populations and between them were obtained as shown in Table 1.

The overall allelic rate (6.44%) was the highest among those obtained for the past five years, but the relationship between allelic rate and distance reported by Wallace (1966) could not be recognized. Almost equal allelic rates between and within populations should be attributed to many clustering and wide spread lethals. Most of them may have been persisting lethals for a long time in the natural populations. Either such wide spread lethals were transplanted from a population to another one by migratory flies or have persisted already in those populations could not be ascertained. However, most of them could be rather persisting ones.

Relationship between the Dispersal of Flies and the Frequency of Allelism of Lethals in a Natural Population

Chozo OSHIMA and Takao K. WATANABE

One day of the beginning of October 1968, Drosophila flies were collected by 10 traps with the mixture of banana and dry yeast. These traps were put almost linearly at intervals of 30 meters from the first one which was put in a large natural population of *D. melanogaster*. This site was located in the locality of Katsunuma.

All flies were caught by net four times between 10 AM and 3 PM from these traps except the first trap and adequate number of flies were caught from the first trap. Flies totaled to 2699. Several species were found among them and their frequencies were as follows; *D. melanogaster* 75.5%, *D. immigrans* 17.9%, *D. hydei* 3.0%, *D. lutea* 2.7% and *D. auraria* 0.7%.

The dispersal of male flies of *D. melanogaster* was assumed to be more active than female flies by the mean sex-ratio (2.16) of flies collected from the second to tenth traps, but the sex-ratio of flies collected in the large population was 1.08. Results of the collection were shown in Table 2.

As B. Wallace (1966 Amer. Nat. 100 : 551—563) discussed on the dis-

Table 2. Number of *D. melanogaster* flies collected from traps and the results of viability test of second chromosomes and of allelism between lethal genes.

Trap	D. melanogaster collected		No. of second chromosomes tested	No. of lethal chromosomes	Allelic rate (%) within (italic) and between traps						
	~	~			1	2	3	4	5	6–7	8–9
1	230	249	88	5	*0.00*						
2	139	342	103	20	1.00	*6.32*					
3	100	245	101	16	2.50	7.19	*5.00*				
4	118	237	95	21	0.95	5.24	5.36	*5.24*			
5	42	91	73	13	0.00	3.46	2.88	4.00	*0.00*		
6	17	33	44	9	0.00	2.22	2.08	1.06	0.83	*0.00*	
7	8	17									
8	26	42	37	1	0.00	3.33	0.00	7.94	2.56	3.70	*0.00*
9	29	72	30	2							
10	0	2	0								
Total	709	1330	571	87 (15.2%)							

Distance between traps	0—30 m	60—90 m	120—150 m
Allelic rate (%)	4.63	3.33	1.64

persal of Drosophila, the dispersal of a natural population decreased linearly with the square root of distance. In our experiments, fairly number of flies unexpectedly came into the eighth and nineth traps, but its cause could not be found out.

Six hundred and eleven male flies among collected flies were mated with virgin *Cy/Pm* female flies and viabilities of homozygotes for each 571 second chromosome could be estimated in the F_3 generation. From the results, 87 chromosomes (15.2%) carried a lethal gene and four chromosomes among them were presumed to have two different lethal genes respectively by the allelism test.

Among a total of 91 lethal genes, 58 (63%) lethals were clustering ones and classified into 14 allelic groups and other 33 lethal (37%) were single ones. In the half diallel cross between these lethal strains, 143 crosses among 3741 crosses were allelic and its frequency was 3.82 per cent.

The allelic rates between lethals isolated from flies collected from the same and different traps were obtained as represented in Table 2. The relationship between the dispersal of flies and the frequency of allelism

of lethals in a natural population of *D. melanogaster* in the locality of Katsunuma was found to be similar to that observed by B. Wallace (1966 Amer. Nat. 100 : 565—578) in a tropical population of the same species.

Frequency of Sterile Chromosomes Concealed in a Natural Population

Chozo OSHIMA and Takao K. WATANABE

Total 473 non-lethal second chromosomes were isolated from about 600 male flies each, collected by 10 traps described in the previous report. They were classified into three grades of viability in homozygous state as follows; 31 semilethal, 36 subvital and 406 normal chromosomes. Then, homozygous male and female flies for these individual chromosomes were crossed with heterozygous female and male flies for *Cy* and the same chromosomes respectively. Such experiments containing 473×2 crosses were repeated for two generations. When no offspring could be obtained, the chromosome was assumed to have a recessive sterile gene. The results were given in Table 3.

Table 3. Frequency of sterile chromosomes among non-lethal chromosomes.

	Classes of viability in homozygote			
	semilethals	subvitals	normals	Total
No. of chromosome	31	36	406	573
No. of sterile chromosome	13	9	36	58
(%)	(41.1)	(25.0)	(8.9)	(12.3)
No. of chromosome manifesting male sterility	7	4	24	35 (60.4)
No. of chromosome manifesting female sterility	3	4	10	17 (29.3)
No. of chromosome manifesting sterility of both sexes	3	1	2	6 (10.3)

From the results, the interrelation between such physiological characters as viability and fertility was thought to be intimate. About 41 per cent semilethal chromosomes were assumed to have also sterile gene and the frequency of sterile chromosomes among subvital and normal chromosomes decreased. It should be analyzed whether the semilethality and sterility are manifested by different genes or by pleiotropic action of the common genes. The number of sterile chromosomes manifesting only

male sterility was about twice that of ones manifesting only female sterility and about six times that of ones manifesting sterility of both sexes.

Egg formation in the ovary of sterile female flies was remarkably reduced, but the testis of sterile male flies could not be distinguished morphologically from the normal one.

Persistence of a Visible Mutant in Natural Populations of *Drosophila melanogaster*†

Takao K. WATANABE

A recessive visible mutant named *reduced bristle* (*rbl*) has been frequently extracted from the natural populations of Kofu-Katsunuma since 1965. Its frequency in the population was 3.8, 3.1 and 3.5 per cent in the successive annual samplings of 1965, 1966 and 1967 respectively. The locus of *rbl* was determined to be 82.3 on the right arm of second chromosome and the mutant gene associated often with an heterotic inversion, *In*(2*L*)*B*, on the left arm. The homozygous flies (*rbl*/*rbl*) had lower viability, developmental rate, female productivity and fecundity than normal heterozygous flies (+/+). However, heterozygous flies (*rbl*/+) showed remarkable heterosis in productivity and fecundity though they were neutral in viability and developmental rate (Table 4). The role of inversion, *In*(2*L*)*B*, was assumed to enhance the heterosis of *rbl* gene at least in the cis-phase (*In*(2*L*)*B rbl*/++). In such a relation we may see the mechanism of persistence of *rbl* gene in the natural populations.

Table 4. Viability, developmental rate, productivity and fecundity of *rbl* bearing flies.

Genotype	Viability	Developmental rate (day)	Productivity	Fecundity
+/+	1.00	10.7	1.00	1.00
rbl/+	1.01	10.6	1.31**	1.19**
rbl/*rbl*	0.36**	12.2**	0.66**	0.64**

** Highly significant.

† Watanabe, T. K., Japan. J. Genetics 44: 15-22 (1969).

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN ANIMALS

Repair in the Mutation Process Studied in Low and High Radiosensitivity Strains of the Silkworm*

Yataro TAZIMA

Based on our experimental results obtained recently with the use of low and high radiosensitivity silkworm strains, the repair of radiation induced premutational damages was briefly discussed.

A direct evidence of repair of premutational damages was obtained by split-dose irradiation to spermatogenic cells, mostly spermatids, of full-grown larvae. Applying similar treatments to several strains differing in radiosensitivity, we discovered that only low sensitivity strains were capable to repair radiation induced pre-mutational damages, while sensitive strains lacked this ability.

In order to gain better insight into the nature of repair, the effects of pre- or post-irradiation treatment were compared between nitrogen and oxygen by subjecting mature larvae to those gases for 25 minutes before (including period of irradiation) or after irradiation. Post-irradiation treatment with nitrogen or oxygen affected only slightly the mutation frequency. In contrast, nitrogen pretreatment and irradiation in nitrogen atomosphere (anoxic irradiation) decreased the frequency to an appreciable extent. The effect of dose-reduction of anoxic irradiation varied depending on the type of mutation, being more pronounced for mosaics than for whole-body mutants.

These findings seem to suggest that most premutational damages produced by anoxic irradiation on one of the double helices of DNA are more likely to be repaired than those produced on both helices.

* Summary of a paper presented at the Small Symposia of the Twelfth International Congress of Genetics, 1968 held in Tokyo.

Comparison of Mutagenic Effects of 14 MeV Neutrons, γ-Rays and Some Chemical Mutagens Upon Silkworm Spermatogenic Cells*

Yataro TAZIMA

Effects of different mutagens can be compared in several ways. Since the double helix model of DNA suggests that there could be two types of mutations concerning the site of occurrence, i.e. those occurring in one of the double helices and those occurring in both helices, comparative studies have been carried out with different mutagens with special regard to the ratio of mosaics to whole mutants.

As reported previously, the mutation response of silkworm germ cells to γ-rays varied markedly with the advancement of the stage of gametogenesis not only with respect to induced frequencies but also with respect to the proportion of mosaic mutants. The incidence of mosaics, when expressed in terms of the ratio of mosaics to whole mutants, was very low before meiosis, reached to the unity around V-4.5 stage and thereafter increased rapidly with the progress of spermiogenesis. Experimental data used for the construction of the curve were obtained, for the most part, from 1,000 R irradiation but some were obtained from experiments, in which higher doses were given: i.e. 3,000 R at mid-pupal stage and 5,000 R at the adult stage. It is known that the incidence of whole mutants increases linearly with increasing dose, while that of mosaics increases more rapidly than linearly. However, since the mutation response is relatively low at such advanced stages as pupa and moth, the curve can be utilized as a convenient standard for the comparison of mutagenic effects among different mutagens.

Mutation spectra have been obtained at several developmental stages by treating the insects with neutrons and chemical mutagens. 14 MeV neutron beams were administered to the insects at V-1 and mid-pupal stages. Radiation doses were 500 rad and 1,000 rad. For both doses calculated ratios of mosaics to whole mutants did not appreciably differ. They were clearly lower than those obtained for γ-rays. Contrary to the effect of neutron, chemical mutagens gave very high figures in the mosaic ratio. The chemicals used were Mitomycin-C and EMS. Those chemicals were administered to the insects by injecting their aqueous solution into the body cavity of the insect at several developmental stages: i.e. III-1, IV-3, V-1, mature larvae and early pupae. The injection was kept

* Summary of a paper presented at the International Symposium on Genetic Effects of Radiation and Radiomimetic Chemicals held in Kyoto during the period of August 30-31, 1968.

constant for its concentration but varied in quantity according to the stage of the insect. Throughout all stages examined the incidence of mosaics was extraordinarily high. For instance, injection of Mitomycin-C into IV-3 larvae (0.04%, 0.01 ml per head) gave the mosaic ratio as high as 11.8 and the ratio increased at more advanced stages. Injection at III-1 satage gave a somewhat lower but still significantly higher than those obtained for γ-rays.

Above results are consistent with the expectation that mosaics induced at postmeiotic stages of spermatogenesis are due to a mutational event occurred in one of the double helices of DNA.

Frequency Pattern of Mosaic and Whole-Body Mutants Induced by Ionizing Radiations in Post-Meiotic Germ Cells of the Male Silkworm*

Yataro TAZIMA and Kimiharu ONIMARU

This work was undertaken in order to investigate the mutation spectra induced by γ- or X-rays in post-meiotic germ cells of male silkworm with special regard to the change in the frequency pattern of mosaic (fractional) and whole-body (complete) mutants.

