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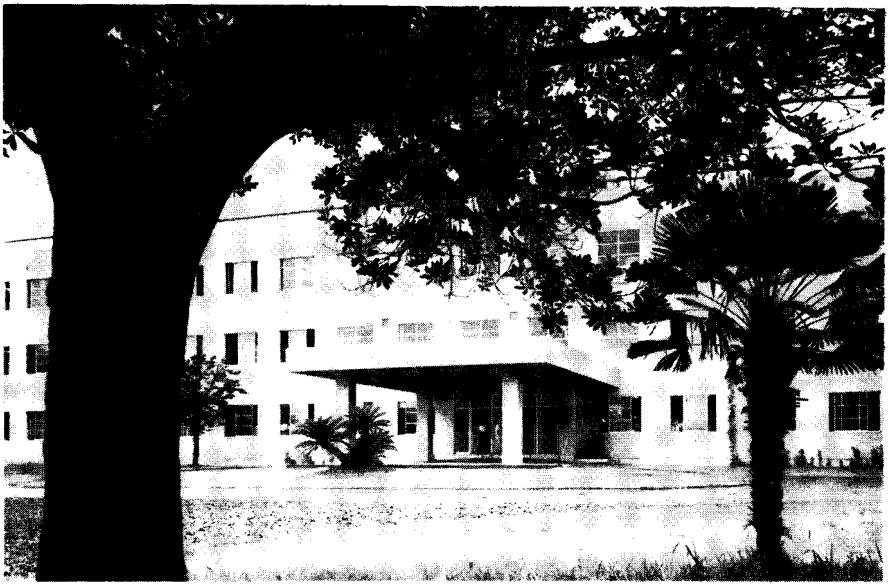

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GENERAL STATEMENT

Dr. Hitoshi Kihara retired from directorship in April. He was the second in the short sequence of our young institute's directors, succeeding Dr. Kan Oguma, the first director, in 1955.

During his service of fourteen years, Dr. Kihara spared neither trouble nor toil in that still difficult time and exerted himself to the utmost trying to put the new institute on a more stable basis. Through his constant endeavors the institute could take long strides toward further developments. Many achievements were attained, the number of departments was steadily growing, the buildings were set up and from the modest beginning an internationally acknowledged National Institute of Genetics emerged. For his most distinguished services, rendered to the institute, Dr. Kihara will be always remembered.

All the more, Dr, Kihara has our great admiration for having not relaxed his pace as an active researcher. At present, he is working on wheat in his own laboratory in the vicinity of our institute. We all wish him the best of luck and continued success in his great lifework.

On the 24th of February His Imperial Highness the Crown Prince visited the institute. This was the second visit of His Highness since 1955. The Prince showed great interest in our recent development presented by several staff members.

This year our institute has attained the age of twenty years. During this period the number of department increased from three to ten. The tenth department, molecular genetics, started this year, and Dr. Kin-ichiro Miura has taken office as Head of the department.

Among the facilities created with the help of new budgets the following two are noteworthy:

(1) Experimental farm comprising 7,082 m^2 in our neighborhood,

(2) Electron computer, TOSBAC-3400.

Dr. M. Kimura, Head of the Department of Population Genetics stayed three months, April 1–July 6, at Princeton University, giving lectures and engaging in cooperative researches.

Among the foreign visitors during the current year, were: Dr. Riojun Kinoshita of City of Hope Medical Center, Los Angeles, Prof. and Mrs. E. L. Tatum of Rockefeller University, New York, Dr. and Mrs. Sui Chuan Hsu of Taiwan Agricultural Research Institute, Taipei, and Prof. L. Tolmach of Washington University, St. Louis.

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SINOTO, Yosito, Manager, Professor of International Christian University
WADA, Bungo, Manager, Emeritus Professor of University of Tokyo

PROJECTS OF RESEARCH FOR 1969

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

Department of Cytogenetics

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Study on incorporation of isolated chromosomes into cultured mammalian cells (KATO, SEKIYA and YOSIDA)
Cytogenetical study on monosomic and trisomic cultured mammalian cells (KATO)
Cytogenetical and biochemical studies on mouse plasma cell tumor (MORIWAKI, YAMASHITA and SAKATA)
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Physiological and population genetics of sterility 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 *Drosophila melanogaster* (OSHIMA and WATANABE)
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Genetical and biochemical studies on Japanese middle size dog (OGAWA)
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Department of Applied Genetics

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Studies on competition in plants (SAKAI, IYAMA and MIYAZAKI)
Genetic studies in natural stands of forest tree species (SAKAI, MIYAZAKI and PARK)
Zymographic studies in forest trees (SAKAI, MIYAZAKI and PARK)
Simulation studies on artificial selection (IYAMA)
Evolutionary studies in wild and cultivated rice species (OKA and MORISHIMA)
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RESEARCHES CARRIED OUT IN 1969

I. CYTOGENETICS

Segregation of Three Types of the Largest No. 1 (A-1) Chromosome in *Rattus rattus* Bred in a Population Room

Tosihide H. YOSIDA and Kimiyuki TSUCHIYA

In order to ascertain the way of segregation in natural condition of three chromosome types (A/A, A/S and S/S)[1] of the largest No. 1 chromosome pair of the Japanese house rat, *Rattus rattus*, some animals were bred in a population room. The size of the room was $250\times430\times300$ cm. Shelters for the nest were partitioned off by board at the side wall of the room. Food and water were administered every day, but no heat was supplied in winter. Breeding of the animals in the population room began in September with one pair having the A/S chromosome type. After one year all animals bred in the room were captured and were sacrificed for chromosome observation. 84 animals thus obtained segregated into 27(A/A), 50(A/S) and 7(S/S). This segregation ratio was significantly different from that of the expected 21:42:21 by χ^2 test ($\chi^2=30.95$, $p<0.001$). Thus the rats with S/S chromosomes seem to be less adaptive to the natural conditions of Misima than those with A/A and A/S chromosome types.

As already reported by us (this Annual Report 18:53, 1968), the segregation of three chromosome types in F_1 hybrids from $A/S\times A/S$, which were bred in usual animal cages did not deviate significantly from the expected ratio, but S/S animals were somewhat fewer than those with A/A and A/S. When S/S females were crossed to A/A, A/S and other S/S males, the litter size of the hybrids was also smaller than that of hybrids between A/S(♀) and S/S(♂). Relation between chromosome types and body size of the animals bred in the population room was examined for the above 84 rats. The body length with S/S chromosome pair was for all under 150 mm, while that of those with A/A and A/S pair attained 200 mm. Thus the rats with S/S chromosome pair do not grow as well as the animals with A/A and A/S pairs in the population room. They seem

[1] In the previous papers we used the term for telocentrics (T), but hereafter we will use for them the term for acrocentrics (A), so A/A and A/S in the present paper correspond to T/T and T/S of the previous reports.

to be less adaptive than those with the other karyotypes, and therefore their number was smaller than that of the others in the population room. From the above results, we must consider a random genetic drift, because the number of animals bred in the room was small. To solve this problem the above experiment will be repeated.

Polymorphism of No. 9 (A-9) and No. 13 (A-13) Autosomes in *Rattus rattus* Collected in Japan

Tosihide H. YOSIDA, Kimiyuki TSUCHIYA and Hiroshi MASUJI[1)]

Polymorphism of the largest acrocentric autosomes (A-1) in relation to acrocentrics (or telocentrics) and subtelocentrics was already reported in the previous communications (Yosida, T. H. *et al.* 1966. This Annual Report 16: 51–52; 1967. Ibid. 17: 61–63; 1968. Ibid. 18: 51–54). In addition we found in the Japanese population also polymorphisms of No. 9 (A-9) and No. 13 (A-13) autosome pairs which were characterized by acrocentrics and subtelocentrics. Most of the animals had acrocentric A-9 and A-13 autosomes, but a few ones had heteromorphic pairs consisting of an acrocentric and a subtelocentric member and a homomorphic pair of subtelocentric chromosomes was also found. A study on the frequencies of A-9 and A-13 polymorphisms in natural population is now in progress.

Due to the polymorphism of three chromosome pairs, the Japanese house rats, *R. rattus*, have very complicated karyotypes. If A-1, A-9 and A-13 chromosomes form homomorphic pairs either acrocentrics or subtelocentrics, theoretically 8 different karyotypes are expected. Among them three are shown in Figs. 1 to 3. In Fig. 1, three chromosome pairs (A-1, A-9 and A-13) are acrocentric, in Fig. 2 A-1 and A-9 pairs are subtelocentric, A-13 pair is acrocentric and in Fig. 3 A-1 pair is acrocentric and A-9 and A-13 pairs are subtelocentric.

1) Okayama University Medical School.

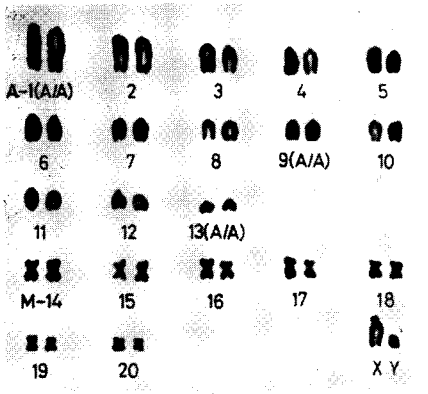

Fig. 1.

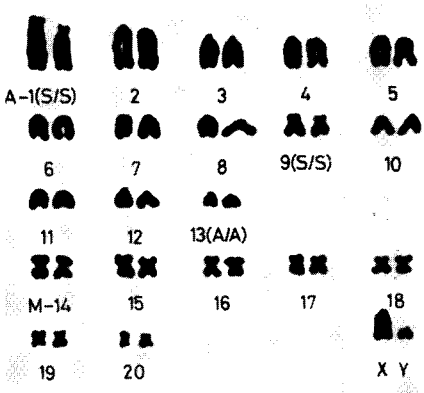

Fig. 2.

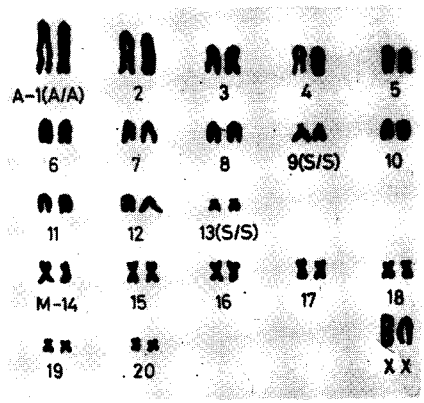

Fig. 3.

Figs. 1-3. Three karyotypes of *Rattus rattus* collected in Japan. 1. A-1, A-9 and A-13 pairs are acrocentric. 2. A-1 and A-9 pairs are subtelocentric, but A-13 pair is acrocentric. 3. A-1 pair is acrocentric, but A-9 and A-13 pairs are subtelocentric.

Frequency of Chromosome Polymorphism in *Rattus rattus* Collected in East and Southeast Asia

Tosihide H. YOSIDA and Kimiyuki TSUCHIYA

In the previous paper (Yosida *et al.* 1969, this Annual Report 19: 11) we had reported only about chromosome numbers and presence or absence of chromosome polymorphism in *Rattus rattus* collected in Southeast Asia.

In the present contribution the karyotypes and frequency of chromosome polymorphism of animals collected in East and Southeast Asia are dealt with. All animals collected in these areas had 42 diploid chromosomes, although fluctuation from 42 to 44 chromosomes was observed in *R. rattus diardi* collected in Kuala Lumpur. As previously reported *Rattus rattus* collected in Japan and Korea showed polymorphism of the largest No. 1 chromosomes in respect to telo- or acrocentric (*A*) and subtelocentric shape (*S*). In the Japanese population about 75 percent of rats had the *A*/*A* pair, about 20 percent the *A*/*S* pair and the remaining 5 percent the *S*/*S* pair.

The animals collected in East Asia (Taiwan and Hong Kong) and the continent of Southeast Asia (Bangkok and Kuala Lumpur) showed the *A*/*A* pair or they were polymorphic having *A*/*A*, *A*/*S* and *S*/*S* pairs. The number of animals collected and the frequencies of *A*/*A*, *A*/*S* and *S*/*S* pairs were different by the locality (Table 1). All rats collected in Hong

Table 1. Frequency of chromosome polymorphism (A-1) of *Rattus rattus* in East and Southeast Asia

Karyotype \ Localities	Japan (19 local.)	Seoul, Korea	Tainan, Taiwan	Hong Kong	Bangkok, Thailand
A/*A*	343	18	3	12	27
A/*S*	90	17	0	0	12
S/*S*	20	4	0	0	3
Total	453	29	3	12	42

Karyotype \ Localities	Kuala Lumpur	Luzon, Philip.	Mindanao, Philip.	Java, Indonesia	Celebes Indonesia
A/A	1	0	0	0	0
A/*S*	3	0	0	0	0
S/*S*	3	25	12	15	20
Total	7	25	12	15	20

Kong and Taiwan showed only the *A*/*A* pair, but the number of animals collected was small. In the Bangkok population the frequencies of rats with *A*/*A*, *A*/*S* and *S*/*S* pairs were similar to those of the Japanese population, while in Kuala Lumpur population the frequencies of *A*/*S* and *S*/*S* were very high although the number of animals collected was very small. This situation is comparable to that found in Hong Kong population. Ac-

cording to the opinion of mammalian systematists, the house rat (*R. rattus flavipectus*) in Hong Kong is a different subspecies from that (*R. rattus diardii*) in Malaysia. A difference between the subspecies is seen also in different frequency of karyotype polymorphism.