When silkworm spermatozoa were irradiated with γ-rays, the frequency of whole-body mutants increased linearly with increasing dose, whereas that of mosaics increased exponentially. The latter relation is in striking contrast to that observed in *Drosophila*, where the incidence of mosaics has been known to reach a plateau at a relatively low dose level. Several possibilities were considered for the interpretation of the difference. Mosaics produced after irradiation of silkworm spermatozoa appeared to form a mixed class of at least two types, single-helix type and chromosome-aberration type. The proportion of both was assumed to vary depending on the irradiated dose.

The incidence of mosaics in comparison to that of whole-body mutants varied considerably with the progress of spermatogenesis. On a per R-basis, spermatogonia yielded the lowest frequency of mosaics; the frequencies of both complete and mosaic mutations increased sharply through meiotic prophase up to V-4.5 (fifth instar larvae, day 4.5). Until this stage relatively more completes than fractionals were produced. Around V-4.5 the ratio of mosaics to completes reached unity. From this stage onward, during spermiogenesis, relatively more mosaics than completes were produced, although the absolute frequencies of their

* Summary of a paper published in Mutation Research 8 (1969): 177-190.

induction was far lower than in V-4.5.

This observation may be interpreted as resulting from a greater capacity for repair of premutational damage leading to mosaic mutations in the early than in the late stages of spermatogenesis.

Mutagenic Action of Mitomycin-C and EMS on Pre-Meiotic Germ Cells of the Male Silkworm

Yataro TAZIMA and Kimiharu ONIMARU

The double helix model of DNA can satisfactorily explain the high incidence of mosaic mutants in the F_1 obtained from the individuals whose germ cells had been treated with Mitomycin-C or EMS at their post-meiotic stage. Results of such experiments had been briefly reported (this Annual Report 17 : 94-95). The present report is concerned with a similar treatment of young germ cells of the male silkworm at pre-meiotic stages, i.e. spermatogonia and spermatocytes. The chemical treatment was performed at the stages of the first, second or third instar. Mitomycin-C and/or EMS were administered to larvae of the wild type strain C108 either by adding the chemical to food (for first and second instar treatment) or by injection (for third instar treatment). After emergence the treated males were mated to *pe re* females which permitted to assess in the F_1 the induced frequencies both for whole and mosaic mutations. The amount of in-take of those chemicals was compared on per gram body weight basis.

The incidence of mosaics was negligibly small in both groups treated at the first or the second instar but it was significantly high in the third instar group. In contrast, the appearance of whole type mutants was highest in the first instar group and decreased as the treatment stage advanced further. The latter observation was closely related to the size of the mutant cluster. The distribution of cluster size, i.e. number of same kind mutants per batch, followed Poisson's equation in Mitomycin-C group but significantly deviated from it showing a long tail in EMS group.

Those results are consistent with the expectations that the chemicals produce mutations by affecting one of the double helices of DNA in dividing cells as well as in non-dividing cells and that the affected cells give rise to whole type mutants instead of mosaics, since in those cells DNA multiplication occurs several times after mutation had taken place. The evidence that the frequency of mosaics increased in the third instar

treatment group seems to suggest that by this stage some fractions of male germ cells have already completed DNA synthesis.

Delayed Mutagenic Effect of Mitomycin-C and EMS Observed in Silkworm

Kimiharu ONIMARU and Yataro TAZIMA

It has been reported by Alderson (1965) that EMS manifests its mutagenic action on *Drosophila* germ cells even at later cell generations following the treatment. In our experiments with the silkworm we have observed a similar effect not only for EMS but also for Mitomycin-C.

Male larvae of wild type strain C108 were injected with 0.05~0.025 ml of physiological saline solution of Mitomycin-C (0.04%) or EMS (0.05%) at V-1 (day-1 of the 5th instar) or V-7. Those males were crossed to *pe re* females after emergence. The F_1 from those crosses comprised, in addition to the expected wild type, a fairly large number of whole-type mutants for *pe* and *re* and mosaic type mutants for *pe*+ and *re*+. Among those F_1, only wild type insects were bred to the next generation. Females of those wild type individuals, were back-crossed again to *pe re* males. Since those females are considered to be ++/*pe re*, the segregation of 1 *pe* : 1+ could be expected in the next generation, because *pe* is epistatic to *re*. Experimental results, however, showed that *re* eggs, in addition to the expected *pe* and +, actually segregated in some batches. The frequencies of appearance of such batches were 14 among 224 total batches observed for MMC-V-1, 12/223 for EMS-V-1, 15/194 for MMC-V-7 and 1/234 for EMS-V-7. The number of *re* eggs segregated varied from 1 to 87 per batch. Further, the number of wild type eggs was observed to decrease below unity, in comparison to *pe* eggs in those batches where a large number of *re* appeared.

Since crossing over does not take place in the female of the silkworm, it seems reasonable to attribute the appearance of *re* eggs among the offspring of F_1 females to the delayed action of the mutagenic agents. The observation that there was a considerable variation in the number of segregated *re* eggs seems to suggest a difference in the time of action of those agents during the successive cell generations of gametogenesis.

The Effect of Post-Treatment with Low Temperature on the Frequencies of Radiation-Induced Mutation on the Silkworm Oocytes

Akio MURAKAMI

Repair of pre-mutational state is terminated by mutation fixation. This process is invariably associated with DNA replication. There are many reports with different organisms that in some experiments post-treatment with metabolic inhibitors lowers the mutation-frequency. These findings were interpreted as an effect of delation of mutation-fixation: the inhibitors, apart from actively affecting repair of premutational damages, slow down the rate of mutation fixation, and thus give more opportunity for repair to occur. There are many metabolic inhibitors known among which low temperature is considered as a typical one. The present experiment investigated the effect of low temperature treatment on the X-ray induced mutation frequencies of the silkworm oocytes.

As the experimantal materials prophase I oocytes in moth are used because the oocytes of this stage were well known to keep for long times under cold temperature conditions. Furthemore, the oocytes in silkworm have characteristics that make it possible to expect that the first DNA replication after irradiation takes place at least 100 to 150 minutes after oviposition (or irradiation): which correspond to stage from the late second meiotic division of oocytes to the first mitotic division stage of zygotes.

The oocytes in wild-type *C108* strain moths which were mated with *pe*; *re* marker strain males for 5 hours after emergence were irradiated by X-rays (180 kVp, 25 mA, 10 mm Al filter and dose-rate at 250 R/min) with three different doses of 500, 1,000 and 1,500 R. Just after irradiation, these moths (or oocytes) were divided into two groups, one of them was subjected to post-treatment with the temperature of 5°C for 5 hours in an ordinary ventilated refrigerator and the other under room temperature of 25°C. All experimental procedures were done at 25°C except for the time of irradiation. The mutational responses were estimated by the egg-colour specific locus method. These data show a linearity on semi-logarithmic plot in the relation between dose and frequency for whole-body mutation. This is in agree with the observation by Tazima (1964). The experimantal data also indicated that radiation-induced mutation frequencies were less in the group treated with low temperature of 5°C then in the non-treated group. By the way, significant differences have not been shown in fractional-body mutation between X-ray alone and post-treatment with low temperature groups because of the frequencies

for this type of mutation found rarely to be observed for both experimental groups. In any case, these findings seem to agree with our expectation suggesting that post-treatment with low temperature can effect both repair and mutation fixation process. Further examinations, for example the changing the duration of cold-treatments and changing the phase of irradiations, are in progress to gain an insight into the mechanisms involved.

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

Akio MURALAMI

In a previous report (Annual Report, No. 17; 98-100, 1967), we showed that there were striking strain differences in radiation induced embryonic killing. These findings indicated that among the 30 strains observed that

Table I. Differences in radiosensitivity of the different silkworm strains

Race	Strain	LD_{50}(R)	Race	Strain	LD_{50}(R)
	Seihaku	560		Kaizyo	1,000
	Shoka	610		Kanko-syaken	1,000
	Kojiki (Beggar)	870		Kohoku	1,080
	Kojiki (Matsumoto)	1,000		Kinto	1,130
	Chiyozuru	1,000		No 5-Shokei	1,140
Japanese	Agurisan	1,020	Chinese	Nanko	1,160
	Kinkoshu	1,050		Shinshocho	1,200
	Onichijira	1,060		Ginto	1,240
	Tanegashima	1,270		Renshin	1,300
	Okusa (Fujisawa)	1,330		Tenmon	1,670
	Kokin	1,420		Tokuishin	1,710
	Amoi-moricaud	690	Southernasin	Canbodge	1,020
	Hakuyoen	710		Bagdad	620
	Hekiren	840		Cévennes	900
Chiness	Sisen-kinsan	850		St. Julian	1,000
	Yoko	900		Szezard	1,070
	Asia-Oken	925	European	Ascoli-oken	1,160
	Shoko-abura	1,000		Spain	1,170

the LD_{50} for the most resistant strain was approximately 1,500 R for *Kansen*; while two strains were extremely sensitive, *i.e.*, LD_{50} were 240 R for *Kojiki* and 140 R for *Ascoli*.

Since we first reported on this subject, we have attempted to obtain more additional information on radiation killing in different strains of the silkworm. Thirty-six new different strains were selected and obtained to investigate their radiosensitivity to embryonic mortality of overwintered eggs. The strains used in this work were generously presented by the National Sericultural Experiment Station, Japan. The experimental methods and conditions were quite the same, except for the experimental year, as those reported in the preceeding paper.

The results obtained are summarized in Table I. It may be seen from the table that there were differences in radiosensitivity among the defferent strains. The average LD_{50} for 36 strains investigated was 1,040 R. It seemed to indicate a somewhat higher LD_{50} than 910 R of the previous data in 1966. From this table it can be selected the two marked radiation resistant strains, *i.e.*, *Tenmon* (1,670 R) and *Tokuishin* (1,710 R) which are more resistant than those strains found in the previous report, *Aojuku* (1,460 R) and *Kansen* (1,580 R). However, It failed to find out the radiosensitive strain as had been found in the previous report, *Ascoli* and *Kojiki*.

Effects of X-Irradiation on Aggregate-Forming Activity and Sorting-Out Property of HeLa Cells in Rotation Culture

Yukiaki KURODA

It has previously been suggested that in liver and heart cells dissociated from chick and quail embryos low doses of X-rays may stimulate the activation of aggregate-forming substances already present on the cell surface and higher doses of X-ray may inhibit new production of these substances through suppression of RNA and protein synthesis (Kuroda, 1968. Ann. Rep. Natl. Inst. Genet. Japan 18 : 96). In the present investigation effects of X-irradiation on the function of HeLa cells were examined by testing them for their aggregate-forming activity and sorting-out property from embryonic quail liver cells after irradiation with various doses of X-ray.

Cell suspensions of HeLa S3 strain were obtained from monolayer cultures at logarithmic growth phase, introduced into Petri dishes and irradiated with 0 R, 400 R, 800 R, 1,200 R, 1,600 R and 2,000 R of X-rays

(175 KVp, 25 mA, distance 40 cm, filter 1.0 mm Al, dose rate 300 R/min). After irradiation each cell suspension containing 10^6 cells in 3 ml culture medium was transferred into a 25 ml Erlenmeyer flask and rotated on a gyratory shaker at 70 rpm at 38°C.

Non-irradiated control HeLa cells formed after 24 hours of rotation culture aggregates with an average diameter of 150 μ. HeLa cells irradiated with X-ray formed aggregates, whose shaps and average diameter were not significantly different from those of non-irradiated control cells. Many cells irradiated with X-ray remained free as single cells in the suspension after 24 hours of rotation culture. The number of these single cells was gradually increasing with the amount of X-rays.