On the other hand all rats collected in islands located in Southeast Asia, such as Luzon and Mindanao of Philippines, Celebes and Java of Indonesia showed only *S*/*S* pairs. Based on the above investigation the rats can be classified by the karyotypes as follows:

Karyotypes	*A*/*A* only	Polymorphic *A*/*A*, *A*/*S* and *S*/*S*		*S*/*S* only
		Few *S*/*S*	Few *A*/*A*	
Population	Northern Japan, Hong Kong	South and Southeast Japan, Korea, Thailand	Malaysia	Luzon, Mindanao, Java Celebes

F_1 and F_2 Hybrids between Asian and Oceanian Black Rats with Different Karyotypes

Tosihide H. YOSIDA, Hatao KATO and Katsumi SAKAKIBARA

In a previous paper (Yosida *et al* 1969, Jap. Jour. Genet. 44: 89) we reported that black rats collected in East and Southeast Asia had 42 chromosomes, while those collected in Australia, New Zealand and New Guinea had 38 diploid chromosomes. The latter karyotype was characterized by having two pairs of large metacentric chromosomes, one larger than the other. The other chromosomes were similar to those of the Asian black rats. F_1 hybrids between Australian (2n=38) and Japanese rats (2n=42) were obtained. They had 40 chromosomes consisting of 19 Australian rat and 21 Japanese rat chromosomes. F_1 hybrids could be obtained by crossing Japanese females (♀) to Australian males (♂) and in reciprocal direction of the cross. Test crosses of F_1 and back crosses to Japanese and Australian rats were carried out. Spermatogenesis in F_1 males seems to be proceeding normally, and normal meiotic figures and spermatozoa were observed. Until now only one F_2 animal was obtained. It was a male and was very healthy. The number of chromosomes in the F_2 male was counted in 73 cells. Among them 70 cells had 39 chromosomes, including 3 large metacentrics, one larger than the other two.

The number of chromosomes in F_2 rats is expected to vary from 38 to 42. One fourth of F_2 rats should have 39 chromosomes. Among them one half should have one large metacentric and two small metacentric elements. The present F_2 rat seems to have that karyotype. From the karyological analysis it is assumed that one large metacentric chromosome came from either of the two parents, but the two smaller metacentric members (A-11) were contributed by both parents. The A-9 pair of the F_2 rat was heteromorphic, although that of the father rat was an *S/S* homomorphic pair. The A-9 pair of the maternal rat should be a heteromorphic pair (*A/S*), although it was not yet observed.

Hybrid between a black rat of New Guinea with 38 chromosomes and that of Malaysia with 42 chromosomes, *R. rattus diardii*, was obtained in the laboratory. It also had 40 chromosomes like the hybrid between Australian and Japanese black rats.

Karyotypes of Black Rats, *Rattus rattus*, Obtained from Honolulu, Hawaii

Tosihide H. YOSIDA and Kimiyuki TSUCHIYA

It was already reported that samples of black rats, *Rattus rattus*, collected in East and Southeast Asia had 42 chromosomes, while those collected in Oceania (Australia, New Zealand and New Guinea) had 38 chromosomes (Yosida, T. H. *et al.* 1969. Jap. J. Genet. 44: 89–91). Recently we obtained black rats from Honolulu, Hawaii, by courtesy of Dr. S. R. Malecha of the University of Hawaii. They had the chromosome constitution of Oceanian type; namely 38 chromosomes including two large metacentric pairs.

According to Bianchi (1969, Experimentia 25: 1111–1112.), the black rats in Argentina have 38 chromosomes like the Oceanian rats. Recently we had two informations about rat karyotypes; one from Texas, U.S.A. (R. J. Barker, personal information) and the other from Rome, Italy (E. Capanna, personal information). They also had 38 chromosomes like the Oceanian black rats. From the above investigations, it may be assumed that the European black rats have 38 chromosomes and had migrated from Europe to Oceania (Australia, New Zealand, New Guinea), North America (Texas, Hawaii) and South America (Argentina). The migrated rats seem to have occupied the new land and made it their territory, so that Asian rats do not penetrate the territory of Oceaeian type rats and *vice versa*. In Japan we collected 453 black rats in 19 different localities, but none had the Oceanian karyotype.

Local Differences in the Frequencies of Chromosome Polymorphism in the House Rat, *Rattus rattus*, in Japan

Tosihide H. YOSIDA, Kimiyuki TSUCHIYA and Jun SONODA

Local differences in the frequencies of the polymorphic largest No. 1 (A-1) chromosome pair which was characterized by consisting of an acrocentric (*A*) and a subtelocentric (*S*) chromosomes were already reported (Yosida 1968, this Annual Report 18: 51.). We have newly obtained 86 animals from 7 localities of Japan, namely Sendai, Mizukaido (Ibaragi), Yokohama, Mikurajima (Izu islands), Okayama, Tsushima (Kyushu) and Okinawa. The frequencies of the three chromosome types of the A-1 pair are given in Table 1. As the table shows, rats collected in Sendai

Table 1. Frequency of three chromosome types of the largest No. 1 pair in *Rattus rattus* newly collected in Japan

Karyotype	Localities						
	Sendai	Mizukaido	Yokohama	Mikurajima	Okayama	Tsushima	Okinawa
A/A	11	10	1	1	2	6	23
A/S		8	2	4	1	3	5
S/S		1	3	2	0	2	1
Total	11	19	6	7	3	11	29

were all of *A/A* type, while those collected in Mizukaido, Yokohama and Mikurajima located in Kanto district showed a higher frequency of *A/S* and *S/S* types. The Okinawan population was rather similar to those of some localities of Kyushu (Okinoerabu and Kusudomari), but in the Tsushima population the frequency of *A/S* and *S/S* types was higher than in the other Kyushu populations, and was rather similar to that of Seoul population in Korea.

In total 453 rats were collected until now in 19 localities of Japan. Among them 343 (75.5%) had the *A/A*, 90 (19.9%) the *A/S* and the remaining 20 (4.4%) the *S/S* pair. All 146 animals collected in northern (Sapporo, Sendai) and northwestern Japan (Niitsu, Obama and Tottori) had the *A/A* type, while those collected in the other localities had *A/A*, *A/S* and *S/S* types. This group was also roughly divided into 3 populations by the frequency of the subtelocentrics; eastern population (79 rats) in Kanto district (Mizukaido, Ooizumi, Yokohama and Mikurajima) showed a higher frequency of *A/S* (46%) and *S/S* types (17%), while the southeastern population (98 rats) collected in Tokai (Hiratsuka, Misima, Hamamatsu) and

Sanyo (Okayama and Ube) showed lower *A/S* (31%) and *S/S* (3%) percentage. In the southern population (100 rats) collected in Kyushu district (Kusudomari, Okinoerabu and Okinawa) the lowest frequency of *A/S* (18%) and *S/S* (1%) types was observed.

From the study of those local differences it is assumed that rats with *A/A* pair are the original type which was the first to propagate widely in Japan, and those with *S/S* pair migrated later secondarily to Kanto district (Tokyo, Yokohama), and are propagating from there to the other parts. This assumption seems to be supported by observations made in Southeastern Asia, and by our crossing experiment in the laboratory as well as a breeding experiment in a population room.

Karyotypes of *Rattus bowersii* Collected in Malaysia

Tosihide H. YOSIDA and Kimiyuki TSUCHIYA

Rattus bowersii collected in Kuala Lumpur, Malaysia, had 40 diploid chromosomes. Their karotypes are remarkable by having one large metacentric chromosome pair. *Rattus rattus* collected in Asia had 42 chromosomes, but those collected in Oceania had 38 chromosomes (Yosida *et al.* 1969, Jap. J. Genet. 44: 89–91.). The latter karyotypes are characterized by containing two large metacentric pairs, one larger than the other. From a comparison of idiograms of these two forms it was assumed that two arms of the large metacentric element might have developed by reciprocal translocation of acrocentric pairs No. 4 and 7, and the smaller one by reciprocal translocation of acrocentric pairs No. 11 and 12. *Rattus fuscipes* collected in Australia had 2n=38, and their karyotypes were very similar to those of the black rats in Oceania.

The karyotypes of *R. bowersii* had one smaller metacentric pair, whose size corresponded to the smaller metacentric pair of the Oceanian black rats. This chromosome seems to have originated from the fusion of No. 11 and 12 acrocentric pairs of *R. rattus*. The other chromosomes of the *bowersii* rat are essentially similar to those of *R. rattus* in Asia; namely 7 small metacentric pairs, 11 acro- or subtelocentric autosome pairs, and an acrocentric sex chromosome pair. The karyotype of *R. bowersii* seems from appearance to be intermediate between that of Oceanian black rats or *R. fuscipes* and that of Asian black rats. Subtelocentrics of No. 1 and No. 9 pairs are similar to those of the other *Rattus* species, but subtelocentric No. 2 and No. 3 pairs are remarkable in this species. From the above investigation it is assumed that the *bowersii* karyotype developed

from that of the Asian black rats by fusion of Robertsonian type of acrocentric No. 11 and 12 pairs, and also a centromeric inversion of No. 2 and 3 pairs.

Protective Effects of Protamine Sulfate on Disintegration of Isolated Metaphase Chromosomes

Hatao KATO, Kunio SEKIYA and Tosihide H. YOSIDA

It has been demonstrated that isolated metaphase chromosomes were readily phagocytized by mammalian cells *in vitro*. Recently Yosida and Sekiguchi (Molec. Gen. Genetics, 103: 253, 1969) presented an evidence for the appearance of some of the incorporated chromosomes in harmony with the host genome. In the present study, we intended to obtain further information on the behavior of the incorporated chromosomes at an early phase of the phagocytic phenomenon and to find appropriate conditions which would protect the engulfed chromosomes from the digestive action of cellular enzymes in the hope to increase the survining rate of incorporated chromosomes.

Metaphase chromosomes were isolated from mouse lymphoma L5178Y cells. The isolated chromosomes were first exposed *in vitro* to DNase dissolved in phosphate buffered saline. A disintegration of chromosomes occurred within 5 min after exposure to the enzyme at 37°C. On the other hand, when protamine sulfate was added to the incubation mixture, chromosome morphology as well as stainability by Feulgen reaction remained intact even 90 min after incubation with DNase. This protective effect of protamine sulfate was constantly manifested at concentrations higher than 10 μg/ml.

When the isolated chromosomes were added to a monolayer culture of Chinese hamster cells, they were phagocytized by the recipient cells. The frequency of cells taking up the exogenous chromosomes reached a peak 4 hours after their addition, and then fell rapidly. This decline appeared to be due to degradation of incorporated chromosomes within the cytoplasm of recipient cells. When chromosomes were labeled with ^{3}H-thymidine prior to isolation and then added to the culture, a uniform labeling of the nuclei of recipient cells was detected 4 hours after their addition, suggesting reutilization by the recipient cells of labeled DNA precursors derived from the degraded chromosomes. The incidence of labeled nuclei increased steadily up to 10.2% by the 14th hour.

Intracellular disintegration of incorporated chromosomes was also

prevented by their pretreatment with protamine sulfate. The incorporation frequency showed a steady rise till 6 hours after the addition of chromosomes and reached a plateau. The percentage of labeled nuclei of recipient cells remained very low until 12 hours (1.3%) after the addition of protamine-treated chromosomes.

A Preliminary Study on the Uptake of Isolated Nuclei by Mammalian Cells *in vitro*

Kunio SEKIYA, Hatao KATO and Tosihide H. YOSIDA

The present report deals with cytological observations of the uptake of isolated nuclei by cultured mammalian cells, and their fate in the recipient cells during an early phase of cultivation. The nuclei were isolated from mouse FM3A cells, which lacked in hypoxanthine-guanine phosphoribosyltransferase activity, and were added to the culture of mouse 3T6-8T cells which were deficient in thymidine kinase activity (TK^-). The recipient cells were grown in plastic dishes as monolayers in Eagle's minimum essential medium supplemented with 10% calf serum.

At various time intervals after incubation with the isolated nuclei, the cells in each dish were fixed in methanol-acetic acid (20:1) after a brief wash with phosphate buffered saline, and stained with Giemsa solution.

Phagocytic activity of the recipient cells was indicated by a gradual increase in the frequency of binucleate cells after the addition of isolated nuclei. In untreated control cultures, the incidence of binucleate cells was only 0.1%, whereas in cultures that had been exposed to isolated nuclei, the frequency increased up to 3.1% by the 4th hour. In these binucleate cells, one of the nuclei was apparently smaller and stained more densely than the other, indicating that it was an engulfed donor nucleus.

Another characteristic feature of the recipient cells was the appearance in their cytoplasm of vacuoles, which sometimes contained densely-stained donor nuclei. The frequency of cells possessing cytoplasmic vacuoles increased steadily after treatment; from 0.8% in untreated cells to 2.4%, 5.0% and 6.5%, after 2, 4, and 12 hour-incubation with the isolated nuclei, respectively.

In autoradiographic studies employing tritium-labeled nuclei, the symptom of disintegration of incorporated donor nuclei, indicated by dispersion of silver grains over the cytoplasm of recipient cells, was first detected 18 hours after the addition of nuclei.

In parallel with these cytological observations, the 3T6-8T cells (TK^-) were cultured in selective medium (HAT) following exposure to nuclei isolated from FM3A cells (TK^+) in the hope of obtaining cells which acquired thymidine kinase activity. So far, no surviving colony has been obtained.

Nondisjunction of Chromosomes in a Synchronized Cell Population Initiated by Reversal of Colcemid Inhibition

Hatao KATO and Tosihide YOSIDA

Stubblefield and Klevecz (Exptl. Cell Res., 40: 660, 1965) reported a method of initiating a synchronized cell population by collecting and replacing colcemid-arrested metaphase cells in fresh medium. Their method appeared to have the definite advantage of obtaining a large yield of mitotic cells as compared with the original selective detachment method developed by Terasima and Tolmach (Exptl. Cell Res., 30: 344, 1963). However, this modified method seems to require a further sure proof that the cells are completely free from lasting effects of colcemid arrest, especially in a long term culture. In the present study, we found that chromosomal nondisjunction occurred frequently in colcemid-reversed cells.

Chinese hamster cells (Don) were grown in 100-mm plastic (Falcon) petri dishes. When the cultures were growing exponentially, cells were treated with colcemid at a concentration of 0.05 μg/ml. The metaphase cells were collected 30 min, 1, 2 and 4 hours after the addition of the drug, respectively, and placed in 60-mm plastic dishes containing pre-warmed normal medium. Some of the cultures were fixed at the first mitotic stage following a release from the colcemid block. Chromosomal examination revealed that nondisjunction had occurred at various frequencies depending upon the duration of colcemid arrest; the percentage of cells possessing the modal chromosome number decreased with increasing duration of the colcemid block (e.g., 88.3% in the untreated control culture, 76.6%, 61.5%, 52.0%, and 29.8% in cultures exposed to colcemid for 30 min, 1, 2 and 4 hours, respectively). Chromosomal monosomies and trisomies were observed frequently. Tetrasomies were also observed. These results appear to suggest that in a certain proportion of colcemid-reversed cells the damages of the spindle fibers and their contact with the centromeres were not completely repaired.

Further cultivation of the remaining cultures and their karyological examination carried out at definite time intervals revealed that cells with

monosomies disappeared rapidly from the cell population. The colonies formed by cells exposed to colcemid for 4 hours were apparently smaller than the colonies derived from those submitted to 2-hour-arrest. The plating efficiencies determined on the 7th day of incubation were 73.0% in the untreated control cultures, 53.3% in those submitted to 2-hour-colcemid arrest, and 44.3% in those that had recovered from 4-hour-arrest by colcemid.

Establishment of Several Chinese Hamster Cell Lines with Specific Chromosomal Trisomies

Hatao KATO

The method of estimating gene dosage effect in trisomic individuals has been used by various workers for the localization of genes in mammalian autosomal chromosomes. Such studies would be much easier if one could induce nondisjunction in cultured cells and isolate a variety of cell clones with chromosomal trisomies.

According to our previous report (Kato and Yosida, 1970. Exptl. Cell Res., 60: 459–464.) nondisjunction can be readily induced by a brief exposure of cells to colcemid. Making use of this technique, seven cell lines with trisomic chromosomes were successfully isolated from cultured Chinese hamster cells.

Chinese hamster cells (Don) have 22 chromosomes. They were treated with colcemid (0.05μg/ml) for 2 or 3 hours, and the metaphase cells were selectively detached from the culture dishes. They were then washed free from colcemid and placed at a concentration of 100 cells per dish in 100-mm plastic culture dishes containing pre-warmed fresh medium. Colonies formed by these colcemid-reversed cells were isolated from the dish by cylinder technique. In the first experiment, twenty five colonies were randomly picked out and chromosome counts were performed. Fourteen colonies were found to have chromosome numbers in diploid range, whereas the other eleven colonies possessed chromosome numbers at tetraploid level. Out of the diploid colonies, three were found to have trisomic chromosomes; trisomy of no. 9, of no. 10, and double trisomies of no. 7 and no. 10 chromosomes. The rest of the diploid colonies showed a karyotype identical to that of the parental cells.

Although the incidence of tetraploid clones was very high among the colonies isolated in the first experiment, they were easily distinguished in the living culture from the diploid ones on the basis of difference in size of metaphase cells. Therefore, in the next experiment, colonies with

small metaphase cells were picked out, all turning out to have chromosome numbers in diploid range. Out of 35 colonies isolated, four had trisomic chromosomes (no. 9 trisomy, no. 9 trisomy accompanied by an extra Y, no. 10 trisomy, and no. 11 trisomy) and three had 21 chromosomes. All other colonies had the same karyotype as the original Don cells. Karyotype analysis of clones with 21 chromosomes revealed that they were not genuine monosomic clones. The reduction of the chromosome number in these clones was due either to translocation of a part of the Y to other chromosomes or to centric fusion of acrocentric homologues. Chromosomes involved in these structural rearrangements were no. 7, M1, no. 10 and the Y. Clones possessing trisomies of large chromosomes (e.g., no. 1, 2, 4, and 5) have so far not been isolated.

Seven trisomic clonal lines have been maintained for approximately two months since they had been isolated without losing their specific chromosome configurations.

Peculiar Marker Chromosomes in Yoshida Rat Sarcoma

Toshiharu MATSUSHIMA and Tosihide H. YOSIDA

In Yoshida rat sarcoma two peculiar marker chromosomes in metaphase cells were recently observed. These two elements, one a large subtelocentric, LS-marker, and the other a small submetacentric chromosome, the SS-marker, were characterized by an abnormally elongated part resembling a long satellite. The SS-marker was more remarkable than the LS-marker. The length of the quasi-satellite was different by the cell. In some cells with the SS-marker it had almost the same length as a long ordinary arm but in some other cells it was shorter. In many cells it showed two parallel daughter threads, but in few cells they formed a ring by joining the ends of the separated daughter chromatids of the satellite.

The occurrence of the two marker elements in the tumor stocks maintained in five different institutes (this Institute, Sasaki Medical Institute in Tokyo, Medical School in Gifu University, Takeda Biological Institute in Osaka, and Medical School in Tohoku University, Sendai) was examined. Almost similar LS- and SS-markers were found in stocks of this Institute, Sasaki Medical Institute, Gifu University and Tohoku University, but the LS-markers in a stock of Takeda Institute had a different shape, as if a small chromosome was united with the satellite by translocation. Frequency of LS- and SS-markers was markedly different by the stock. Higher

frequency of the markers was observed in stocks of this Institute, Sasaki and Takeda, slightly lower in Gifu and a very low in Tohoku.

The SS- and LS-markers had sometimes the appearance of a satellite association. From the figures the long threads seem to have a relation to nucleolar organization. Late replication of DNA in the thread region of LS-markers was observed by labeling with H^3-thymidine, but was not found in that region of SS-markers.

Synthesis Rate of Intracellular Gamma-Globulin in Dipliod and Tetraploid MSPC-1 Myeloma Cells[1)]

Kazuo MORIWAKI and Hirotami T. IMAI

It was previously reported that no significant difference was found in the rate of gamma-globulin synthesis between diploid and tetraploid MSPC-1 myeloma cells when it was determined in the excreted globulin fraction (Moriwaki, K. *et al.* Symp. Cell Chem. 20: 195-204 1969). To exclude a possibility that this phenomenon was caused by a difference between di- and tetraploid cells in the ability to excrete the protein, the rate of intracellular myeloma protein synthesis was assayed in both sublines of the tumor.

The tumor cell suspension was incubated for 2 hours at 37°C with C^{14}-leu. The soluble fraction was extracted from sonicated cells followed by precipitation at pH 5. Proteins soluble at the acidic pH were chromatographed on DEAE-cellulose column to separate the gamma-globulin fraction to which anti-MSPC-1 myeloma protein rabbit serum was added for the precipitation of the specific protein. Radioactivity in the antiserum-precipitated fraction represented the rate of intracellular myeloma protein synthesis. No significant difference was observed between the synthesis rates of cells of both sublines (Table 1), suggesting the possibility that an "allelic exclusion" mechanism functioned in the tetraploid myeloma cells producing gene dosage compensation, which did not take place in the total protein synthesis of these tumor cells (Moriwaki, K. and H. T. Imai. 1969. This Annual Report 19: 15-16).

In order to determine the synthesis rate of light and heavy chains separately, the myeloma protein precipitate was further reduced and alkylated by mercaptoethanol and monoiodo-acetic acid, followed by separation in Sephadex G-100 column with 1 N acetic acid. The ratio of light

1) Supported by Public Health Service grant CA-07798-06 from the National Cancer Institute, U.S.A. (T. H. Yosida).

Table 1. Rate of myeloma protein synthesis in diploid and tetraploid MSPC-1 myeloma cells

Fractions	C^{14}-leu incorporated (cpm/10^6 viable cells/120 min.)	
	Diploids	Tetraploids
Total protein	2634 (5)	4552 (5)
Myeloma protein	190 (5)	216 (5)
Light/Whole ratio	0.29	0.29

Figures in parentheses indicate the number of tumor-bearing mice used for the assays.

chain to whole molecule in the synthesis rate of the diploids was almost the same as that of the tetraploids (Table 1). This finding indicates that inhibition of myeloma protein synthesis in tetraploid cells is not restricted either to light or to heavy chains.

Exchange between Cell Populations during Serial Transplantation of MSPC-1 Mouse Myeloma[1]

Kazuo MORIWAKI and Junkoh YAMASHITA

During a 2-year serial transplantation of MSPC-1 mouse myeloma the modal chromosome number has shifted from 40 in the early generations to 39 with 3 marker chromosomes, A, B, and C, in the late generations. The present study was undertaken to reveal a possible mechanism of such an exange between two tumor cell populations. The tumor cells with modal number 40 in early transplant generations designated as subline P-40 have been stored in liquid nitrogen for several months and used simultaneously with another subline, NP-39 ABC, of a later transplant generation, for mixed inoculation experiments. As the growth rate of NP-39 ABC subline was considerably higher than that of P-40 subline, 10^3 cells of the former and 10^6 cells of the latter developed tumors of the same size in two weeks, when they were inoculated separately on both sides of abdomen of a host mouse. On the other hand, when cells of both sublines were inoculated as a mixture of 10^3 NP-39 ABC cells and 10^6 P-40 cells, more than 98% of the tumor cells developed after two weeks consisted of NP-39 ABC cells (Table 1). In order to know what cellular function plays a role in the inhibition of P-40 cells, NP-39 ABC cells irradiated by 15,000r gamma-rays which had completely blocked

[1] Supported by a Grant in Aid (Nos. 9151 and 4051) for Fundamental Scientific Research from the Ministry of Education in Japan.

Table 1. " Allogeneic inhibition "-like phenomenon between two syngeneic tumors, namely P-40 and NP-39 ABC sublines of MSPC-1 myeloma

Subcutaneous inoculation		Chromosome constitution	
		Inoculum	Grown tumor (2 weeks old)
Separately			
Left abdomen,	10^6	40	40>98% (10/10)
Right abdomen,	10^3	39 ABC	39 ABC>98% (10/10)
Mixed	10^6 + 10^3	40 + 39 ABC	39 ABC>98%(10/10)

Figures in parentheses represent the number of mice developing tumors, in relation to the total number inoculated.

proliferation of the cells, were inoculated mixed with viable P-40 cells. Comparing the control which contained a mixture of heavily irradiated and untreated P-40 cells, the growth of the tested tumor seemed to be fairly retarded.

Those findings suggested that an " allogeneic inhibition "-like mechanism may exist in the inhibitory action of NP-39 ABC cells against P-40 cells, though they are syngeneic to each other in histocompatibility.

Cytological Studies of Mouse Gliomas

Junkoh YAMASHITA

The techniques of studying metaphase chromosomes have been well established in the study of ascites tumors and *in vitro* cultivated cells, but one has not been always successful in obtaining reliable chromosome preparations directly from solid tumors without an eventually selective action of tissue culture. We found that a direct drying method following enzymatic digestion with extremely diluted trypsin (0.02%) is satisfactory for the karyological study of glial tumors in mice.

Three transplantable methyl-cholanthrene-induced mouse gliomas were studied.

1) 203 glioma (obtained through the kindness of Dr. Y. Ishida, Department of Pathology, Gumma University). A wide distribution of chromosome numbers ranging from 63 to 132 was observed at the 23rd transplant generation. Among 100 cells analysed, the frequency of cells with 67 chromosomes was the highest (30 cells). Four kinds of marker chromosomes

were observed; large metacentrics (M1), small metacentrics (M2), median submetacentrics (SM) and minutes (m).

2) No. 4 glioma (originally induced). Chromosome numbers were distributed from 39 to 82 at the second transplant generation. The highest frequency (52 among 100 cells) had cells with 40 chromosomes. No marker chromosome was observed.

3) No. 5 glioma (originally induced). Among 100 cells examined at the first transplant generation, chromosome numbers ranged from 37 to 84 with the highest frequency at 39 (71 cells). No marker chromosome was observed.