Irradiated HeLa cells were intermixed with liver cells dissociated from 7-day quail embryos, then tested for their sorting-out activity in rotation culture for 24 or 48 hours by the standard procedure. Non-irradiated control HeLa cells became sorted-out from embryonic quail liver cells. Both types of cells formed aggregates separately from each other and no chimaeral or mosaic tissues were formed. HeLa cells which were irrradiated with various doses of X-ray were gradually losing their sorting-out property with the increase of X-ray dose. HeLa cells irradiated with 400 R still maintained their ability to form aggregates which were composed of HeLa cells alone. HeLa cells irradiated with 800 R or 1,200 R formed aggregates in which many quail liver cells were interspersed. HeLa cells irradiated with 2,000 R lost completely their sorting-out property from quail liver cells and formed chimaeral, or mosaic aggregates composed of both types of cells.

These changes in sorting-out activity of HeLa cells after irradiation with X-rays may be useful as strictly controllable and allowing quantitative cell function to be detected after relatively short term cultures following X-irradiation.

Postspermatogonial Rate for 14.1 MeV Neutron-Induced Dominant Mutations Affecting the Skeleton of Mice

Kiyosi TUTIKAWA

The present study was designed primarily to obtain an estimate of frequency of 14.1 MeV neutron-induced dominant mutations affecting the skeleton of mice. A method devised by Ehling was used (Ehling, U. H., 1966, Genetics *54*: 1381). The present experiment is an extension of an earlier study (Tutikawa, K., 1967, Ann. Rep. Nat. Inst. Genet., Japan, No. *18*: 98).

The methods were given in the previous report. In the present paper, the progeny were selected for sampling gametes irradiated in postspermatogonial stages. Since the examination of the skeletons is still in progress, some of the prepared specimens have been examined in detail. The assumed mutations are briefly described as follows; 1) Control: (C188) Anomaly of the skull (hydrocephalus); 2) Neutrons 242.5 Rad: (F73) Longitudinal shortening of the skull, shape anomalies of the interfrontal bone and foramen ovale, presphenoid-basisphenoid fusion; (F117) Abnormal frontals, presphenoid-basisphenoid fusion, dyssymphysis of the posterior of C7 and Th1; (F161) Curvature and shortening of the radius and ulna in both forelimbs; (F162) Absence of the left upper molar, dyssymphysis of the posterior of C5 and C7; (F230) Foramina transversaria imperfecta in the C3 (both sides), C4 (both sides), C5 (both sides) and C6 (left side); (F245) Malformation of the sternum associated with whole intrasternal fusions and irregularities of the costosternal articulation, shape anomaly of the foramen ovale, shortening of the right tubercula anterius in the C6; (F272) Imperfect ossification of the right frontal bone, interfrontal-frontal fusion, right squamosal-parietal fusion. 3) Neutrons 485 Rad: (G5) Shape anomalies of the foramen ovale on both sides, foramina transversaria imperfecta in the C5 (both sides) and C6 (left side), fusion of cervical ribs to the transverse process and centrum in the C7 (both sides); (G16) Imperfect ossification of the basisphenoid, shape anomaly of the foramen ovale (right side); (G17) Shape anomalies of the skull, interfrontal bone and foramen ovale, abnormal elongation of incisor, basisphenoid-occipital fusion, presphenoid-basisphenoid fusion, dyssymphysis of the C4, foramina transversaria imperfecta in the C3 (both sides), C4 (left side) and C5 (both sides); (G23) Longitudinal shortening and imperfect ossification of the skull, absence of the left tubercula anterius in the C6; (G133) Foramen ovale open posteriorly (left side) Th10-11 fusion; (G144) Shortening metacarpal and metatarsal bones, fusion of II-III metacarpal (both manus) and II-III metatarsal (left pes), malformation of all phalanges.

So far, one of the deviations assumed to be mutations (G144), has been confirmed as such by breeding test. The estimate for the induced dominant mutation rates was 7.3×10^{-5} per gamete per Rad for neutrons and 4.6×10^{-5} for X-rays.

Lack of Effect of Urethane on the Induction of Dominant Lethal Mutations in Male Mice

Kiyosi TUTIKAWA

In the earlier studies (Tutikawa, K. and A. Akahori, 1964, Ann. Rep. Nat. Inst. Genet., Japan, No. *15*: 62; Tutikawa, K. and A. Akahori, 1965, Cong. Anom. *5*: 166), a marked strain difference was found in the frequency of a certain kind of induced malformation between inbred strains CBA and C3H$_e$B/Fe, when the female mice were injected once with 1.5 mg per g of 10% urethane after 8.5 days of pregnancy. However, the changing pattern of urethane levels in blood withdrawn at each time up to 15 h after injection of urethane did not reveal any difference between those strains.

From the viewpoint that the active principle appears to be a metabolite of urethane, an attempt was made for N-hydroxyurethan analysis, but failed to reveal any N-hydroxyurethan in blood withdrawn after the injection of 1.5 mg urethane per g.

Table 1. Analysis of matings of urethane treated male mice by dissection 14 days after mating

Strain		Week						Controls
		1	2	3	4	5	6	
CBA	Corpora lutea (CL)	137	110	131	122	115	36	218
	Implantations (IMP)	132	104	116	104	107	35	204
	Early deaths (ED)	8	8	8	3	6	4	18
	Late deaths (LD)	2	2	3	1	2	0	2
	Living embryos (LE)	122	94	105	100	99	31	184
	ED/IMP %	6.1	7.7	6.9	2.9	5.6	11.4	8.8
	(ED+LD)/IMP %	7.6	9.6	9.5	3.9	7.5	11.4	9.8
	LE/CL %	89.1	85.5	80.2	82.0	86.1	86.1	84.4
C3H$_e$B/Fe	Corpora lutea (CL)	52	35	63	26	34	—	323
	Implantations (IMP)	43	33	54	25	33	—	292
	Early deaths (ED)	5	2	7	2	5	—	29
	Late deaths (LD)	1	0	1	1	1	—	0
	Living embryos (LE)	37	31	46	22	27	—	263
	ED/IMP %	11.6	6.1	13.0	8.0	15.2	—	9·9
	(ED+LD)/IMP %	14.0	6.1	14.8	12.0	18.2	—	9.9
	LE/CL %	71.2	88.6	73.0	84.6	79.4	—	81.4

The purpose of the present experiment was to provide an information on mutagenic action of urethane in mice, using the dominant lethal technique. Male mice of two inbred strains, CBA and C3H$_e$B/Fe, were injected once intra-peritoneally with 1.5 mg per g of 10% urethane aqueous solution. After injection, each male was mated to 2 BALB/c females each week for the next 5 to 6 weeks. The females were dissected on the 14th day of pregnancy.

The results obtained from these males are given in Table 1. The weekly frequencies of dominant lethals did not increase significantly over the control values. Thus, it seems that urethane may lack the ability to induce dominant lethals. I took notice of Bateman's report (Bateman, A. J. 1967, Mut. Res. *4*: 710) after my experiment started. His findings are in agreement with mine.

VIII. RADIATION GENETICS IN MICROORGANISMS AND PLANTS

A Mutant of *Bacillus subtilis* Possessing Modified Specificities to Irradiated Transforming DNA

Yoshito SADAIE and Tsuneo KADA

A mutant named Sb-36 was isolated by treatment with N-methyl-N′-nitroso-N-nitrosoguanidine of a substrain named H-17 derived from a Marbourg strain of *Bacillus subtilis*. The parent H-17 and the mutant Sb-36, both requiring arginine for their growth, have similar cellular radio-sensitivities to gamma-rays or ultraviolet light. However, when transforming DNA extracted from a wild strain was irradiated with increasing doses of gamma-rays and donated to H-17 or Sb-36, inactivation curves as to the arginine marker were much steeper by titrating on Sb-36 than H-17. The observation is explained by assuming that the strain Sb-36 lacks the repairing capacity of irradiated DNA whereas the strain H-17 has it. This capacity was concerned with the transformation process but not with radiation-induced cellular lethality. Ultraviolet irradiation acted similarly to gamma-irradiation.

Similar specific sensitivities were also found with transforming DNA treated with certain enzymes relating to DNA metabolism. Ando, T. and his collaborators (J. Biochemistry, Tokyo, *66* (1969) 1) have recently isolated from T4 infected cells of *E. coli* B two enzymes A and B having nicking actions on DNA strands. Treatment of transforming DNA with enzyme B reduced much more the yield of transformants using Sb-36 as the recipient strain than using H-17. Such specificity was more moderated with enzyme A. Thus these enzymes acted in a similar way to irradiations.

Above results indicate that gamma-irradiation as well as ultraviolet irradiation may involve DNA damages of the similar nature as those due to nicking action. It is supposed that the strain Sb-36 might lack a repair capacity on this point.

^{32}P-Decay and Mutations in *Escherichia coli* B/r WP2 try$^-$

Masaru HAYASHI and Tsuneo KADA

Tryptophane reversions were studied in *Escherichia coli* B/r WP2

heavily labelled with ^{32}P. The bacteria were grown in a modified H-medium (Stent, G. and C. R. Fuerst, J. Gen. Physiol. *38* (1955) 441), contaning a low level of phosphorus supplemented with hot H_3PO_4, for more than several generations, washed to eliminate extra-cellular material, and stocked at $-20°C$ in H-medium suspensions without glucose but with a 12.5% glycerine supplement.

The following points suggest that induction of mutations occurred in consequence of decay events in addition to those induced by β-irradiation.

a) To observe mutations, labelled bacteria were washed and stocked for different periods. Mutation inductions in such a stock were compared with a non-labelled control stock in which the bacteria were added only to obtain radioactivity for example, approximately 300 μCi per ml which is similar to that of the previous suspension of labelled cells. Higher rate of mutation induction was observed in the course of stocking the labelled cells.

b) When bacteria in stock were thawed and plated on minimal agar plates with and without 2.5% supplementation of Difco broth, proportions of mutants observed on these two kinds of media were different for β-ray exposed cells and for ^{32}P-incorporated cells. Labelled cultures gave usually over five times more mutants on broth-supplemented plates than on minimal agar. Cultures exposed to β-irradiation gave only twice as many mutants with supplementation as those without it.

Many revertants were isolated and purified to study their genetic nature. Approximately 70% of mutants derived from a ^{32}P labelled population could support the growth of the T4 mutant possessing an ochre nonsense codon. No mutant permitted the growth of amber mutants alone. This indicates that ochre suppressor mutations are predominant as Bridges, B. A. (Harwell) showed for mutations induced by ultraviolet light or X-ray.

When efficiencies of decay events were calculated as to their lethal as well as mutagenic effects it was found that the killing efficiency decreased with increasing rate of labelling. On the other hand, the efficiency of mutation induction was constant throughout the experiments when cells were labelled with different rates. The higher was the level of labelling, the more frequently were the reversions observed.

Mechanisms of Radio-Sensitization with Iodine Compounds

Tsuneo KADA and Mitsuo NAMIKI*

Bridges, B. A. (Nature *188* (1960) 415) was the first to show that lethal effects of irradiations are enhanced in the presence of certain chemicals. Since then compounds containing iodine atoms such as iodoacetic acid (Dean, C., and Alexander, P.; Nature *196* (1962) 1324), potassium iodide (Namiki, M., Okazawa, Y., and Matsuyama, A.; Agr. Biol. Chem. *25* (1961) 108) or potassium iodate (Kada, T.; Int. J. Rad. Biol. *15* (1969) 271) have been ascertained to be especially effective. In order to find if the sensitization involves enhancement of radiation damages in deoxyribonucleic acid, our studies have been carried out in the transformation system of *Bacillus subtilis*.

Cells of *B. subtilis* 168 T^- can be inactivated to the 50% survival level by 15 kR of gamma-rays of ^{137}Cs. With this dose of irradiation the survival fractions were reduced to 1% in the presence of iodoacetic acid (0.3 mM), potassium iodide (2.5 mM) or potassium iodate (1.0 mM) in cellular suspensions in phosphate buffer solution (pH 7.0) prepared for irradiation. Detailed studies were carried out with potassium iodide. Deoxyribonucleic acid was extracted by phenol method from irradiated cells and the transforming capacity was measured as to the arginine marker. No remarkable difference in genetic capacity of DNA was observed whether the bacteria were irradiated under nonsensitized or sensitized conditions. Observations with alkali sucrose gradient technics showed that the number of single strand breaks of DNA of irradiated cells was not enchanced by addition of the sensitizer during irradiation. When transforming DNA was irradiated *in vitro*, inactivation of the tryptophane marker was protected very much by addition of potassium iodide. Protective effect of the iodine compound was also found in radiolysis of four DNA bases dissolved in water. These observations suggest that radiation induced damages in DNA molecules were not enhanced by the presence of the sensitizer, whether DNA was irradiated *in vitro* or *in vivo*. Therefore it is supposed that the radio-sensitization may not be due to the enhancement of non-specific damages in cellular DNA. These studies, briefly reported here, had been initiated in the Institute of Physical and Chemical Research, Tokyo (Radiation Biology Lab.) and then continued in this institute.

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High Bacteriocidal Action of Iodine Compounds Irradiated in Acidic Solution

Tsuneo KADA

It is known that the cellular lethality induced by ionizing irradiation is very much increased by the presence of certain iodine compounds at non-toxic levels in the bacterial solution. Most previous studies have been carried out with neutral buffer solutions to which bacterial cells and chemical reagents, such as KI, KIO_3 or $I \cdot CH_2 \cdot COOH$, were added and altogether irradiated. Under these conditions, the presence of chemicals at the time of irradiation was required in order to observe the radiation sensitizing effect and the reagent solutions previously irradiated had little toxicity. These observations indicated that radio-chemical reaction between some cellular key substances and the reagent were terminted in a very short time under irradiation.

Chemical reagent (1 mM)	pH of reagent solution	Number of viable cells per ml
None	5.6	2.6×10^5
	7.1	3.0×10^5
KI	5.6	0
	7.1	2.4×10^5
KIO_3	5.6	0
	7.1	1.2×10^5
$I \cdot CH_2 \cdot COOH$	5.6	0
	7.1	1.2×10^5

We have observed that iodine compounds irradiated in acidic solutions were highly toxic. As Table indicates, bacteria cells were completely inactivated at 0°C by 1 mM reagent solutions previously irradiated with 10 kR of gamma-rays at pH 5.6. On the other hand no lethal action was found at pH 7.6. Additional experiments have shown that the radiation-produced toxicity disappeared by heating to 37°C.

It was necessary to elucidate if similar mechanisms are actually involved in the radiation-sensitization phenomena where cells are usually irradiated in the presence of chemicals in neutral solutions. Studies are being carried out on genetic effects of radiation products of certain stability as found above.

Action Spectrum of Photoreactivating Light in UV Irradiated Maize Pollen

Etsuo AMANO

To study the mechanism of photoreactivation in maize pollen, kind of mutation, and wave length dependence of UV induced mutation had been investigated (Ikenaga *et al.* 1966, Amano *et al.* 1966 and Amano 1967, the two last in the Ann. Rep. N.I.G. of 1966 and 1967). Both fractional and entire endosperm mutations and both single and multiple loci mutations of linked marker genes were induced by 250 mμ UV light and photoreactivated by post-irradiation with visible light.

The present report deals with the wave length dependence of photoreactivation of UV irradiated maize pollen. The materials used were the same genetic stocks as used in the study on wave length dependence of UV damages. Pollen of the multiple dominant stock, C^I *Sh Bz Wx*, was first irradiated in a single layer with 6×10^3 erg/mm^2 of UV from four 4 W germicidal lamps at the intensity of 100 erg/mm^2·sec. Then followed irradiation by monochromatic output of a UV monochrometer (Bausch and Lomb). A 500 W super high pressure mercury lamp (Philips, SP-500) was used as a light source. PR light intensity as measured by a thermopile (Eppley) varied from 330 erg/mm^2·sec at 334 mμ to 753 erg/mm^2·sec at 365 mμ. As a standard PR treatment, some of the pollen grains were exposed to the PR light as in the previous experiments, i.e. 30 minutes, 6,000 lux of visible light from a high pressure mercury fluorescent lamp. Variously treated pollen was used to pollinate an appropriate multiple recessive stock. Except during the treatment, pollen was handled in a dark room illuminated by a yellow sodium lamp. Pollinated ears were wrapped in alminum foil to prevent unnecessary photoreactivation.

After harvest and drying, kernels showing mutations of any of the linked marker genes (C^I *Sh Bz Wx*) on more than 1/8 of the endosperm surface were classified according to the expression pattern, area affected and number of genes involved. Of the four near UV wave lengths tested, none showed and ability to induce mutation in my experiment of 1967. At the comparable PR light dose (3×10^5 erg/mm^2) photoreactivation was not significant at 365 mμ and 405 mμ. More effective photoreactivations were observed at 334 mμ and 436 mμ, the latter being the most effective.

Present results show the same spectrum for total mutant frequency, single and multiple loci mutations, and entire and fractional mutations.

Wave Length Effect on the Photoreactivation of UV-Induced Mutations in Maize

Taro FUJII

Photoreactivation of mutation induced by UV in maize pollen has been recently observed (Ikenaga & Mabuchi 1966, Rad. Bot. 6: 165, Matsumura & Mabuchi 1966, Seiken Ziho 18: 1, Fujii 1968, Ann. Rep. 18: 102, Amano p. 75 in this issue). In order to check whether the PR of UV induced mutation in maize pollen is of the direct or indirect type, the kinetics of the PR involved was compared at 3341 and 4047Å. The method used in this experiment is basically the same as that reported in the previous paper. Pollen grains of a dominant line were exposed to 8000 erg/mm^2 of UV-light from a germicidal lamp emitting primarily 2537Å. Immediately after the UV irradiation, pollen grains were divided into four parts; the first and the second parts were exposed to 3341Å or 4047Å light obtained from a Bausch-&-Lomb Monochrometor. The third part was exposed for 30 minutes to visible light, emitting light from 4000 to 7000Å, and the last part was held for 30 minutes in the dark. After these post-

Table 1. Decreasing UV-induced whole and partial mutations after photoreactivation with luminescent-lamp light, 3341 or 4047 Å.

UV-treatment (erg/mm^2)	PR-treatment (erg/mm^2)	Number of seeds	Mutation frequency (%±S.E.)		
			Whole	Partial	Total
—	—	7964	1 (0.01±0.01)		1 (0.01±0.01)
8000	—	4126	34 (0.82±0.14)	103 (2.50±0.24)	137 (3.32±0.28)
〃	Visible* 6.5×10^4	2303	9 (0.39±0.13)	27 (1.17±0.22)	36 (1.56±0.26)
〃	3341Å 3×10^4	2324	15 (0.65±0.17)	46 (1.98±0.29)	61 (2.60±0.33)
〃	〃 1×10^5	2753	23 (0.84±0.17)	68 (2.47±0.30)	91 (3.31±0.34)
〃	〃 3×10^5	1680	7 (0.42±0.16)	36 (2.14±0.35)	43 (2.56±0.39)
〃	4047Å 3×10^4	4014	20 (0.50±0.11)	103 (2.57±0.25)	123 (3.06±0.27)
〃	〃 1×10^5	2707	13 (0.48±0.13)	56 (2.07±0.27)	69 (2.55±0.30)
〃	〃 3×10^5	3016	9 (0.30±0.10)	48 (1.59±0.23)	57 (1.89±0.25)

* Luminescent lamp light.

treatments, the pollen grains were dusted on the silks of the recessive female stock in a dark room. The experimental results are summarized in Table 1. Two types of mutations, whole and partial, occurred and the frequency of the latter was higher than of the former. As may be seen from the table, frequencies of whole and partial mutations at 8000 erg/mm^2 UV-exposure with dark treatment amounted to 0.82 and 2.50%, respectively, and both kinds decreased by about 50% by visible light treatments. In the region of monochromatic light doses studied, it seemed that 4047Å treatment reactivated the mutagenic lesion more steadily with increasing doses than 3341Å treatment. Further studies are required to ascertain this point.

Relative Biological Effectiveness of 14 MeV Neutrons on Mutation Frequency in Maize

Taro FUJII

The frequency of gamma-ray and 14 MeV neutron induced endosperm mutations (*Bz*-gene) was already reported by the present author (cf. Ann.

Table 1. Mutated seedlings after pollen irradiation.

Treatment (rad)	Number of seedlings (germination rate)	Number of mutants (%)
0	1472 (97.7)	4 (0.27)
Gamma-rays		
290	2601 (97.0)	8 (0.31)
580	1660 (96.1)	13 (0.78)
870	1370 (86.5)	19 (1.39)
1450	2749 (79.9)	76 (2.76)
Neutrons		
69	364 (87.5)	4 (1.10)
127	2454 (89.8)	25 (1.02)
154	4277 (92.1)	50 (1.17)
284	5894 (83.6)	115 (1.95)
295	4397 (82.3)	101 (2.30)
545	6160 (82.7)	157 (2.55)
2034	339 (10.9)	40 (11.80)
3700	9 (0.8)	5 (55.56)
10735	0 (0.0)	

Rep. 18: 103-105). In addition to *Bz*-gene, the materials used in that experiment had the dominant and the recessive Yg_2 and yg_2 genes (yellowish green character) in male and female stocks, respectively. Mutation from Yg_2 to yg_2 was examined in F_1 seedlings and the results are given in Table 1. In 1450 rad of gamma-ray treatment, germinability was only slightly decreased, however a drastic effect on germinability was observed in the neutron treatments beyond 545 rad.

It is obvious that the mutation frequency after neutron irradiation is very much higher than that after gamma-ray irradiation: by gamma-ray irradiation 1.4% mutation was induced at 870 rad, while it could be induced at less than 284 rad by neutron irradiation. From these results considering the preceding results (endosperm mutation), the RBE value of 14 MeV neutrons after pollen irradiation was roughly assessed to be about 5. Thus, a relatively low RBE value was observed in this material, since it was pollen, being as such haploid in nature in contrast to the other materials which were diploid in nature somatic cells. Although the environmental variation is very small in high LET radiation, the low RBE value of neutrons was also observed in the killing effect on presoaked *Arabidopsis* seeds (cf. Ann. Rep. 18: 107-108). Ploidy or water content thus seems to be influence of RBE value.

Fractionation Experiments with Gamma-Rays and Neutrons in Maize

Taro FUJII

To investigate the fractionation effect of radiation treatments, 1400 rad of gamma-rays and 600 rad of 14 MeV neutrons, both adequate doses for mutation experiments with maize pollen, were delivered as a single fraction or in two fractions with 30, 60 and 120 minute intervals. Irradiated by gamma-rays and neutrons maize pollen with *Bz*-gene was crossed to the recessive stock. Mutation from *Bz* to *bz* could be observed in the F_1 seeds with bronzy aleurone color and the results are given in Table 1. In the gamma-ray lot, mutation frequency at single dose of 1400 rad was 2.25%, and frequency at two fractions each of 700 rad with 30 minute interval was almost the same as that of a single dose. However, decrease of mutation frequency was observed with the increase of interval to 60 and 120 minutes as the table shows. In the neutron lot, on the other hand, mutation frequencies did not change within 30 to 120 minute interval with 300 rad as first and second dose; frequencies in these lots are almost the same as that after a single dose of 600 rad. The results suggest that some of premutational damage induced by gamma-

Table 1. Relation between mutation frequency and fractionation of gamma-rays and neutrons.