In younger No. 4 and No. 5 gliomas with small number of transplant passage, chromosomal abnormalities were less frequent and the proportion of cells in the diploid range was higher than in the older 203 glioma. It was reasonably assumed that the chromosomal aberration was not an essential factor in carcinogenesis but a secondary product of stepwise genetic changes during tumor progression. These results were consistent with the documented fact on most murine tumors that they have a common tendency to tetraploidy after a period in the diploid range. Nevertheless, it should be emphasized that the chromosome analysis will be of great importance as a visible indicator of invisible genetic changes in tumors.

Aggregate-Promoting Substance from HeLa Cells in Rotation Culture

Yukiaki KURODA

It has been previously found by the author that in mouse spontaneous mammary gland tumors, mouse plasma cell tumors and chick Rous sarcoma characteristic changes in aggregate-forming activity of dissociated cells were exhibited in the process of carcinogenesis (Kuroda, 1968. Gann 59: 281; Kuroda, 1969. Ann. Rep. Natl. Inst. Genet. Japan 18: 11). In embryonic chick liver and retina cells some cell-free materials showing aggregate-forming activity were obtained from a culture medium in which the cells were cultured for a few hours (Kuroda, 1968. Exptl. Cell Res. 49: 626; Lilien, 1968. Develop. Biol. 17: 657). These findings suggest that the characteristic alteration of aggregate-forming activity of neoplastic cells may depend upon the qualitative or quantitative changes in their aggregate-promoting substance(s).

In the present experiment the author attempted to isolate cell-free

materials showing aggregate-forming activity from HeLa cells in comparison with those from normal human diploid cells. When HeLa S3 cells were cultured in Eagle's basal medium supplemented with 10% calf serum at 20°C at 70 rpm on a gyratory shaker for 24 hours, the characteristic aggregates which were formed at 38°C under otherwise identical conditions were not detected. It was found that a cell-free supernatant obtained from culture medium in which HeLa cells were previously rotated at 38°C for 2 hours showed aggregate-forming activity on newly liberated HeLa cells at 20°C in rotation culture.

The supernatant which was obtained from 2-hour rotation cultures of 3×10^6 cells in 3 ml culture medium was diluted with the culture mudium into various concentrations and tested for their effect on the aggregation of HeLa cells at 20°C. Culture medium containing fifty per cent supernatant had the highest activity among various concentrations examined. Isolation of the corresponding supernatants from normal human diploid cells and comparison of the activity between those from HeLa cells and normal cells are now under investigation.

Pachytene Pairing in Translocation Heterozygotes Having a Huge Knob Close to the Breakpoint

Yasuo OHTA

An exceptionally huge knob ($3L^{111}$) was found by the present author on the long arm of chromosome 3 (position .58) in an inbred line, N. C. Inb. 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, two translocation heterozygotes having the breakpoint close enough, one proximal and the other distal, to the knob position, were selected for hybridization to the inbred line. Strains T3-8h, the breakpoint at .53, and T3-9f, at .63, were obtained from Dr. E. B. Patterson, for which the author is grateful to him. They were crossed to the inbred line, $3L^{111}$, and the F_1 hybrids were examined cytologically.

Pachytene pairing in a translocation heterozygote, $3L^{111}$/T3-9f, was analysed. Three major types were found:

1) complete asynapsis or asynapsis in each of the four arms of the cross-shaped configuration in segments adjacent to the breakpoint, was observed in about 50 per cent of the cells analysed although the lengths of asynaptic arms varied, 2) asynapsis in two or three arms of the

cross-shaped configuration was observed in about 35 per cent of the cells and, 3) complete homologous pairing throughout the cross-shaped configuration in the rest of the cells, in which two cases existed: either the huge knob paired with the knobless homologue or it folded down itself and U-shaped (partly non-homologous pairing in a strict sense). In the most extreme case of 1) the asynaptic segment was as long as about two-fifths of the length of the long arm of chromosome 3, but no case of asynapsis beyond the centromere was observed.

Pachytene pairing in another translocation heterozygote, $3L^{111}/T3\text{-}8h$, was also analysed. Three major types were found:

1) complete asynapsis or asynapsis in each arm of the cross-shaped configuration in the segments adjacent to the breakpoint, 2) complete homologous pairing throughout the cross-shaped configuration and, 3) partly homologous pairing and partly asynapsis. The observed frequency of each of the three types was about 1:1:1.

From the above observation the following conclusion is drawn: Heterochromatin when exists in huge amount heterozygously does prevent pairing of homologous chromomeres or ordinary chromosome segments in the region adjacent to it, but it does not for homologous centromeres, indicating that different mechanisms are involved between synapsis of chromomeres and that of centromeres during meiotic prophase.

II. PHYSIOLOGICAL AND DEVELOPMENTAL GENETICS

An Attempt to Obtain Long-Term Culture Cells from *Drosophila melanogaster*

Yukiaki KURODA

To obtain some long-term culture cells from *D. melanogaster*, the author has been searching for the most suitable conditions under which the cells of this insect can be maintained in luxuriant growth. Embryonic tissues and larval imaginal discs, gonads, intestines, Malpighian tubes, salivary glands and ganglions were cultured in various chemically defined media with or without supplementation of some macromolecular substances.

For the cultivation of embryonic tissues, it was found that embryos at the time of gastrulation (4 hours after egg laying) were a better source of materials for cultivation than those at earlier or later stages. Attachment of explanted tissues to the glass surface of the culture bottles was an essential factor for the maintenance of cells in active living state. It was found that the preincubation of explants in physiological salt solution for 60 minutes resulted in an enhancement of their attachment to the glass surface.

Various chemically defined media which have been previously devised for cultivation of insect tissues by many investigators were tested for their suitability in culturing *Drosophila* tissues. None of them were found to be more efficient in sustaining cell growth than medium K-6 and its modified medium K-6′ which was devised by the author for organ culture of *Drosophila* imaginal discs.

Supplementation to medium K-6′ of 10% calf serum was found to be the best among sera from various sources and at various concentrations examined. The addition to this supplement of heat-treated blood collected from fifth instar larvae or pupae of silkworm exhibited no growth improvement in the culture of embryonic *Drosophila* tissues. Among ecdysone analogues tested ecdysterone at the concentration of 0.01 μg/μl had a slight growth-promoting effect, whereas inokosterone at as low concentration as 0.0001 μg/μl had an inhibitory effect.

Dodecyl methyl ether, a substance acting as juvenile hormone, was tested at various concentrations for its growth-promoting effect on embryonic *Drosophila* tissues. The result is shown in Table 1.

It was found that 0.1 μg/μl dodecyl methyl ether stimulated the growth

Table 1. Effects of dodecyl methyl ether (DME) on the growth of embryonic *Drosophila* tissues in culture

Concentration of DME (μg/μl)	No. of explants tested	No. of explants in which growth was observed	Per cent growth
Control	16	12	75
10.0	13	2	15
1.0	2	1	20
0.1	8	7	88
0.01	22	18	82

of tissues, whereas with higher concentrations than 1.0 μg/μl an inhibitory effect was observed as compared with the control cultures without it. Fetuin, a fraction of fetal calf serum (Grand Island Biol. Co.) and phytohemagglutinin P (Difco) were also tested for their growth-promoting activity. The results are shown in Table 2.

Table 2. Effects of fetuin and phytohemagglutinin (PHA) on the growth of embryonic *Drosophila* tissues in culture

Substance	No. of explants tested	No. of explants in which growth was observed	Per cent growth
Control	34	28	82
Fetuin, 0.1 μg/μl	24	18	90
PHA, 10 μg/μl	14	14	100
PHA, 100 μg/μl	31	23	74

A marked stimulatory effect on the growth of explanted tissues was found with 0.1 μg/μl fetuin or 10 μg/μl phytohemagglutinin. For obtaining cell strains throughout a long-term cultivation of *Drosophila* tissues further improvements of culture conditions and attempts to subculture the primary explants are now under investigation.

Clonal Analysis of Embryonic Limb Cartilage Cells in *Creeper* Mutant of Chicken

Yukiaki KURODA and Toru SHIBUYA

It is known that the *Creeper* (*Cp*) gene is a dominant gene on the C chromosome of chicken. Embryos homozygous for *Cp* (*Cp*/*Cp*) are lethal at an early stage of embryonic development, whereas embryos heterozygous

for *Cp* (*Cp*/+) continue to live for a long time after hatching showing deteriorating growth of their hind limbs. Among offsprings from a cross between *Cp*/+ and +/+ parents, *Cp*/+ embryos can be morphologically distinguished from +/+ embryos on the eighth day of incubation.

In the present experiment chondrocytes were dissociated by trypsinization of hind limbs of *Cp*/+ and +/+ embryos at various stages of development, and the growth rate, identification of colony-forming cells and tissue-reconstructing activity of dissociated chondrocytes from both strains were investigated.

The growth rate of chondrocytes from 8-day or 10-day old +/+ embryos was 1.3~1.6 times higher than that from *Cp*/+ embryos at the corresponding stages. Two morphologically distinguishable kinds of colonies were observed when dissociated chondrocytes from embryos of both genotypes were inoculated on 60 mm plastic Petri dishes: cartilage-making cell colonies (CMC) and fibroblastic cell colonies (FC). Percentages of each type to total colonies formed from single cells of both genotypes are given in Table 1.

Table 1. Percentages of cartilage-making cell colonies (CMC) and fibroblastic cell colonies (FC) formed from dissociated limb chondrocytes of *Cp*/+ and +/+ embryos

Stage of embryos	Genotype	No. of colonies counted	No. of CMC	No. of FC	Per cent CMC
8-day	*Cp*/+	575	366	209	63.7
	+/+	424	257	167	60.6
10-day	*Cp*/+	525	265	260	50.5
	+/+	401	178	223	44.4
12-day	*Cp*/+	344	146	198	42.4
	+/+	257	96	161	37.0

Percentages of CMC to total colonies formed from dissociated cells of *Cp*/+ embryos were significantly higher than the corresponding values of +/+ embryos regardless of age of embryos from which the cells were obtained. In reconstructed tissues from dissociated *Cp*/+ cells after rotation culture, a well differentiated cartilage matrix which was metachromatically stained with toluidine blue was found, whereas reconstructed tissues from dissociated +/+ cells exhibited less differentiation of cartilage matrix which was faintly stained with toluidine blue.

From the above findings it is suggested that limb chondrocytes of *Cp*/+ have differentiated precociously as was indicated by their retarded growth

rate, higher percentage of CMC, and more differentiated cartilage matrix formation in comparison with the chondrocytes of +/+ embryos.

Effect of Molting Hormone on the Cell Cycle in Insect Epidermis

Kiyoshi MINATO

In a series of experiments dealing with the analysis of cell kinetics during the formation of larval cuticle at the molting stage of *Philosamia cynthia ricini*, it has been previously found that there was a long G_2 phase (about 48 hours) between the DNA synthetic phase and the following mitotic phase through the molting. To elucidate further the correlation between the characteristic duration of the long G_2 phase and the phenomenon of molting taking place during this phase, an experimental induction of larval molting was attempted by injecting ecdysterone, a substance having molting hormone activity.

When ecdysterone at the concentration of 20 μg/g body weight was injected into larvae at the initial stage (6~7 hours after molting) or an early stage (12 hours after molting) of the fourth instar which seemed to be under no influence of molting hormone, the larvae entered precociously into the pause prior to molting 12~17 hours after injection. However, most of them died before the molting was completed.

In the same way when larvae at the middle stage (26 hours after molting) of the fourth instar were injected with ecdysterone, a precocious pause prior to molting was also induced 12~17 hours after injection, in contrast to the non-injected control larvae in which no pause was detected at this time. In this case most of the injected larvae proceeded normally into the fifth instar through molting. The time between the injecting of ecdysterone and the initiation of molting was 12~17 hours, indicating that it was constant regardless of the age at which the larvae were injected. The relation between the concentrations of ecdysterone used for injection and the time length following injection before molting remains to be examined. The effect of these experimental inductions of molting on each phase of the cell cycle of larval epidermis is now under investigation.

Cytoplasmic Male Sterility and Virus Infection in *Capsicum annuum* L.

Yasuo OHTA

A phenotypical similarity is known to exist between cytoplasmic and virus-induced male sterility in certain plants. In some cases it is probable that viruses behave as cytoplasmic agents or in some way react with them.

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*).

The first and second inoculation experiments have been described in the previous papers (Ohta 1968, 1969), so that only the results are now given in Table 1. The inocula were broad-bean wilt virus (BWV), three strains of cucumber mosaic virus, i.e. ordinary (CMVo), leguminous (CMVl) and necrotic spot (CMVn), ordinary and yellow strains of tobacco mosaic virus (TMVo and TMVy) and a mottle strain of potato virus X (PVXm). With these previous results only, the cause of reduced pollen fertility could not be determined. Therefore, the third and the fourth experiments were performed. They are listed also in Table 1. The inoculated plants were (1) *S*-Fresno Chile×Fushimiamanaga F_1, (2) Fresno Chile× Fushimiamanaga F_1, (3) Fushimiamanaga×Fresno Chile F_1 and (4) Fushimiamanaga. The above three F_1 hybrids possessed an identical genotype (*Rfrf*) and the same genetic background, the only difference being in the cytoplasm between the first (*S*) and the second (*N*) or the third (*N*), and practically no difference in the influence of cytoplasm between the second and the third. As inocula a hydrangea isolate of CMV(CMVh), a tomato strain of TMV (TMVt) and a tomato ringspot virus (TMRV) were also employed.