Treatment			Number of seeds	Mutation frequency (%±S.E.)
First dose (rad)	Interval (min)	Second dose (rad)		
—	—	—	10682	0
Gamma-rays				
1400	—	—	8574	2.25±0.16
700	30	700	7557	2.30±0.17
700	60	700	7341	1.99±0.16
700	120	700	15858	1.62±0.10
Neutrons				
600	—	—	11086	3.34±0.17
300	30	300	9285	3.01±0.18
300	60	300	11056	3.13±0.17
300	120	300	15340	3.04±0.14

rays could be repaired during the interval, and recovery was enhanced when the interval was extended. But such recovery did not occur when neutron exposure was fractionated with up to 120 minute interval.

IX. MICROBIAL GENETICS

Determination of the Length of Flagella in *Salmonella*

Tetsuo IINO

On *Salmonella* cells grown for 2 to 3 hr in a medium containing p-fluorophenylalanine, the percentage of flagella with different numbers of newly-formed curly waves was calculated for each group which had different numbers of pre-existing normal waves. All distributions fitted a Poisson distribution. The average numbers of curly waves per flagellum decreased with the increase in normal wave number and reached zero for 5 to 6 normal waves. The lines plotted at different incubation times were not parallel but convergent. The lower was the number of normal waves in a flagellum the smaller was its whole length. The decrease in curly wave number in the flagella with greater normal wave number is attributed to the decline of growth rate with the increase in length. When flagella were shortened by mechanical shaking of bacteria before p-fluorophenylalanine treatment, the growth rate of flagella was not decreased as compared with that of intact ones of similar length. Thus the decline of the growth rate is explained by a decrease in transportation efficiency of flagellin molecules with increasing length rather than by ageing of the flagellum forming apparatus.

The length of flagella grown on bacterial bodies has an upper limit. In *Salmonella*, the greatest number of waves seen on the wild type strain was between 5 and 6 normal waves in ordinary culturing conditions. This maximum corresponds to that at which the growth rate of flagella reached zero in the present experiment. It is likely that the mechanism controlling the decline in growth rate of flagella also plays an important role in determining flagellar length in growing bacteria. (The detail submitted to J. gen. Microbiol.)

Polarity of Flagellar Growth in *Salmonella*

Tetsuo IINO

The phenomenon whereby *Salmonella* cells produce curly flagella in media containing *p*-fluorophenylalanine was used in an investigation of the polarity of flagellar growth. *S. typhimurium* LT2 in logarithmic growth in broth was transferred to minimal medium or saline. Then, after 2 to 3 hr at 37 C in a medium containing p-fluorophenylalanine the

distributions of number, length and shape of their flagella were observed. Under the condition where newly formed flagella were all curly, curly waves appeared at the distal portions of flagella, indicating that a flagellum grows at the end distal from the bacterial body. This agrees with the polarity of flagellar growth *in vitro*.

A normal flagellar strain and a curly mutant strain of *S. abortus-equi* were grown together in broth. Numbers of both types of cells and flagella increased to 1.5 fold in 3 hr. At this stage, neither heteromorphous cells nor single flagella having both normal and curly waves were detected. Hence, flagellin molecules reach the top of a growing flagellum without being excreted into the culture medium; the remaining pathway by which flagellin molecules can reach the tip may be the tubular hole of the flagellum. (The detail submitted to J. gen. Microbiol.)

A Method for Concentrating Paralyzed Mutants from Non-Flagellated Cells of *Salmonella*

Masatoshi ENOMOTO

A method was invented for concentrating flagella-paralyzed (*mot*$^-$) cells occurring in a large number of non-flagellated cells of *Salmonella*. The *mot*$^-$ cells expressing the phase-1 flagellar antigen *i* were agglutinated by anti-*i* serum with carriers, the dead cells of the wild-type TM2 expressing the phase-1 antigen *i*; only the agglutinated cells were harvested by low speed centrifugation and incubated in broth. About 10 fold concentration was achieved per one tretment which was repeated for further concentration. By this method, the *motA*$^-$ *motC*$^-$ double mutant which was produced by transduction at a frequency of about 3×10^{-6} per a non-flagellated recipient cell (*motA*$^-$ *ah1*$^-$) was isolated from the broth culture concentrated five times, in which about 30% of the cells were the double mutant. (The detail was submitted to Genetical Research, Vol. 14, 1969).

Further Studies on Different Frequencies of Cotransduction in *Salmonella*

Masatoshi ENOMOTO and Shigeru YAMAGUCHI

The frequency of cotransduction of *motC* (motility) and *H1* (phase-1 flagellin) gene in *Salmonella* was investigated, using four *Salmonella* species (*S. dublin*, *S. abortus-equi*, *S. abony*, and *S. typhimurium*) and many serotypic recombinants as donors and *S. typhimurium motC*

mutant as recipient. The frequency varied with the four species from 7 to 52%. By comparing these frequencies with those obtained from serotypic recombinants, it was suggested that the difference in frequency arose not only from differences in genetic homology between the chromosome of the recipient and the fragment from the donor, but also from differences in genetic composition of the chromosome fragments carried by the phage. The frequency of serotypic recombinants selected for *mot*$^+$ and *H1* gene of the donor was generally higher than with recombinants selected for *flaK*$^+$ and *H1*. The difference in genetic homology between *S. typhimurium* and other species was more marked in the region between *H1* and *flaK* than between *motC* and *H1*. (The detail was submitted to Genetical Research vol. 14, 1969).

Stability and Phenotypic Expression of Mutation to Arginine Sensitivity in *Salmonella*

Jun-ichi ISHIDSU

To elucidate the mechanisms of phenotypic expression of *Salmonella* mutants which show sensitivity both to arginine and to uracil, the pattern of back mutation and the characters of the back mutants were examined under various culture conditions.

Arg-s-3 was first grown in liquid minimal medium supplemented with arginine and uracil. After washing, cells were transferred into the following media: 1) minimal, 2) minimal plus arginine, 3) minimal plus uracil, or 4) minimal plus arginine and uracil. Incubation was continued for one to three hours, then after washing, samples were plated on minimal agar plates containing arginine or uracil. Number of colonies which appeared after incubation was largest in the case of 1), where both arginine and uracil biosyntheses had been derepressed beforehand, and was smallest in the case of 4), where the two pathways had been simultaneously repressed. When one of the two pathways had been repressed (in the case of 2 or 3), the number was intermediate.

Such "revertant" colonies appearing on minimal medium supplemented with either arginine or uracil were picked up and their characters were further examined. In either case, only less than 10 per cent were true revertants, losing sensitivity to both chemicals simultaneously. Many of such "revertants" had lost the sensitivity to only one of the two chemicals or had gained auxotrophic requirement for uracil. Unexpectedly, most of the colonies that appeared on arginine-supplemented minimal agar plates showed the original, unchanged property on further tests,

namely they still carried the sensitivity to both chemicals.

These phenomena altogether suggest that the mechanisms of phenotypic expression of this mutation is very complicated and greatly affected even by slight changes in culture conditions. More detailed tests are being carried out and will be reported elsewhere.

High Frequency Mutations, Drug Sensitivity and λ-Lysogenization in *Escherichia coli* K12

Tsuneo KADA

Previous studies of high frequency reversions in a threonineless strain of *Escherichia coli* K 12 *Hfr H* have shown that revertants due to a plural suppression had increased sensitivities to irradiations and the majority of cells formed filaments during post-irradiation culture. Observations of the growth of T 4 nonsense mutants on typical bacterial strains indicated that two suppressor genes involved, one (*mod*) locating in the *leu* region and the other (*fgr*) near *gal*, were neither of amber nor of ochre type. It was especially noted that mutations $fgr^- \rightarrow fgr^+$ were very frequent (more than 10^{-3}) in mod^+ strains.

In certain streptomycin-resistant and poly-auxotrophic F^- strains having mod^- and fgr^- genes, approximately 30% of lysogenized strains with phage λ became sensitive to the antibiotic. In these strains, reversion rates increased considerably simultaneously at many loci. Many kanamycin-resistant and neomycin-resistant strains were isolated from a typical above mentioned F^- strain and lysogenized with phage λ. Approximately 10% of lysogenic strains became sensitive to kanamycin. Reversion-rates also increased drastically at many loci in those strains whose sensitivities to antibiotics were modified. Phenotype of revertants was that of fgr^+.

Above observations were interpreted as follow: Phage λ might have transduced specifically certain suppressor gene into the lysogenic strains, and the rate of mutation $fgr^- \rightarrow fgr^+$ increased very much in its presence as was the case of mod^+ strains of *Hfr H*. Functions of these suppressor genes may concern the ribsomes.

X. HUMAN GENETICS

Delayed Fertilization and Down's Syndrome

Ei MATSUNAGA and Takeo MARUYAMA

German proposed that decreasing frequency of coitus with advancing age which predisposes to delayed fertilization could account for the correlation of increased incidence of Down's syndrome with advancing maternal age. This hypothesis is especially interesting because it points out a possible biological consequence of human sexual behaviour.

In order to test the hypothesis, we first analyzed the original data by Kinsey *et al.* on the frequencies of marital coitus for females of different age groups and found that their variation among individual couples for a given age group can be approximated by a Poisson distribution. Although this finding does not necessarily imply that coitions themselves follow a Poisson process within individual couples, we could hypothesize an "average" couple whose coitions follow a Poisson process with the mean frequency of that group, the resulting variability within this couple in the interval between coitions representing the corresponding variability from couple to couple belonging to the group. It is further assumed that ovulation occurs independently of the occurrence of coitus, so that the interval between an ovulation and the following or the preceding coitus, t, also follows a Poisson process, its probability density being expressed by

$$p(t)=\lambda e^{-\lambda t}$$

where λ is the inverse of the mean interval between coitions for the group concerned.

The above assumptions led us to construct the following formula which expresses the expected frequency of non-disjunction for a given age group:

$$F(\lambda)=\frac{\left\{1-\int_0^{\alpha}\lambda e^{-\lambda t}f_1(t)dt\right\}\int_0^{\beta}\lambda e^{-\lambda t}f_2(t)g(t)dt}{\int_0^{\alpha}\lambda e^{-\lambda t}f_1(t)dt+\int_0^{\beta}\lambda e^{-\lambda t}f_2(t)dt-\int_0^{\alpha}\lambda e^{-\lambda t}f_1(t)dt\int_0^{\beta}\lambda e^{-\lambda t}f_2(t)dt}$$

where α and β are the maximum life spans of the sperm and the secondary oocytes in the female reproductive tube respectively, $f_1(t)$ and $f_2(t)$ are respectively the probabilities that the male and female gametes remain fertilizable, and $g(t)$ the frequency of non-disjunction. The values of α and β may be taken, according to German, as 48 h and 24 h respec-

tively. Assuming that both $f_1(t)$ and $f_2(t)$ decrease linearly while $g(t)$ increases either linearly or exponentially with t, various values of λ calculated from the data of Kinsey *et al.* were applied to the above formula. It was shown that the expected frequency of non-disjunction with advancing age was rather slow; the frequency in the oldest group was less than twice as high as that in the youngest group, indicating that German's hypothesis cannot account for the well known steep rise in the incidence of Down's syndrome in older mothers. The details of this work were published in *Nature* 221: 642-644, 1969.

It may be worth while noting that although we have made several different models for $f_1(t)$ and $f_2(t)$ other than those given above, the final results were essentially the same. For example, if we assume that both $f_1(t)$ and $f_2(t)$ decrease exponentially while $g(t)$ increases in similar fashion, i.e.,

$$f_1(t)=\frac{e^{\alpha}-e^{t}}{e^{\alpha}-1},\ f_2(t)=\frac{e^{\beta}-e^{t}}{e^{\beta}-1}\ \text{and}\ g(t)=\frac{e^{t}-1}{e^{\beta}-1}k$$

Table 1. Frequency of non-disjunction for different age groups, as expected from delayed fertilization; the maximum life spans of sperm and oocytes are assumed to be 48 h and 24 h, respectively, their capacity for fertilization decreasing exponentially while the frequency of non-disjunction increases exponentially

Kinseys' data on marital coitus of females		Mean interval (in hours)	λ	Expected frequencies of non-disjunction
Age group	Mean frequency per week			
				$k\times10^{-2}$
16—20	3.7	45.4	0.022	0.304
21—25	3.0	56.0	0.018	0.360
26—30	2.6	64.6	0.016	0.391
31—35	2.3	73.0	0.014	0.424
36—40	2.0	84.0	0.012	0.459
41—45	1.7	98.8	0.010	0.496
46—50	1.4	120.0	0.008	0.535
51—55	1.2	140.0	0.007	0.556
56—60	0.8	210.0	0.005	0.598
			0.000	0.714

where k is a constant, we obtain

$$F(\lambda)=\frac{\lambda k}{(2-\lambda)\{e^{\lambda(\alpha+\beta)}(1-\lambda)-1\}}$$

and its limiting value as λ approaches to zero will be

$$F(\lambda\to 0)=\frac{k}{2(\alpha+\beta-2)}\,.$$

Table 1 summarizes the results of computation of $F(\lambda)$ according to $\alpha=$ 48 h, $\beta=24$ h and different values of λ based on the data by Kinsey *et al.*

Maternal Age of Chromosome Translocations with Down's Syndrome

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

It is well established that the condition of the majority of patients with Down's syndrome is due to trisomy for chromosome no. 21, and that the maternal age at the birth of the patients is, on the average, higher than in the control population. On the other hand, cases with (DqGq) or (GqGq) translocations are less frequent, and information of maternal age for the translocations has so far been very scarce.

Thirty one cases of Down's syndrome with such translocations have been collected from our consecutive survey. As shown in Table 1, mean maternal ages for 22 sporadic, 7 maternally inherited and 2 paternally inherited translocations were 27.8 years, 29.6 years and 25.5 years, respectively.

Table 1. Mean maternal ages for the translocation types

Translocation type	Number of cases examined	Mean maternal age
Sporadic	22	27.8±3.83
Inherited from mother	7	29.6±3.25
Inherited from father	2	25.5±2.50

The maternal age distribution for 22 sporadic cases is given in Table 2. The expected number for each maternal age group, classified by five-

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

Table 2. Maternal age distribution of cases with sporadic translocation

Maternal age (years)	Observed	Expected
15—19	0	0.26
20—24	4	6.06
25—29	9	10.05
30—34	7	4.30
35—39	2	1.11
40～	0	0.22
Total	22	22.00
Mean age	27.82	27.14
S.D.	3.83	4.61

year's interval, was constructed from vital statistics data and was corrected for the year of birth of the patients. According to this procedure, however, the 6 cases born in the years 1966-1968 were included in the group born in 1965. Using the median value of each quinquennium, the mean maternal age was estimated to be 27.1 years for the general population, which is almost the same as the mean age (27.8 years) for the mothers of the patients. There was also no difference in the distribution of the maternal age between the observed and the general population.

The above results may suggest that the occurrence of sporadic translocation Down's syndrome is independent of maternal age at the patient's birth. It is, however, necessary to separate cases of (DqGq) translocation from those of (GqGq), so that more data are needed in order to arrive at a definite conclusion.

Clinical Conditions of Patients with Apparently Normal Chromosomes, V.

Hidetsune OISHI, Yasumoto KIKUCHI, Masaru KUMAGAI and Kunihiko SHIBATA

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

	Name	*Age*	*Legal sex*	*Clinical conditions*
a) Multiple deformations with mental retardation				
84	J.M.		M	Osteogenesis imperfecta; high-arched palate; malformed ears; transverse palmar creases
85	M.Y.	1 month	F	Hypertelorism; antimongoloid slant eyes; malformed and low-set ears
86	M.S.	2 months	F	Brachycephaly; hypertelorism; low-set ears; short neck; congenital heart disease
87	T.O.	2 months	F	Exophthalmos; epicanthus; low-set ears; high-arched palate; transverse palmar creases
88	H.T.	6 months	F	Cleft palate; congenital heart disease; mild coarctation of aorta; overlapped fingers
89	Y.O.	9 months	F	Microcephaly; hemangioma; galactosemia
90	Y.Y.	1 year	M	Antimongoloid slant eyes; low-set ears; micrognathia; transverse palmar creases; retentio testes
91	K.T.	1 year	M	Cleft palate; malformed ears; congenital heart disease; umbilical hernia
92	S.H.	9 years	M	Hypertelorism; antimongoloid slant eyes; large deformed ear lobes; low-set ears
93	M.W.	4 months	F	Microcephaly; lacunal skull; exophthalmos; overlapped toes; lipochondrodystrophy
94	K.K.	7 years	F	Antimongoloid slant eyes; coloboma of the iris; nystagmus; epicanthus
95	S.S.	4 months	M	Microphthalmia; incurved fifth finger with clinodactyly; transverse palmar creases
96	H.I.	4 years	F	Prominent occiput; strabismus; nystagmus; hypomyotonia; logopathy
b) Sex anomalies				
97	Y.K.	7 months	F	Female pseudohermaphroditism
98	K.T.	25 years	F	Male pseudohermaphroditism
99	T.K.	30 years	M	Slender body; short neck; shield chest; gynecomastia; small penis; atrophic testes (Klinefelter's syndrome ?)
100	M.S.	4 days	F	Webbed neck; excessive hair-growth (Turner's syndrome ?)
101	S.A.	3 years	F	Female pseudohermaphroditism; high-arched palate; excessive hair-growth

102	I.M.	5 months	F	Female pseudohermaphroditism

c) Hereditary disease

103	—.S.	19 days	F	Dysostosis cleidocranialis
104	K.K.	1 year	F	Dysostosis cleidocranialis; hypertelorism; webbed neck; saddle nose

Cytogenetical Studies of Human Ova, I. A Preliminary Note on the Oocytes Liberated from the Ovary

Hidetsune OISHI and Yasumoto KIKUCHI

In order to investigate the meiotic chromosomes of human ova, ovarian oocytes were collected in collaboration with some university hospitals. Six oocytes were obtained from 5 ovaries by puncturing large follicles during gynecological operations of the patients for various clinical conditions. In other instances when whole ovaries were removed in the operation, comparatively large follicles were dissected under a dissecting microscope, and 53 oocytes were collected from 7 ovaries. These oocytes were surrounded by several cell layers of corona radiata, and were similar to those obtained by puncture. However, 4 of these collected from ovariotomy showed signs of degeneration; they were partially devoid of corona cells, and the vitellus was deformed though the shape of zona pellucida was spherical. An ovum with blastomeres was also found in 4-cell stage; it was possibly formed by parthenogenetic cleavage.

Thirty oocytes were used for the examination of meiosis. In order to remove the corona cells, the oocytes were treated with hyaluronidase in hypotonic solution. Air-dried preparations were made with a modification of Tarkowski's method (Tarkowski, A. K. 1966. Cytogenetics 5:394) and stained with carbol fuchsin. All had a germinal vesicle in dictyotene stage.

In vitro cultivation of the ova is under examination.

Studies on Several Genetic Hematological Traits of the Japanese

Tomotaka SHINODA

Polymorphic genetic systems recognized in man have revealed striking differences from one population to another, not only in specific gene frequencies but also in the presence or absence of particular genes.

Since 1963, I have analyved several polymorphic hematological traits

of the Japanese. The results were partly already reported.

Acid phosphatase in red cells determined by three autosomal allelic genes, namely P^a, P^b and P^c, occurs in six types. However, occurence of phenotypes associated with the P^c gene, such as AC, BC and C, seems to be very rare in the Japanese population. With regard to this finding analyses of a total of 612 samples collected from unrelated persons have been carried out.

Of 612 samples examined 26 (4.2%) of type A, 192 (31.4%) of type AB and 394 (64.4%) of type B were identified. Accordingly, the frequencies of the genes P^a and P^b may be estimated as 0.199 ± 0.011 and 0.801 ± 0.011, respectively. No single case of types AC or BC was found. A complete lack (or an extreme rarity) of the gene P^c seems to be a characteristic feature of the Japanese population. The weighted mean enzyme activity attributable to each of the two alleles was estimated approximately as for P^a 56.2 units and for P^b 91 units, the ratio being close to 2 : 3.

Phosphoglucomutase (*PGM*) occurs in multiple forms and a number of inherited variants have been described. They appear to be controlled by alleles at three distinct and not closely linked autosomal loci, each of which determines a separate group of *PGM* isozymes.

A total of 932 blood samples collected from unrelated adults have so far been analyzed and the result is summarized in Table 1. From the

Table 1. Observed and Expected Numbers of PGM_1 Types among the Japanese

	Observed		Expected*	
	No.	%	No.	%
1	567	60.84	556.9	59.75
2—1	307	32.94	327.1	35.09
2	58	6.22	48.0	5.16
Total	932	100.00	932.0	100.00

* On the basis of Hardy-Weinberg law with allele frequencies: $PGM_1^1 = 0.773$ and $PGM_1^2 = 0.227$.

data of Table 1, the frequencies of the genes PGM_1^1 and PGM_1^2 may be estimated as 0.773 and 0.227, respectively. The above value of PGM^1 appeared to be somewhat higher than that of British whites, but the difference was not significant. No variation was found in the PGM_2 isozyme region. Although variation was observed in the PGM_3 isozyme region, the data were not sufficient for the estimation of gene frequency.

The haptoglobin, a serum protein which binds with hemoglobin in vivo and in vitro occurs in three common types, namely 1-1, 2-1 and 2-2, which are determined by a pair of allelic genes Hp^1 and Hp^2. A total of 629 samples collected from unrelated adults were tested by starch gel electrophoresis. From the data summarized in Table 2, the frequencies of the

Table 2. Observed and Expected Numbers of Haptoglobin Types Assuming a Hardy-Weinberg Equilibrium with Gene Frequencies $Hp^1=0.242$ and $Hp^2=0.758$ among the Japanese

Hp type	Observed		Expected	
	No.	%	No.	%
1—1	42	6.68	36.8	5.86
2—1	220	35.14	230.8	36.67
2—2	367	58.18	361.4	57.47
Total	629	100.00	629.0	100.00

genes Hp^1 and Hp^2 were estimated to be 0.242 and 0.758, respectively. The frequency of the Hp^1 gene observed for the Japanese is somewhat higher than that of the Indian population, but is significantly lower than those of American whites and Negroes. No incidence of a modified type, such as *Hp* 2-1 (Johnson) often reported for the Negroes was found in this survey.

Polymorphism of Prealbumin Fraction in Human Serum

Yoshito OGAWA and Norio ODAKI

When human serum is subjected to zone electrophoresis in starch gel with a sodium acetate-ethylenediaminetetraacetic acid buffer of pH 4.95, several bands migrate in front of the albumin fraction. These rapidly migrating fractions were named collectively "prealbumin" and five different phenotypes were reported in Norway. According to the genetic theory of Fagerhol *et al.* (1964), three codominant alleles, designated by symbols Pr^F Pr^M and Pr^S, are responsible for the observed phenotypes.