Markedly reduced pollen fertility appeared only in plants having the (*S*) cytoplasm, indicating that this specific cytoplasm was responsible for the reduction in pollen fertility by inoculation with certain viruses.

Progeny tests have provided more evidence indicating that pollen fertility in plants having (*S*) cytoplasm and *Rfrf* genotype, and should be pollen fertile, was reduced following infection with specific viruses.

It is concluded that a certain interaction between specific viruses and a particular cytoplasm (*S*) is responsible for the reduced pollen fertility. In other words, the specific cytoplasm (*S*) modifies the action of *Rf*-gene by interacting with specific viruses.

Table 1. Pollen fertility (%) of virus-inoculated *Capsicum annuum*

Exp. no*	Inoculated plants		Cont.	Inoculated with+										
				BWV	CMVo	CMVl	CMVh	CMVn	TMVo	TMVy	TMVt	TMRV	AMV	PVXm
1st	(1) S-F.C.×Fu.	(S)*Rfrf*	93.5	64.2	68.0				92.7	92.7				
	(4) Fushimiama.	(N)*RfRf*	93.9	86.7	93.8				83.4	94.3				
2nd	(1) S-F.C.×Fu.	(S) *Rfrf*	91.2	75.3	72.5	77.6		89.3					86.9	87.3
	(4) Fushimiama.	(N) *RfRf*	92.6	88.0	90.8	84.9		88.3					91.0	93.6
3rd	(1) S-F.C.×Fu.	(S) *Rfrf*	88.0	65.2	64.1	69.2				74.6	85.6			
	(2) N-F.C.×Fu.	(N) *Rfrf*	90.8	85.1	82.1	80.7				88.8	80.1			
	(3) Fu.×N-F.C.	(N) *Rfrf*	90.5	77.0	71.6	80.4				84.7	83.6			
	(4) Fushimiama.	(N) *RfRf*	84.3	78.7	87.4	81.8				84.5	88.2			
4th	(1) S-F.C.×Fu.	(S) *Rfrf*	91.6	70.9	63.2		63.9					75.2		
	(2) N-F.C.×Fu.	(N) *Rfrf*	94.7	86.4	89.9		89.3					85.7		
	(3) Fu.×N-F.C.	(N) *Rfrf*	90.9	85.5	89.9		90.0					86.9		
	(4) Fushimiama.	(N) *RfRf*	92.0	86.3	90.5		90.4					90.2		

* 1st: 1967. 2nd: 1968 3rd: 1969a. 4th: 1969b.

\+ See text for the abbreviation of virus name.

Since it is evident that a cytoplasmic entity reacts in this way with specific viruses, its nature and origin were discussed, and the following speculation was proposed. A cytoplasmic entity for male sterility in *Capsicum* could have originated from an exogeneous virus in the course of evolution followed by the loss of infectivity. Then it may be supposed to have arrived at a state of a kind of plasmon or plasmid of RNA nature. (The details were submitted to Japan. J. Genetics Vol. 45.)

A Variant Found in the Progeny from Grafting in *Capsicum annuum*

Yasuo OHTA

If a hereditary trait could be altered by grafting, how could such an event take place. One may speculate that the mechanism would be a kind of transformation or transduction. There are evidences which indicate that transformation does take place in insects such as *Bombyx* (Tsujita and Nawa 1969) and *Ephestia* (Nawa and Yamada 1967, 1968). Recently alteration of certain Mendelian traits by grafting was reported in red peppers (*Capsicum annuum* L.) (Kasahara 1967, 1968). My curiosity was aroused and I repeated Kasahara's experiment.

My grafts were performed between two cultivars of *Capsicum annuum* L. provided by Dr. Kasahara: Tochigisantaka (abbrev. T) and Kiiro (K). The material has been maintained by selfing for more than five generations. Three fruit characters were chosen for analysis: erect vs. pendent, fasciculate vs. non-fasciculate fruiting, and yellow vs. red ripe pericarp. Cultivar T is erect, fasciculate, and red (*up*, *fa*, $y^+c_1^+$); and K is pendent, non-fasciculate, and yellow (up^+, fa^+, yc_1). Crossing experiments made for control revealed that three characters are Mendelian traits, the pericarp color being inherited as dihybrid, and that the four genes concerned are inherited independently.

Grafting was made by usual cleft-graft method in reciprocal combinations. Young plants, two to three months old, having 20 to 30 leaves and already initiating flower buds, were used as stocks, while about three weeks old seedlings served as scions. To be sure that the scions depended mainly on the metabolism of the stocks, leaves of the scions were removed twice a week during their growth except for two or three leaves at the top. Several flowers of each scion and stock were artificially self-pollinated. Seeds thus obtained were sown and grown into the G_1 generation. Several plants of each family were randomly selected for selfing and their seeds

gave rise to the G_2 generation.

No particular phenomenon was observed either in the course of grafting (G_0) or in the G_1 generation. As to the G_2 generation, in the progeny of a T scion grafted onto K, a single variant was found whose fruits were red and erect but not fasciculate, indicating a change from recessive *fa* to dominant fa^+. The possibility of a mistake by contamination is refuted by the fact that the plant still possesses another recessive character, namely erect. To obtain more evidences about this variant, whether was it originated by a spontaneous mutation or by grafting, for instance, selfing as well as backcrossing with both parents were made. Also the sister plants were selfed for further investigation.

Induction and Inhibition of Nucellar Embryo Development in *Citrus*

K. FURUSATO aud Y. OHTA

From the viewpoint of citrus breeding it is important to obtain hybrid seedlings from polyembryonic seeds which usually contain nucellar embryos. However, true hybrids can be obtained when the nucellar embryos are eliminated by certain treatments. The present paper deals with experiments aiming at inducing artificial development inhibition of nucellar embryos in the Rutaceae genera, *Citrus*, *Fortunella* and *Poncirus*.

Neither legitimate nor nucellar embryos developed when no pollination was carried out after emasculation in the parthenocarpic and polyembryonic *C. unshiu*. To examine the direct effect of pollination on the number of embryos in a seed, the following crosses were made: polyembryonic (poly-)×poly-species, poly-×monoembryonic (mono-) species and mono-×poly-species. In the case of *C. unshiu* as the female parent, the number of embryos in a seed seemed to be slightly higher when pollen of monoembryonic species was applied than when that of polyembryonic species was used.

Small amounts of 0.01% solution of α-naphthalene acetic acid (αNAA) were applied to the stigmata of 100 emasculated flowers of *C. unshiu*. Of 15 fruits obtained, none had set seeds. When injected into young fruits of both mono- and polyembryonic species, no change was observed in the number of embryos in a seed. In the case of *C. aurea*, a monoembryonic species, young seeds were excised from treated fruits and examined microscopically. No nucellar embryos were found. Maleic hydrazide (MH) solution was either injected into young fruits or sprayed on the leaves of

fruit bearing branches. Generally speaking, the mean embryo number in the treated group decreased slightly in *C. natsudaidai* and *F. japonica*, whereas it showed an increase in *C. unshiu*. In the remaining three species no change was seen.

It was attempted to prevent transportation of nutrients in branches bearing young fruits. Bark ringing produced no change in the number of embryos. Incisions on one side of the branches produced a slightly higher embryo number in *C. unshiu*, *C. sinensis* (Valencia) and *C. pseudoparadisi*, whereas it produced a lower embryo number in *C. leiocarpa* and *F. japonica*, while no change was seen in *C. grandis* and *C. natsudaidai*. Effect of fertilizer and soil was examined with potted three-year-old *F. japonica*. When lime superphosphate was used, the mean embryo number was slightly higher than in the control. This is interesting since it is well known that much phosphate is required for seed formation. Another experiment was carried out to ascertain the effect of fertilizer. 1.0% urea solution was sprayed on the leaves. The embryo number was not affected by the treatment.

Potted plants of *F. japonica* were placed either in a phytotron kept at 30°C constantly day and night or in a glasshouse without heating. The experiment lasted from the end of September, when the plants were in full bloom, to the end of December. During that period the temperature in the glasshouse was below 30°C in daytime and much lower at night. The embryo number was the same at the temperatures used. (The details were published in Seiken Zihô No. 21. 1969)

III. BIOCHEMICAL GENETICS

Syntheses of Drosopterins

Saburo NAWA

The possibility of syntheses of Drosophila eye pigments, drosopterins, on the reaction with reduced pteridine and anion from α-keto acid has been already suggested by Nawa and Forrest (this Annual Report, No. 13). In co-operation with Sugiura and Goto of University of Gakushuin, a large quantity of two kinds of red pigments sufficient for various kinds of analyses were obtained in the reaction between reduced pteridine and *d*, *l*-β-hydroxy-α-keto-butyric acid. Combustion analyses, UV-spectra, ORD-curves, Rf-values and electrophoretic mobilities of the synthetic pigments were identical with those of drosopterin and isodrosopterin respectively. The incubation of 7, 8-dihydro-2-amino-4-hydroxypteridine with *d*, *l*-α-hydroxyacetoacetic acid gave also the same products. Thus, synthesis of drosopterins in this manner was completely proved to be achieved. Furthermore, these synthetic procedures suggest strongly a possibility that *in vivo* drosopterins are derived from the reaction between reduced pteridines and keto-acids. (For details, see Tetrahedron Letters No. 34, 1969).

Genetic Effects of DNA in *Bombyx*

Saburo NAWA, Masa-Aki YAMADA and Mitsuo TSUJITA

It has been shown that treatment of Ephestia larvae or eggs with a recessive eye-color genotype with DNA from wild-type produced wild-type individuals in the treated generation and in subsequent generations (Nawa and Yamada, 1968. Genetics 58: 573). In co-operation with Sakaguchi, the experiment has been designed for inducing DNA-mediated genetic transformation in silkworm. There are many advantages in the use of silkworms in this kind of experiment. For example, the egg color of white-1 is determined not only by its own genotype but reflects that of its mother. Of most important is that eggs are deposited on a paper by a female placed in a separate box (moth funnel) within a short time, making contamination of an egg absolutely impossible.

When larvae of *w*-1/*w*-1 (white eyes and white egg color) were treated

with DNA prepared from wild-type larvae, black-eyed moths were obtained in the treated generation and in the progeny by backcross to untreated *w*-1/*w*-1 moths. The change of *w*-1 to *w*-1$^+$ was detected in eggs in some cases by their color, but there were cases when the change was detected only after raising to adults. Several unexpected phenomena were observed in subsequent backcross generations of these mutants. 1) More than half of the black-eyed moths behaved as if their genotype was *w*-1$^+$/*w*-1$^+$. 2) A few behaved as if their genotype was *w*-1$^+$/*w*-1, but some of them produced significantly higher number of black-eyed adults than would be expected, when crossed to untreated *w*-1/*w*-1. 3) Egg color produced by some of these mutants was unusual and the abnormality was also found even in later generations. 4) Sometimes, repeated appearance of mutations in later generations of outcrossing of mutants was observed. Mechanisms for these unusual feature were considered in terms of replication of the effect of incorporated DNA. (The details were submitted to Genetics).

A triple recessive genotype, *lem*; *oc*; *pe* was also treated with +DNA. Both in the progeny by cross of the treated animals to untreated *lem*; *oc*; *pe* and in the 2nd backcross generation, several individuals having the genotype *lem*/*lem*; *oc*/*oc*; *pe*$^+$/*pe* were obtained. It was observed that the gene *pe*$^+$ has persisted for several generations and segregated like a normal allele at the *pe* locus. So far no instance for the change of *lem*→*lem*$^+$ or *oc*→*oc*$^+$ has been obtained.

Characterization of Incorporated DNA in Ephestia Eggs Treated with DNA

Masa-Aki YAMADA and Saburo NAWA

Evidences of DNA uptake by Ephestia eggs have been reported previously (Annual Report No. 18). DNA was extracted from eggs treated with ^{3}H-DNA of Salmonella for 4 hours using SDS-phenol method. Preparative cesium chloride density gradient centrifugation of the DNA solution was carried out in SW 39 rotor of Spinco L centrifuge. The patterns of DNA fractionation showed that the peaks of radioactivity were found at the positions of double stranded and single stranded DNA of Salmonella. This was verified by analyzing the mixture of native and denatured Salmonella DNA. When DNA extracted from the eggs immediately after the treatment with labelled DNA was fractionated, single stranded DNA was detected as a shoulder of the double stranded peak,

but when eggs were left standing for 15 hours after treatment with DNA single stranded DNA was obtained in a separate peak higher than that of the double strand. These results indicate that DNA was incorporated into the eggs at double stranded state and was denatured gradually into the single strand.