The present writer found antitrypsin activity in some fractions of these rapidly migrating region in starch gel and confirmed the identity of these active fractions with α_1-antitrypsin in the serum by immunochemical examination. Furtheremore, six different phenotypes were recognized in Japan. These results agree with the finding of α_1-antitrypsin variation found in Sweden by Eriksson *et al.* in 1963.

This finding shows that α_1-antitrypsin fractions of human serum often migrate into the so-called prealbumin fraction when starch gel electrophoresis is applied.

Allo-albumin F Mishima, a Variant of Human Serum Albumin

Yoshito OGAWA and Norio ODAKI

A second case of heritable allo-albumin F (a fast type of bis-albumin), an electrophoretic rapidly migrating variant of human serum albumin, was found among thousand and fifty nine sera chosen at random from specimens available in the departments of clinical pathology of the Mishima hospitals.

Cellulose acetate (Separax) electrophoretic technique was performed at 0.8 mA/cm for 33 minutes at 28°C in 0.06 M barbiton buffer of pH 8.6. The abnormal (fast) band accounted for approximately half of the total albumin fraction when densitometric scanning of cellulose acetate strip stained by Ponceau 3R was applied.

The propositus was a 48 yr old man treated in the hospital after accidental injury. He was otherwise healthy and so were the other members of his family. From the results of the family study, this albumin variant seems to be inherited as a codominant autosomal allele.

The allo-albumin F Mishima, might be identical with one of the rapidly migrating albumins demonstrated by Weitkamp *et al.*, 1967, who used bis-albuminaemic sera from nineteen unrelated families.

XI. APPLIED GENETICS

Cluster Distribution of Related Individuals in a Natural Forest of *Thujopsis Dolabrata*

Kan-Ichi SAKAI and Yasusada MIYAZAKI

Variability in peroxidase isozyme pattern was investigated in a natural forest of *Thujopsis dolabrata*. The forest consisted of forty five trees, sporadically mixed with broad-leaved trees. The trees were of different ages, the range being several hundred years from the youngest to the oldest. Leaves were sampled from each of the trees whose location had been mapped on a graph paper. The collected samples were examined electrophoretically according to the method developed by Smithies in 1965 and by Endo in 1968.

In the present species, more than fifty bands of peroxidase isozymes were superficially identified. On the basis of those isozyme bands, each tree was investigated for its resemblance to other trees in the forest. It was found from this comparison that trees growing in proximity were often very similar with each other in the isozyme pattern, whereas trees distant from each other were always very dissimilar. If we assume that environmental variation in the forest does not affect the isozyme pattern of the tree and that variation in the isozyme pattern is genotypic, we may interpret our finding as indicating that trees genetically related are likely to grow rather in clusters than to be randomly distributed in the forest. Without doubt, the trees which are vegetatively propagated from one tree should show approximately or exactly the same pattern of isozymes. A few of supposedly such cases have been noticed. Most of the trees we are interested in are, however, those which are more dissimilar in isozyme pattern than their vegetative sibs, but highly similar in comparison with other trees in the forest. The occurrence of trees more or less similar in isozymes growing in proximity may indicate that they are produced from seeds of single trees.

A Study on Oligogenic and Polygenic Mutations in the Flower Organs of Citrus

Kan-Ichi SAKAI, Teruo NISHIDA and Kihachiro OHBA

The present report deals with the results of an investigation of a short-styled mutation in a clone of Iyokan-orange, *Citrus iyokan*. Pro-

pagules from the clone were divided into two groups. One of them was steadily irradiated by gamma-rays for 184 days in 1965, while another was left non-irradiated as the control group. The dose rate applied was 50, 100 and 200 R per day. The treated and control plants were grown in pots under glass for two and a half years before they were investigated in the spring of 1968. The investigation consisted in measuring petal length, style length, stamen length and length of the peduncle with the aim to detect the effect of irradiation. Examined were five to ten flowers collected from each twig on each branch of irradiated and control trees.

It was found that in the irradiated plots, the flower organs were highly variable in length in comparison with the control. Of special interest was a distinctly bimodal distribution of style length in the irradiated trees. The average length of the mutated style was 2 to 3 millimeters, whereas that of the normal style was 9–10 mm. The frequency of mutant flowers was about 8 percent in the 100 R and 200 R treatments though the rate of spontaneous mutation in the control plot was only about 1%. This mutation is understood to involve a major gene responsible for style length. This gene, however, is found to influence pleiotropically also other flower organs, *i.e.* petals, stamens and peduncles. The pleiotropic effect was found to be shortening of petal, stamen and peduncle by 23%, 19% and 14% respectively in comparison with their normal size.

In addition to this major gene, mutated polygenes were found to participate in shortening the size of flower organs. Their effect was to reduce primarily style length and the length of other organs.

Use of Zymography for Clone Identification in Forest Trees

Yasusada MIYAZAKI and Kan-Ichi SAKAI

Some species of forest trees are propagated vegetatively, yielding superior cultivars for artificial plantation. The sugi-tree or *Cryptomeria japonica* is one of those forest trees and we have at present a number of cultivars, either of single clones or clone complexes consisting of several clones each. Since coniferous trees have apparently less variable genotypes concerning the vegetative characters than broad-leaved trees, it is often necessary to develop some techniques allowing us to discriminate easily and reliably one cultivar from others. This study deals with an electrophoretic variation in the peroxidase among the genotypes

of sugi-trees with the objective of establishing a zymographic technique for clone identification.

We have a famous clone-variety of *Cryptomeria japonica* named Kumotooshi which was originated from a single mother tree detected in the early beginning of this century by a late forester in Kyushu. This clone shows a rapid growth in the early stage after planting, due to which it meets with a favourable reception of forest people. The materials for the present study were "propagules" from this clone which are grown in various districts of Japan.

At first, needle-leaves were collected from different parts of a single tree or from three trees which were genuine propagules of a Kumotooshi tree in order to see to what extent isozyme patterns of a homologous organ, for instance needle-leaves, vary among different parts of a single individual or within a single clone. After confirming that the isozyme patterns is accurately the same within a tree or a clone, needle-leaves were collected from 51 trees which were more or less firmly believed to be derivatives from the Kumotooshi cultivar. They were grown in eleven localities widely distributed in Kyushu island and Japan proper. Sampling was made from May through October, 1968. Samples collected were stored in a freezer kept at $-20°C$ until they were examined electrophoretically. The electrophoretic technique adopted was that developed by Smithies in 1965 and by Endo in 1968.

The electrophoretic test of the 51 trees showed that 45 of them were genuine but nine were false. Thus, it is concluded that the electrophoretic variation of peroxidase is useful for determining if a given tree is a member of a given clone or not.

When Do Trees Start Competing in a Forest?

Shigesuke HAYASHI and Kan-Ichi SAKAI

Intraspecific competition in a seed propagated forest of *Cryptomeria japonica* is investigated on the basis of correlation of stem diameter at breast height between the adjoining trees: Positive and high correlation indicates none or a slight, if any, competition, whereas low positive, zero or negative correlation is indicative of the occurrence of competition. Based on this observation, it may be possible to answer the question "When do the trees start competition?", if the correlation of annual stem growth between adjoining trees is investigated in successive years. The method of investigation consists in mapping the location of each tree on a graph paper, on the one hand, and measuring the annual

growth of the stem diameter of individual trees, on the other. The measurement of annual growth was performed by measuring disks for yearly ring width in eight directions, from which the mean annual growth was estimated. The total number of investigated trees was a little less than 200, all of which being 50 years of age.

The correlation was calculated for total growth and annual growth of the radius and total and annual growth of the basal area. It was found from the result of computation of correlation coefficients that among the first six or seven year old seedlings no competition appears to occur, the correlation coefficient being some +0.60. Later, however, the trees start to compete against each other, with increasing intensity year after year until they are about 30 years old, after which the competition remains the same.

X-ray Induced Variation in the Quantitative Characters of Rice

Shin-ya IYAMA

Seeds of rice variety Norin No. 8 were irradiated by 2.5 kr, 5 kr, 10 kr and 20 kr of X-ray in 1959. Each treated population together with untreated control was maintained until the X_8 generation by one-parent-one-offspring method in order to avoid as much as possible a bias due to natural selection and sampling. At the X_8 generation, when most of the mutations induced were supposedly fixed, variation in the experimental populations was investigated on individual plant basis. Genetic variances of these populations in respect to the examined characters were estimated by subtracting environmental variance from their variances (Table 1).

Table 1. Observed variances and estimated genetic variances in some quantitative characters of rice in control and X-ray treated populations

Treatment	d.f.	Plant weight		Plant height		Panicle wt./plant	
		Variance	σ_g^2	Variance	σ_g^2	Variance	σ_g^2
Control	961	75.1484	10.8221	15.3563	5.5924	19.8094	2.9149
2.5 kr	614	81.1936	16.8673	17.0010	7.2371	20.6469	3.7524
5 kr	677	79.0052	14.6789	16.8181	7.0542	21.3889	4.4944
10 kr	1071	77.3715	13.0452	18.5513	8.7874	21.5177	4.6232
20 kr	792	87.6930	23.3667	24.7647	15.0008	24.0640	7.1695
Environmental variance	2576	64.3263		9.7639		16.8945	

Environmental variance was estimated from the variance within lines which were derived from 150 X_7 plants. The characters investigated were plant weight (g), plant height (cm), panicle number and panicle weight per plant (g). The results showed that 1) genetic variance was revealed even in the control population, 2) genetic variance was increased by X-ray irradiated except for panicle number and 3) induced genetic variance increased with increasing dose of irradiation. The regressions of induced genetic variance (Y) in the characters investigated on the doses of irradiation (X kr) were

Plant weight: $Y=0.4898X+12.0823$,
Plant height: $Y=0.4549X+5.3229$,
and Panicle weight per plant: $Y=0.1973X+3.1112$.

Though the difference between population means were not significant (Table 2), a comparison of the frequency table of control population with those of the treated ones suggested that the irradiation treatments were likely to induce mutations in the minus rather than in the plus direction.

Table 2. Means of some quantitative characters of rice in control and X-ray treated populations

Treatment	No. of plant	Plant weight	Plant height	Panicle number	Panicle weight per plant
Control	968	47.35 g	112.90 cm	12.35	23.40 g
2.5 kr	620	46.98	113.00	12.00	22.92
5 kr	683	45.88	112.50	11.64	22.77
10 kr	1077	46.69	112.60	11.84	22.66
20 kr	798	45.47	110.60	11.51	22.84

A Comparative Analysis of Productive Traits in Wild and Domesticated Japanese Quails

Takatada KAWAHARA and Akihiko MITA

Two hundred and sixty-eight wild quails captured at the foot of Mt. Fuji were successfully propagated in cages. F_1 hybrids between wild and domesticated strains and backcrosses to the parental strains have been bred for our experiment. The birds were kept in a heated battery brooder for the first 3 weeks after hatching and then were removed to an unheated one. After sexing at 5 weeks of age, the birds were transferred to individual or colony cages. They were given *ad libitum* a commercial feed and were kept under 24 hour light without dark period

conditions throughout the experimental period. Traits observed were body weight and external shank length at various developmental stages, age at sexual maturity, fertility, hatchability, viability, egg production rate and egg weight.