DNA Specific in Early Development in *Ephestia*

Masa-Aki YAMADA

DNA extracted from eggs at an early development stage was found to be heterogeneous. Eggs were collected for 0-18 hours after spontaneous laying in plastic boxes. DNA was extracted from the eggs with Tris-SDS-phenol method, treated with RNase and deproteinized with chloroform. The density of DNA was analyzed by using Spinco E analytical centrifuge. Preparative cesium chloride density gradient centrifugation of the DNA was performed in an angle rotor #40 of Spinco L centrifuge. It was found that the eggs at the early stage of development contained at least two DNA fractions which were sensitive to DNase. The values of their buoyant densities were 1.696 (the same as that of adult DNA) and 1.672. The distribution of the main 1.696 fraction in density gradient was asymmetrical, indicating that the fraction was heterogeneous. When this DNA was denatured at 100° for 20 minutes, two bands with density of 1.723 and 1.702 were obtained, but in the adult DNA denatured at 100° only one band with density 1.723 was detected. Therefore the 1.696 fraction may have consisted of two components. The minor component with 1.672 density was specific at the early development stage, since it was not found in late stage eggs. Further study of these components is in progress.

Incorporation of ^{14}C-phenylalanine or ^{14}C-tyrosine into the Hypodermal Cuticle of the Silkworm. I. Lethal Lemon Larvae

Mitsuo TSUJITA and Susumu SAKURAI

Incorporation experiments with ^{14}C-phenylalanine or ^{14}C-tyrosine as metabolites into the hypodermal cuticle of normal, lemon and lethal lemon larvae were carried out. It was found from the experimental results that in lethal lemon larvae the radioactivity of alkali-insoluble protein containing as principal component of hard cuticle protein, sclerotin, is conspicuously weaker in both head and body than in normal larvae.

Incorporation of ^{14}C-phenylalanine or ^{14}C-tyrosine into the Hypodermal Cuticle of the Silkworm. II. Lethal Albino Larvae

Mitsuo TSUJITA and Susumu SAKURAI

Incorporation experiments with ^{14}C-phenylalanine or ^{14}C-tyrosine as metabolites into the hypodermal cuticle of normal and lethal albino larvae were carried out. It was found that in lethal albino larvae also the alkali-insoluble protein containing as the principal component of hard cuticle protein, sclerotin, is conspicuously weaker in both head and body parts than in normal larvae.

Sepiapterin Reductase in the Silkworm

Mitsuo TSUJITA and Susumu SAKURAI

The occurrence of two different reductases, sepiapterin reductase and dihydrofolate reductase, in the silkworm was demonstrated (Matsubara *et al.* 1963. Nature 199: 808–809 and Katoh *et al.* 1969. Handbook of IVth Intern. Cong. Pteridine: 32–33). The former reductase catalyses sepiapterin to dihydrobiopterin and the latter catalyses dihydrofolate to tetrahydrofolate.

In our previous paper (Tsujita 1961. Jap. J. Genet. 36: 337–346) the enzyme reducing sepiapterin in the reaction solution containing crude enzyme solution prepared from normal larvae, NADPH and phosphate buffer at pH 6.2–7.0 was called pterin reductase. For the catalytic reaction by this enzyme, the requirement for NADPH as a cofactor is quite specific and the optimum rate of the reaction occurred at about pH 6.8. The enzyme activity in the reaction solution is not inhibited by aminopterin. The appearance and increase of dihydrobiopterin in the reaction solution was detected.

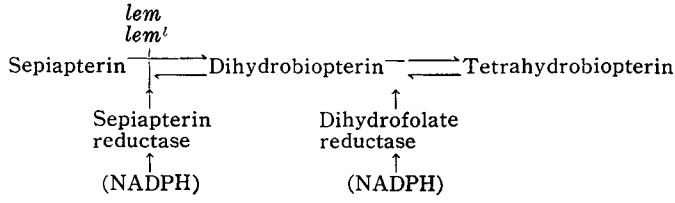

Fig. 1. Main pteridine metabolic reaction in the silkworm.

It may be safely said from these experimental results that the enzyme reducing sepiapterin in our experiments is really sepiapterin reductase. This enzyme activity is strong in normal larvae, but in lemon larvae a

much lower activity is detected than in normal larvae. Furthermore, in lethal lemon larvae the enzyme activity is almost lacking.

Each crude enzyme solution prepared from normal, lemon or lethal lemon larvae showed almost the same activity of dihydrofolate reductase.

It is considered from these experimental results that the main pteridine metabolic reaction as shown in Figure 1 was taking place by the two kinds of reductase.

Characterization of Pteridine Granule Membrane in Larval Skin Cells of the Silkworm

Susumu SAKURAI and Mitsuo TSUJITA

Using pteridine granules isolated from the larval hypodermis of a normal silkworm strain and various abnormal oily mutants the following chemical analysis was carried out.

Isolated pteridine granules were solubilized by adding 1% sodium deoxycholate in 0.01 M $NaHCO_3$-0.01 M ammonium formate buffer (pH 9) at room temperature. Dialysis was carried against 0.01 M $NaHCO_3$-1 mM EDTA-1 mM 2-mercaptoethanol-0.01 M ammonium formate buffer (pH 9) in a cold room at 4°C. The dialysed sample was chromatographed on a 4×55 cm column of Sephadex G 200 equilibrated with 0.01 M $NaHCO_3$-1 mM EDTA-1 mM 2-mercaptoethanol-0.01 M ammonium formate buffer (pH 9). The protein eluted at the void volume was dialyzed overnight at 4°C against 0.01 M $NaHCO_3$-EDTA-mercaptoethanol-ammonium formate buffer (pH 9) and the sample was precipitated between pH 4.0 and 5.6, indicating a low isoelectric point typical of acidic protein.

The lipid content by weight of the chloroform-methanol soluble fraction amounted to 35–40% of dry weight. Protein content was 50–60% of membrane dry weight. Carbohydrates seemed to be low accounting for 0.3–1.0% of dry weight. These results show that pteridine granule membrane protein was composed almost entirely of protein and lipid.

The hydrolysates by 6 N HCl at 110° of lipid-free membrane proteins obtained from oily mutants and normal strains were analyzed by an automatic amino acid analyzer. The results indicated that membrane proteins were replete with hydrophobic amino acids, such as alanine, leucines, phenylalanine, methionine and valine. Cysteine content was the lowest and that of acidic amino acids, aspartic acid, glutamic acid was high in comparison with the basic amino acid content. The hydrophobic and acidic amino acid composition of the membrane proteins of w^b oily mutant and C-124 normal strain were very similar. On the

contrary, lysine content of normal membrane protein was somewhat low in comparison with w^b oily mutant.

Immunochemical Study of Pteridine Granule Membrane in Hypodermal Cells of Silkworm Larvae

Susumu SAKURAI

As a part of investigations planned to clarify the relationship between normal and abnormal proteins, an immunological analysis of membrane proteins was carried out with the use of Ouchterlony double-diffusion reaction.

C-124, *E lem* and W-C were used as normal strains with non-transparent larval skin, and w^a, w^b, w^{oh} (Aojyukuhakuran oily) and *oa* were used as mutant strains with semi-transparent or transparent larval skin. It has been known that among those mutants w^a, w^b and w^{oh} belong to w_3 multiple allelic series located on chromosome 10, but that *oa* is located on chromosome 14.

Immunization was done in rabbits by eight intravenous injections of 80 mg membrane protein, each in 1% aluminium sulfate solution over a period of one month. Precipitin lines were allowed to develop at room temperature. Using antiserum to normal membrane protein or using antiserum to abnormal membrane proteins obtained from oily mutants, several membrane preparations from different oily mutants were compared in double diffusion analysis.

On immunodiffusion, anti-*E lem* normal membrane protein antibody gave a single precipitin band with its antigen, normal membrane protein of *E lem*. Anti-w^{oh} abnormal membrane protein antibody reacted against its antigen, and abnormal membrane protein of w^{oh} oily mutant displayed several precipitin bands. On the other hand, membrane protein of *oa* oily mutant gave a precipitin line with anti-*E lem* membrane antibody, indicating an identity pattern with membrane protein of *E lem*. When membrane proteins of *E lem* and w^a oily mutant were allowed to react with anti-*E lem* membrane antibody a reaction showing a complete difference occurred. Similar results were seen on immunodiffusion when anti-*E lem* membrane antibody reacted with membrane protein of *E lem* and membrane proteins of w^{oh} and w^b oily mutants. These facts suggest that transparency of larval skin of w_3 multi-allelic oily mutants is due to a defect of membrane proteins themselves.

Aldolase Isozyme Patterns of Brain and Brain Tumors in Mammals

Junkoh YAMASHITA and Kazuo MORIWAKI

It has already been reported that there were three molecular species of aldolase in mammalian tissues; aldolase A in muscle, aldolase B in liver and aldolase C in brain, and that the aldolase B found in normal rat liver disappeared in fast-growing rat hepatomas and instead aldolase A was produced.

1) The brain of the Japanese monkey (*Macaca fuscata*), was studied to ascertain whether or not aldolase patterns have topographic characteristics within the brain. The sample was homogenized with one volume of 20 mM tris-HCl buffer (pH 7.4) containing 0.15 M KCl and 5 mM EDTA. The aldolase of the supernatant fraction obtained after centrifugation at 105,000 g for 60 min was separated electrophoretically on a cellulose acetate membrane and stained as described by Matsushima *et al.* (Biochem. Biophys. Res. Comm. 30: 565–570, 1978). All parts of the brain showed five distinct bands of A-C type. There were aldolase C(C4) on the cathodal side, aldolase A(A4) on the anodal side and three hybrid bands (A3C1, A2C2 and A1C3) between them. The patterns in the cerebral cortex, corpus callosum, hippocampus, thalamus, midbrain, pons and medulla were similar and A3C1 activity was the most pronounced among the five bands, while both in the cerebellar vermis and hemispheres the most pronounced was C4 band. No gross difference was detected between the gray and the white matter. The peripheral nerve obtained from the sciatic nerve also showed five bands of A-C type, among which A4 was predominant, although the overall activities were weaker than in the brain.

2) The aldolase patterns of three transplantable mouse gliomas were investigated. No. 4 and No. 5 gliomas showed five bands of A-C type, with more pronounced activities at A1C3 and A2C2 instead of A4 and C4 of normal mouse whole brain. 203 glioma, on the other hand, presented four bands lacking in C4, but definitely carried C components such as A1C3, A2C2 and A3C1. In contrast to No. 4 and No. 5 gliomas which were recently induced, 203 glioma had been serially transplanted through 23 generations. The findings indicated that all three tumors had their origins in the brain, although the present aldolase patterns somehow deviated from the original form.

3) One case of human brain tumor (glioblastoma) obtained as a surginal specimen was studied in similar manner. Comparison was made with the normal cerebral cortex which had to be sacrificed in the surgical procedures. This tumor showed five bands of A-C type, but C4 activity

was extremely weak.

These results coincided with observations made in hepatomas, namely that the aldolase isozyme patterns have a common trend to become closer to the muscular (A) type during tumor progression. It is very likely that the aldolase isozyme patterns of brain tumors might be new diagnostic tools helping to evaluate their origin and the degree of malignancy, histological diagnoses being equivocal.

Biochemical Characters of Serum Transferrin in Several *Rattus* Species Collected from South-East Asia and Oceania

Kazuo MORIWAKI, Kimiyuki TSUCHIYA and Harumi SAKATA

Electrophoretic pattern and molecular weight of serum transferrin of several wild *Rattus* species collected from South-East Asia and Oceania were compared. Identification of transferrin bands in starch gel electrophoresis was carried out by labelling serum transferrin with Fe^{59} and by autoradiography on non-screen type X-ray film. Approximate molecular weight of transferrin was determined by thin layer gel-filtration on Sephadex G-200 plates. Fe^{59} labelled serum was applied on these plates, followed by 20 hours elution. Separated serum proteins were taken up on a filter paper on which the mobility of transferrin, that is its approximate molecular weight, was determined by autoradiography. The data thus obtained are summarized in Table 1.

Table 1. Electrophoretic pattern and molecular weight of serum transferrin in several *Rattus* species collected from South-East Asia and Oceania

Species	Place of collection	Electrophoretic bands of Tf		Approx. molecular weight	Chromosome number (2n)[1)]
		Between origin and $S\alpha_2$	Between $S\alpha_2$ and Hp.		
Rattus rattus	Okinawa, Japan	0	2	70,000	42
〃	Tainan, Taiwan	0	2	〃	42
〃	Khao Yai, Thailand	0	0	〃	42
〃	Luzon, Philippines	0	2	〃	42
〃	Bogor, Java	0	2	〃	42
〃	Makasar, Celebes	0	2	〃	42
R. Bowersii	Kuala Lumpur, Malaysia	0	1	〃	40*
R. sabanus	Kuala Lumpur, Malaysia	0	1	〃	42

Table 1 (continued)

Species	Place of collection	Electrophoretic bands of Tf		Approx. molecular weight	Chromosome number (2n)[1]
		Between origin and $S\alpha_2$	Between $S\alpha_2$ and Hp.		
R. jalorensis	Kuala Lumpur, Malaysia	0	2	70,000	42
R. argentiventer	Kuala Lumpur, Malaysia	0	2	〃	42
R. exulans	Makasar, Celebes	0	2	〃	42
Bandicota indica	Tainan, Taiwan	0	2	〃	44
R. rattus rattus	Cairns, Australia	2 1	0 1	〃	38
〃	Brisbane, Australia	2 1	0 1	〃	38
〃	Wellington, New Zealand	2 1	0 1	〃	38
〃	Port Moresby, New Guinea	2 1	0 1	〃	38
R. Conatus	Cairns, Australia	1	1	〃	32
R. fuscipes	Brisbane, Australia	2	0	〃	38(39)
R. muelleri	Kuala Lumpur, Malaysia	1	0	〃	42
R. diardi	Kuala Lumpur, Malaysia	2	2	〃	42(43,44)
R. rattus (Japan) × *R. rattus rattus*	F_1	2	2	〃	40

1) Yosida, T. H. *et al.* 1969. Mam. Chrom. News. 10: 217-219.

* This data will be reported in this Annual Report, 1970. Abbreviations: Tf; transferrin, $S\alpha_2$; Slow α_2-macroglobulin, Hp; Haptoglobin.

Organ-Specific Expression of Allelic Peroxidase Isozymes in Heterozygotes of *Oryza perennis*

Toru ENDO

Two allelic peroxidase isozymes, 2A and 4A, were zymographically detected on starch gels in an annual strain W107 (P_1) and a perennial strain W1294 (P_2) of a wild rice, *Oryza perennis* Moench, respectively. Both isozymes were specified by codominant alleles, Pe^{2A} and Pe^{4A} at the *Pe* locus (Shahi *et al.* 1969. Japan. J. Genent. 44: 321). The heterozygote Pe^{2A}/Pe^{4A} produced a hybrid isozyme 3A together with both parental isozymes in some plant organs, *i.e*, leaf blade and leaf sheath as well as in lemma and palea. An organ-specific difference in the intensity ratio of isozyme bands was observed. In the leaf blade and leaf sheath, the

intensity ratio seemed to follow the distribution expected from random association of the monomer components of 2A and 4A isozymes. However, the ratio of the three bands in lemma and palea deviated from expectation as the intensity of 2A was very weak and that of 3A did not correspond to the sum of the intensities of 2A and 4A bands. The same fact was also found in 24 out of 25 heterozygous F_2 segregants.

The zymograms of 60 F_2 plants were classified into P_1, F_1 and P_2 types. The ratio was considered to be 1:2:1, under the assumption that it was distorted due to gametic selection whose coefficient was calculated to be 0.857. One particular segregant was found which had an F_1 type zymogram but the intensity ratio of the three bands corresponded to the expected distribution from association of the monomers, *i.e.*, the intensity of 3A was approximately the sum of those of 2A and 4A bands. In leaf sheath zymograms, however, no difference in the intensity ratio was detected among the 25 F_2 heterozygotes, including that particular plant. Such an organ-specific profile of the allelic isozymes in the heterozygotes was tentatively explained by a competitive activation of the alleles due to two kinds of inducing systems each carried by the parental strains. In the particular plant, one of the inducing systems might have been deleted or inactivated by mutation, resulting in the activation of both alleles at a similar level.

IV. EVOLUTIONARY GENETICS

Intraspecific Differentiation of Genome in *Aegilops caudata*

Hitoshi KIHARA and Yasuo OHTA

Aegilops caudata L. includes two varieties, *typica* and *polyathera* Boiss. These two varieties are characterized by awnedness at the lateral spikelets. The following three strains were used in our experiment:

	Variety	Origin	Characters
No. 1	*polyathera*	Turkey	awned, waxy, early
No. 2	*typica*	Volos, Greece	awnless, non-waxy, late
No. 3	*typica*	Hama, Syria	awnless, non-waxy, early

Reciprocal hybrids between any two of the three strains were easily obtained. The chromosome behavior in the F_1's was normal throughout meiosis, but the hybrids were almost completely pollen sterile. For the combination of no. 1 with no. 2, for instance, the reciprocal hybrids backcrossed successively in great numbers to both parents as pollen providers gave rise to two substitution backcross lines (SB) and two restoration backcross lines (RB) as follows:

$$(\text{no. 1 } ♀ \times \text{no. 2 } ♂)\ F_1 \begin{cases} \times(\text{no. 1 } ♂)^n \cdots\cdots RB_n \\ \times(\text{no. 2 } ♂)^n \cdots\cdots SB_n \end{cases}$$

$$(\text{no. 2 } ♀ \times \text{no. 1 } ♂)\ F_1 \begin{cases} \times(\text{no. 1 } ♂)^n \cdots\cdots SB_n \\ \times(\text{no. 2 } ♂)^n \cdots\cdots RB_n \end{cases}$$

In the same way the other two combinations were carried out so that in all six SB lines and six RB lines were obtained.

In the combination of no. 1 with no. 2, both pollen fertility and seed setting began to increase from B_1 and normal fertility was recovered by B_2 in both SB and RB lines when backcrossed to no. 2 as the male parent (Figure 1). On the contrary, when no. 1 was the male parent, pollen and seed fertility remained almost at zero until B_2, then began to increase from B_3 and normal fertility was recovered by B_5 in both SB and RB lines. Such delay in the repair of fertility by successive backcrosses was termed "delayed effect" by Kihara (Kihara, H. 1968. Proc. III Internatl. Wheat. Genet. Symp.: 125–134). The delayed effect was observed in the SB line with cytoplasm of no. 2 and in the RB line with cytoplasm of no. 1. The latter case is most interesting since the delayed effect appeared in no. 1's own cytoplasm.

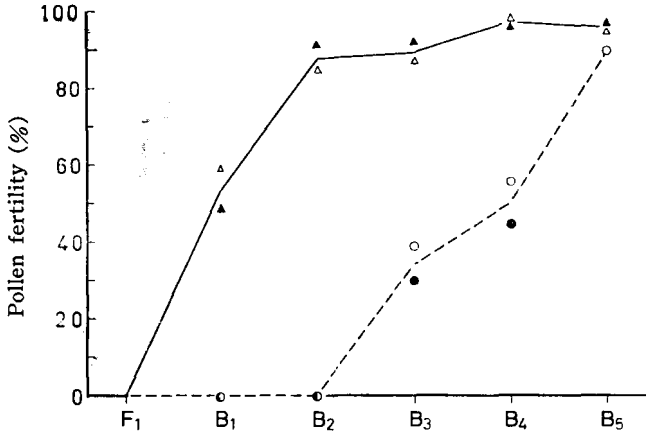

Fig. 1. Pollen fertility in successive backcrosses in the restoration and substitution lines of reciprocal hybrids, *Aegilops caudata* no. 1×no. 2 (1964-1969).
○: RB to no. 1. ●: SB to no. 1.
△: RB to no. 2. ▲: SB to no. 2.

In the combination of no. 2 and no. 3, only the B_2 backcross genera tion is available until now but essentially the same tendency may be already noticed concerning the recovery of fertility. While in the combination of no. 1 and no. 3, pollen fertility was about 10 and 13 per cent in the reciprocal F_1's, it was about 69 and 58 per cent in B_1, about 70 and 65 per cent in B_2 backcrossed with pollen of no. 3 and no. 1, respectively. Distinct delayed effect was not observed in this combination but no. 1 was slightly inferior to no. 3 as pollen parent in recovering fertility.

The real cause of the delayed effect is still unknown. From our investigations, it may be concluded i) that a delayed effect was observed between no. 1 and no. 2 and also between no. 2 and no. 3 even in RB lines having their own cytoplasms, but not between no. 1 and no. 3, ii) that no. 2 recovered fertility quicker than no. 1 or no. 3 in the order of

$$\text{no. } 2 \gg \text{no. } 3 > \text{no. } 1,$$

indicating that the three strains of *Ae. caudata* are slightly different in their genomes but, probably not in their cytoplasms. The situation is a little different from our previous expectation.

Comparison of the Developmental Pattern between Cultivated and Wild Types of Rice

Hiroko MORISHIMA and H.I. OKA

To look into difference in the developmental patterns between wild and cultivated types and their variation due to growing condition, three *sativa*, two *perennis* and one *glaberrima* strains were grown in gravel culture (using Matsushima's formula) and in paddy, and were measured for dry matter weight, length of various organs and other yield characters. Measurements were taken three times during the growing period and at maturity. The results may be summarized as follows: 1) The wild type has a lower growth rate in the early stage than the cultivated type. 2) Its growth rate rapidly increases at a certain stage before heading, but also rapidly declines after the maximum is attained. In contrast, improved cultivated varieties were found to keep up a relatively low growth rate for a long period. 3) In cultivated types the panicle and the third internode (from top) elongate simultaneously about ten days before heading, and the elongation of first and second internodes results in heading. In the wild type, the elongation of the third internode results in heading, and the first and second internodes elongate after heading. Probably an amount of nutrients may be consumed in this process reducing the deposit of carbohydrates in the endosperms. 4) Examining the variation in size of various organs due to growing conditions, wild plants appeared to be phenotypically more plastic than cultivated plants. They showed a large variability in the length of panicle and internodes which were much less variable in cultivated types.

A Survey of Genetic Variations in the Populations of Wild *Oryza* Species and their Cultivated Relatives

Hiroko MORISHIMA and H.I. OKA

The first-generation plants raised from population seed samples belonging to *Oryza perennis*, *O. sativa*, *O. breviligulata* and *O. glaberrima*, each collected from its natural habitat or a farmer's field, were observed for various metric characters, and their intrapopulational genetic variability was estimated for each character and also in terms of generalized variance. In *O. perennis*, perennial and allogamous forms generally had in their populations more genetic variations than the annual and autogamous forms, while the latter were more diversified from population to population.

The level of heterozygosity was estimated from the ratio of within-line to between-line component of genetic variance. Perennial and allogamous forms were more heterozygous than annual and autogamous ones. Diallel analysis of a *perennis* population showed appreciably large mean squares due to dominance for certain characters. Further, intrapopulational variability in spikelet size was observed using the original seeds collected from various sites. Though cultivated forms (*sativa* and *glaberrima*) were largely homozygous, their populations in primitive agricultural conditions contained as much genetic variations as wild forms. This was considered to be due to introgression and disruptive selection in the environment.

V. MATHEMATICAL AND STATISTICAL STUDIES ON POPULATION GENETICS

The Rate of Molecular Evolution Considered from the Standpoint of Population Genetics

Motoo KIMURA

The rate of amino acid substitutions was computed for hemoglobins in various lines of vertebrate evolution. The method of calculation was as follows. Let n_{aa} be the total number of amino acids in each of two homologous polypeptide chains being compared with each other and let d_{aa} be the number of sites in which they are different. Then the mean number (K_{aa}) of substitutions per amino acid site over the whole evolutionary period that separated these two polypeptides may be estimated from

$$K_{aa}=-2.30\log_{10}(1-p_d)\,,$$

where $p_d=d_{aa}/n_{aa}$ is the fraction of differing sites. The standard error of this estimate is

$$\sigma_K=\sqrt{p_d/\{(1-p_d)n_{aa}\}}\,.$$

The rate of substitution per amino acid site per year may then be computed from

$$k_{aa}=K_{aa}/(2T)\,,$$

where T is the number of years that have elapsed since divergence from a common ancestor.

The estimated rates of amino acid substitutions based on comparisons of the alpha hemoglobin chains of various mammals with that of the carp are about the same as those based on comparisons of the carp alpha and mammalian beta or the alpha and beta chains in mammals. All these comparisons give roughly 10^{-9} for k_{aa}. In addition, comparison of human beta hemoglobin with lamprey globin gives a similar value.

These remarkable uniformities in the rate of amino acid substitutions in evolution support the hypothesis that a large fraction of amino acid substitutions that occurred in these proteins are result of random fixation of selectively neutral or nearly neutral mutations.

This leads to the conclusion that random genetic drift is playing an important role in determining the genetic structure of biological

populations and the prediction that genes in "living fossils" will have undergone as many DNA base substitutions as corresponding genes in more rapidly evolving species.

A new term *pauling* was proposed for a unit of evolutionary rate at the molecular level, defined as the rate of substitution of 10^{-9} per amino acid site per year.

For details, see Kimura (1969. PNAS 63:1181-1188).

The Number of Heterozygous Nucleotide Sites Maintained in a Finite Population Due to Steady Flux of Mutations

Motoo KIMURA

In the present study the following model was assumed: The number of nucleotide sites making up the haploid genome is so large, while the mutation rate per site is so low, that whenever a mutation occurs it represents a mutation at a previously homallelic site, that is, a site in which no mutant forms are segregating in the population.

Since a mutant that appears in a finite population is either lost from the population or fixed in it within a finite length of time, under continued production of new mutants over many generations, a statistical equilibrium will be reached between the production of new mutants and their random extinction or fixation. In such an equilibrium, a stable distribution will be realized with respect to frequencies of mutants among different sites, if we restrict our consideration only to those sites in which mutant forms are currently segregating.

We will denote by $\Phi(p, x)$ the frequency distribution at equilibrium such that $\Phi(p, x)dx$ is the expected number of sites in which the frequencies of mutants lie in the range $(x, x+dx)$, and p is the frequency at the time of the first occurrence of the mutant at each site.