Results of the experiment are summarized as follows: The wild strain was consistently poorer in all traits than the domesticated line. Growth curves of body weight showed that the wild line was always lighter than the domestic one, the differences being 14.5% for females and 13.1% for males on the average of various stages. The growth pattern of the F_1 hybrids and backcrosses almost agreed with the logical curves on the assumption that gene effects on the growth curve are additive. The external shank length showed the same genetic nature, the genetic control of this character being clearer than for the body weight. There was a fairly large difference between the body size of wild birds when captured and the plateaued size of the wild line bred in the laboratory, the former being 97.6 g. for females and 92.6 g. for males and the latter 93.4 g. for females and 83.0 g. for males. The external shank length of captured birds was longer than that of the laboratory wild strain. Such differences may be due to the differences in ecological conditions. A remarkable difference was found in the age at sexual maturity. Average number of days to attain sexual maturity was 48.3 days for domesticated and 117.0 days for wild lines. In addition, the appearance of nonlayers over 20 weeks after hatching amounted to 0% for the domesticated strain, whereas it was 47% for the wild line. Other differences between wild and domesticated strains were: 51.7% in viability (up to 20 weeks after hatching), 20.9% in hatchability, 22.5% in fertility, 44.1% in egg production rate (up to one month after sexual maturity) and 0.6 g. in egg weight (at sexual maturity), the wild strain always showing poorer performance than the domestic one. Backcross tests between wild and domesticated strains showed a tendency to approach the performance of the recurrent parent in all traits investigated except fertility. These results suggested that there are distinct differences in genetic constitutions between wild and domesticated quails and such differences may be produced in the course of domestication, though the mechanism of domestication is not yet known.

Directional and Fluctuating Asymmetry of Ribs of Cervical Vertebrae in Fowls

Takatada KAWAHARA and Kan-Ichi SAKAI

It was demonstrated in the previous report (Sakai, K. I. and T. Kawahara, 1964, Ann. Rept. Nat. Inst. Genet. 15 : 52) that the bilateral asymmetry of ribs of the cervical vertebrae of the fowl, measured by the absolute difference between sinistral and dextral rib length, was significantly different among strains. This paper describes the results of a further study on this subject. The asymmetry was classified into two categories: directional and fluctuating. The directional asymmetry was measured by the difference in the length of ribs, that is, sinistral minus dextral. The fluctuating asymmetry, however, was defined as asymmetry due to chance fluctuation in the development of sinistral and dextral ribs and was measured by the bilateral asymmetry remaining after subtraction of the directional asymmetry. Let the degree of fluctuating asymmetry be a, then, $a=|$(sinistral−dextral)−average (sinistral−dextral)| on a single bird basis. Since a was found not to distribute normally, we have employed the transformation, $A=\log_{10}[(a+0.1)^2]$. The birds investigated were White Leghorn females of six inbred, one closed and 5 F_1 hybrid strains, each at 16 months of age. Measured were length of sinistral and dextral ribs of four sixth to ninth cervical vertebrae.

Results of this study are summarized as follows:

1) The directional asymmetry was found to be statistically significant for the vertebrae order, but not among strains. The sinistral rib was usually longer than the dextral one, the mean difference being 0.13 mm.
2) With regard to the fluctuating asymmetry, variation among strains was highly significant. The F_1 hybrids were less asymmetric regarding fluctuating asymmetry than their parental inbreds. If we assume that the fluctuating asymmetry in rib length of cervical vertebrae is a result of developmental instability in the bird, it may be reasonable to expect that heterozygosity might reduce the instability of the fluctuating asymmetry as was found in this study.
3) Directional and fluctuating asymmetries were positively correlated with each other ($r=0.472$). In other words, those vertebrae which showed higher directional asymmetry had simultaneously high fluctuating asymmetry. Both asymmetries, however, were not significantly correlated with rib length. Correlation between body weight and rib length was estimated to be 0.789, whereas body weight was not correlated with either directional or fluctuating asymmetry.

Developmental Stability of American and Japanese Rice Varieties Tested in Kathmandu

B. B. SHAHI and H. I. OKA

From the data for one native, six American and six Japanese rice varieties tested at Kathmandu, Nepal, standard deviations of individual plants were studied with regard to panicle number per plant, single panicle weight, total panicle length per plant, panicle weight per unit length, and grain yield per plant. Among the American and native varieties, some showed stability of panicle number and plasticity of single panicle weight, while others showed the relation in opposite. A similar variation pattern was also found in the relative stability of total panicle length per plant and panicle weight per unit length. This indicates that the stability-plasticity pattern of yield characters differs according to varietal genotypes. Some Japanese varieties had a large variability for all these characters, and among Japanese varieties stability was correlated to high character value. Varieties showing large variability in many characters were considered inadaptive. It was suggested that the stability-plasticity pattern of character development could be a factor conditioning yield stability. (Published in SABRAO Newsletter 1: 103–111)

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ABSTRACTS OF DIARY FOR 1968

March	12	80th Biological Symposium
	15	158th Meeting of Misima Geneticists' Club
	16	81st Biological Symposium
April	26	159th Meeting of Misima Geneticists' Club
May	17	160th Meeting of Misima Geneticists' Club
June	15	28th Meeting of the Board of Councillors
	28	161st Meeting of Misima Geneticists' Club
July	19	162nd Meeting of Misima Geneticists' Club
	26	82nd Biological Symposium
September	3	83rd Biological Symposium
	9	84th Biological Symposium
	11	85th Biological Symposium
	20	163rd Meeting of Misima Geneticists' Club
October	18	164th Meeting of Misima Geneticists' Club
November	16	Public Lectures on Genetics (at the National Science Museum, Ministry of Education, Tokyo)
December	24	165th Meeting of Misima Geneticists' Club

FOREIGN VISITORS IN 1968

Jan.	15	DASS, M., Central Silk Board, Ministry of Commerce, India
		ULLAL, S. R., Central Silk Board, Ministry of Commerce, India
		SHAH, S., Central Silk Board, Ministry of Commerce, India
Mar.	11	SOBELS, F. H., State Univ. of Leiden, Netherlands
Mar.	16	LATARJET, R., Radium Institute, France
Mar.	21	ANOS, A., Center of Corn Breeding, National Institute of Research in Agronomy, Spain
May	7	RUPER, E. A., The Rockefeller Foundation, U.S.A.
June	4	DASS, H., Research Branch, Canada Dept. of Agriculture, Canada
Sept.	2–5	BARIGOZZI, C., Università degli Studi di Milan, Italy
	2–3	PETRAS, M., University of Windsor, Canada
		FOSTER, M., University of Michigan, U.S.A.
	2–4	AUERBACH, C., University of Edinburgh, U.K.
	3	RIZKI, T. M., University of Michigan, U.S.A.
		SUZUKI, D. T., Univ. of British Columbia, Canada
	3–5	KIMBALL, R. F., Oak Ridge National Laboratory, U.S.A.
		SOBELS, F. H., State Univ. of Leiden, Netherlands
	4	DONN, S. J., University of Toronto, Canada
		FISZEARLE, M., University of Toronto, Canada
		NIDDER, R., University of Toronto, Canada
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	5	OFTEDAL, P., Norsk Hydros Institute for Cancer Research, Norway
		SENGBUSCH, P. V., Max-Planck-Institut, Germany
	5–7	CHOWDHURY, S. N., Sericultural Experiment Station, India
Aug.	13	CROW, J. F., University of Wisconsin, U.S.A.
Aug.	16–19	FELSENSTEIN, J., Institute of Animal Genetics, Scotland
Aug.	25	SEARS, E. R., University of Missouri, U.S.A.
		SMITH, H. H., Brookhaven National Laboratory, U.S.A.
Aug.	28	BURDICK, A. B., Adelphi University, U.S.A.
Aug.	29	ARSENIEVA, Institute of Developmental Biology, U.S.S.R.
		WILLIAMSON, D., Philadelphia University, U.S.A.
		POULSON, D. F., Yale University, U.S.A.
		PAIK, Y. K., Yonsei University, Korea
Aug.	30	CORDEIRO, A. R., Univ. do Rio Grande do Sul, Brazil
		LEE, W. J., Seoul University, Korea

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Sept. 11 SCHADE, H., University of Düsseldorf, Germany
Nov. 12 Hadders, G., Institute for Forest Improvement, Sweden
13–26 PETRAS, M., University of Windsor, Canada

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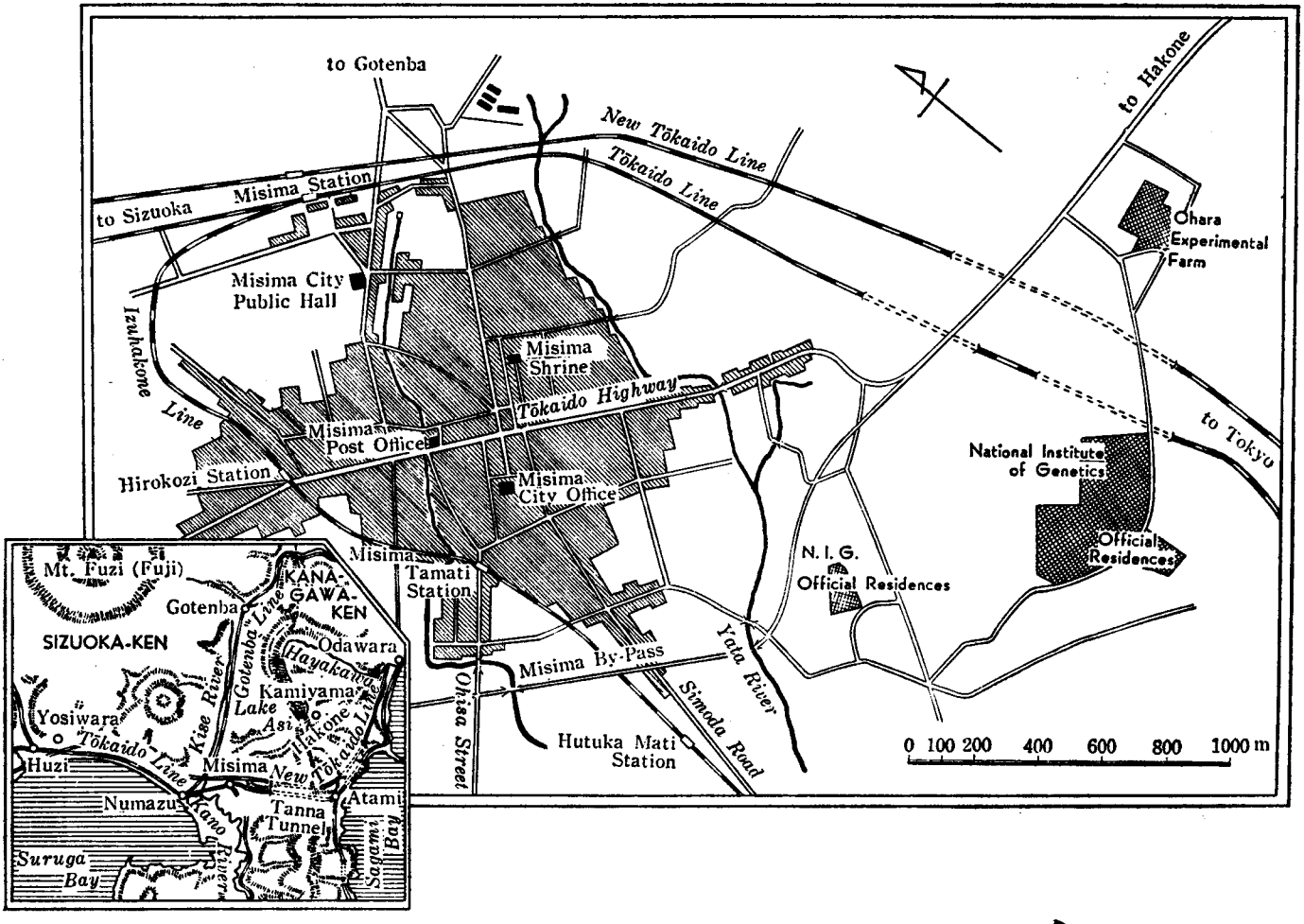
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