Let ν_m be the number of sites in which new mutants appear in the population each generation, and let

$$I_f(p)=\int_0^1 f(x)\Phi(p, x)dx$$

be the expection of an arbitrary function $f(x)$ with respect to this distribution. Integration in the right hand side of the above equation is over the open interval (0, 1). This is an approximation to the summation over all frequency classes from $x=1/(2N)$ to $x=1-1/(2N)$. It can

then be shown that I_f is given by

$$I_f(p)=\{1-u(p)\}\int_0^p \psi_f(\xi)u(\xi)\,d\xi+u(p)\int_p^1 \psi_f(\xi)\{1-u(\xi)\}d\xi\ ,$$

where

$$\psi_f(\xi)=2\nu_m f(\xi)/\{V_{\delta\xi}u'(\xi)\}\ ,$$

in which $u(p)$ is the probability of eventual fixation, $u'(\xi)=du(\xi)/d\xi$, and $V_{\delta x}$ is the variance in the rate of change in mutant frequency x per generation.

The number of heterozygous nucleotide sites per individual at equilibrium (denoted by $H(p)$) is given by $I_f(p)$ with $f=2x(1-x)$. In the special case of neutral mutations, $u(p)=p$ and the above formula for I_f reduces to

$$H(p)=4N_e\nu_m p(1-p)\ ,$$

where N_e is the effective population number.

In a mammalian species having an effective population number of 10,000, if the majority of molecular mutations due to base substitutions are almost neutral with respect to natural selection, and if they occur at the rate of 2 per gamete per generation, the average number of heterozygous nucleotide sites per individual becomes about 8×10^4. Mutation is used here to designate any change in the nuclear DNA whether it codes protein or not.

For details, see Kimura (1969. Genetics 61:893-903).

The Average Number of Generations until Extinction of an Individual Mutant Gene in a Finite Population

Motoo KIMURA and Tomoko OHTA

Since a majority of mutant genes are lost from the population by chance within a small number of generations, it is desirable to know the average number of generations until extinction of an individual mutant gene in a finite population, excluding those cases where it is eventually fixed. Using the method of diffusion equations, we have obtained approximation formulae for the cases of selectively neutral, semidominant deleterious, and recessive deleterious mutations.

Let t_0 be the mean number of generations until extinction of an individual mutant gene (excluding the cases of eventual fixation) in a

population with "variance" effective number N_e and actual population number N. When the mutant is selectively neutral, we have

$$t_0=\frac{4N_e}{2N-1}\log_e(2N)\approx\frac{2N_e}{N}\log_e(2N)\ . \qquad (1)$$

When the mutant is deleterious and semidominant with selection coefficients s' and $s'/2$ against the mutant homo- and heterozygotes respectively,

$$t_0=\frac{2N_e}{N}\{\log_e(2N)-\log_e(2N_es')+1-\gamma\}\ , \qquad (2)$$

where γ is Euler's constant, 0.577, and $2N_es'$ is assumed to be much larger than 1.

When the mutant is recessive with selection coefficient s' against the mutant homozygotes,

$$t_0=\frac{2N_e}{N}\left\{\log_e(2N)-\frac{1}{2}\log_e(2N_es')+1-\frac{\gamma}{2}\right\} \qquad (3)$$

Monte Carlo experiments were performed to check these three formulae. The agreement between the theoretical and experimental results was satisfactory.

For details, see Kimura and Ohta (1969. Genetics 63:701-709).

Linkage Disequilibrium at Steady State Determined by Random Genetic Drift and Recurrent Mutation

Tomoko OHTA and Motoo KIMURA

Linkage disequilibrium caused by random genetic drift in a finite population was investigated using diffusion models, assuming that a steady state has been reached under recurrent mutation and random sampling of gametes.

We will assume that a pair of alleles A_1 and A_2 are segregating in the first locus and alleles B_1 and B_2 are segregating in the second locus. Let g_1, g_2, g_3 and g_4 be the frequencies of the 4 chromosome types A_1B_1, A_1B_2, A_2B_1 and A_2B_2 in the population. Then, the coefficient of linkage disequilibrium is $D=g_1g_4-g_2g_3$, the frequency of A_1 is $p=g_1+g_2$ and the frequency of B_1, $q=g_1+g_3$. By solving the equations for the moments of the distribution the expected value of D^2 was obtained in terms of N_ec and N_ek, where N_e is the effective population number, c is the

recombination fraction between the two loci and k is the sum of the mutation rates in all directions.

Since the value of D depends heavily on gene frequencies, a more stable measure of linkage disequilibrium, $\sigma_d^2=E\{D^2\}/E\{pq(1-p)(1-q)\}$ has been used, and σ_d was termed the standard linkage deviation. It was then shown that, at equilibrium, we have

$$\sigma_d^2=1/\{3+4N_e(c+k)-4/(5+2N_ec+4N_ek)\}\ .$$

The standard linkage deviation, σ_d, is approximately equal to the correlation of gene frequencies between the two loci. For a large N_ec, σ_d^2 becomes about $1/(4N_ec)$, and this also holds for the case of steady decay without mutation.

As long as all the segregating loci are selectively neutral, linkage disequilibrium has no effect on the behavior of individual genes. However, when selection occurs, the effect of linkage disequilibrium may become important.

Apparent overdominance may arise at an intrinsically neutral locus by the presence of overdominant or ordinarily dominant loci tightly linked to it. The magnitude of such overdominance is roughly proportional to the sum of D^2 between the neutral and the remaining selected loci.

For details, see Ohta and Kimura (1969. Genetics 63:229–238).

Some Relationships between Local and Global Genetic Variabilities

Takeo MARUYAMA

Consider a typical natural population occupying a geographically structured habitat in which migration occurs. Let f_0 be the probability that the homologous genes in actually mated gametes are identical by descent, and let $\bar{f}$ be the same probability for two homologous genes chosen randomly from the whole population. Then for neutral genes at equilibrium, the following relationship holds,

$$\bar{f}=\frac{(1-f_0)(1-2u)}{4Nu}$$

where u is the mutation rate and N is the total population size.

If $u=0$, we have transient processes in which f_0 and $\bar{f}$ approach zero as time increases. The rate at which these quantities approach zero is of genetical interest. Let t indicate the generation, $\bar{h}(t)=1-\bar{f}^{(t)}$ and

$h_0(t)=1-f_0^{(t)}$. Then the following relationships hold,

$$\frac{\bar{h}(t+1)}{\bar{h}(t)}=1-\frac{h_0(t)}{2N\bar{h}(t)} \qquad (1)$$

and

$$h_0(t)=-2N\frac{d\bar{h}(t)}{dt}\ .$$

Formula (1) due to Robertson (1964).

Speed of Gene Substitution in a Geographically Structured Population

Takeo MARUYAMA

In evolutionary theory, the number of generations (T) required for a new gene arisen by mutation to become fixed in the population plays an important role. This problem was first solved by Kimura and Ohta (1969) for a random mating population of finite size. Later Kimura (1970) obtained the distribution for this quantity. Many natural populations, if not all, have a geographical structure and matings in a population depend on the distance between the two individuals. Thus as a whole the population may not be a random mating unit and this fact should be taken into account in the calculation. Using the one- and two-dimensional stepping stone models, this problem was investigated. The result can be expressed as follows,

$$\text{mean of } T=\frac{2}{\lambda}$$

$$\text{second moment of } T=\frac{6}{\lambda^2}$$

in which

$\lambda\approx\frac{10m}{4n^2}$ (for large n), $\lambda\approx\frac{1}{2Nn}$ (for large N) for one-dimensional habitat,

$\lambda\approx\frac{m}{4n}$ (for large n), $\lambda\approx\frac{1}{2Nn}$ (for large N) for two-dimensional square habitat,

where n=number of colonies, N=colony size and m=migration rate. It was also shown that the distribution of T from simulation experiments fits well with Kimura's distribution obtained for a random mating

population. This is illustrated in Table 1.

Table 1. Frequency distribution of $T/E[T]$ where E indicates the mean value of T. The parameters are $n=10$, $N=1$ and $m=0.1$. The number of the simulations from which the table was constructed was 100

$T/E[T]$	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.5<
Simulation	.01	.06	.06	.11	.12	.13	.05	.07	.04	.05	.04	.04	.02	.20
Kimura's formula	.01	.04	.07	.10	.11	.10	.09	.08	.07	.06	.05	.04	.03	.15

On the Fixation Probability of Mutant Genes in a Subdivided Population

Takeo MARUYAMA

Since all natural populations are geographically distributed, it is an important problem to consider the ultimate fixation probability for mutant genes in a population with geographical structure. Although populations may be usually continuously distributed, the two-dimensional stepping stone models is often a good approximation to their structure. Here we will consider a population consisting of a finite number of partially isolated colonies and obtain an approximation formula for the fixation probability for one locus with two alleles A_1 and A_2 which are additive in fitness.

Let N_i be the size of colony i, $Y_i^{(t)}$ be the number of A_2 alleles in colony i at the t-th generation and $Y^{(t)}$ be the total number of A_2 alleles at the t-th generation, i.e. $Y^{(t)}=\sum_i Y_i^{(t)}$ and $N=\sum_i N_i$. Assume that the sampling of gametes from one generation to the next is done independently in each colony. Then we have,

$$E[\exp(-2sY_1^{(t)})]\cdot E[(\exp(-2sY_2^{(t)})]\cdot\ \cdots=E[\exp(-2sY^{(t)})]\ , \qquad (1)$$

where E stands for the expected value. Now suppose that selection occurs independently in each colony. Then

$$E[\exp(-2sY_i^{(t)})]\approx E[\exp(-2sY_i^{(t+1)})]\ , \qquad (2)$$

for all i. Thus

$$E[\exp(-2sY_1^{(t+1)})]\cdot E[\exp(-2sY_2^{(t+1)})]\cdot\ \cdots=E[\exp(-2s(\sum_i Y_i^{(t+1)}))]$$
$$\equiv E[\exp(-2sY^{(t+1)})]\approx E[\exp(-2sY^{(t)})]\ ,$$

follows from (1) and (2). Therefore $E[\exp(-2sY^{(t)})]$ is invariant over

population subdivision and selection within colonies. The invariance still holds over migration of individuals between colonies since this does not change the total number of A_2 alleles. If each colony can be reached from any other colony by migration in one generation or several generations either A_1 or A_2 will be eventually lost from the whole population. For such a situation, as above, we have

$$\exp(-2sY^{(0)}) \approx U(\bar{p}) \exp(-2s2N) + (1-U(\bar{p})) ,$$

in which $U(\bar{p})$ is the fixation probability of A_2 in the population with the initial frequency,

$$\bar{p} = \frac{1}{N} \sum_i N_i (Y_i^{(0)}/2N_i) = \frac{1}{N} \sum_i p_i N_i ,$$

where p_i is the initial frequency in colony i. Therefore we have

$$U(\bar{p}) = \frac{1-\exp(-4Ns\bar{p})}{1-\exp(-4Ns)} . \qquad (3)$$

This is equal to Kimura's fixation probability in a panmictic population of size $N = \sum_i N_i$. It should be noted that, in the derivation of (3), neither a fixed migration pattern nor fixed colony size is assumed, though the total population size is assumed to be constant.

The Variance Effective Number of Human Population

Norikazu YASUDA

A method is devised for estimating the variance effective number of a population based on gene frequency data. Let us suppose that the gene frequencies of two alleles A_1 and A_2 in the parental population are p and q, respectively. The frequency of gene A_1 in the filial generation (x) will be $x=i/2N$, where $2N$ is the number of gametes which contribute to the filial generation and which are considered as a random sample from an infinite number in the gamete pool produced by the parents, and i is the number of A_1 genes in the filial population. The mean $\bar{x}$ and variance V_x of x will be respectively $\bar{x}=p$ and $V_x=pq/2N$.

The change of gene frequency between two generations, $\delta p=x-p$, has an expectation of zero and a variance $pq/2N$; *i.e.* $\delta p=0$ and $V_{\delta p}=pq/2N$. The variance effective number (or size) of population is thus defined by

$$N_e = pq/2V_{\delta p} .$$

By applying an angular transformation, the variance will be independent of the gene frequency of the parental generation: that is, $p=\sin^2\theta$ gives $V_{\delta\theta}=1/8N$, where $\delta\theta=\theta'-\theta$ and the prime indicates the filial generation. In practice the variance of $\delta\theta$ may be computed from

$$nV_{\delta\theta}=\sum(\delta\theta)^2\ ,$$

in which n is the number of loci studied.

For a multiple allelic locus, $\delta\theta_i$ can be measured for each allele separately (considering the others being pooled). In this case, however, the contribution of each gene to $V_{\delta\theta}$ will be $(m-1)(\delta\theta)^2/m$ where m is the number of alleles. Thus the variance in general will be

$$nV_{\delta\theta}=\frac{1}{2}\sum_{k_2}\{(\delta\theta_1)^2+(\delta\theta_2)^2\}+\frac{2}{3}\sum_{k_3}\{(\delta\theta_1)^2+(\delta\theta_2)^2+(\delta\theta_3)^2\}+\cdots$$
$$+\frac{m-1}{m}\sum_{k_m}\{(\delta\theta_1)^2+\cdots+(\delta\theta_m)^2\}\ ,$$

where $n(=k_2+2k_3+\cdots+(m-1)k_m)$ is the number of independent genes examined, and k_i the number of loci with i alleles. The variance effective

Table 1. Variance effective number in some human populations

Population		N	N'	N_e	N_e/N'	f_r	n
Japan[1]							
Yashiro		48	18	8	0.44	0.06477	6
Kurodani		71	10	9	0.90	0.06166	4
H-group[2]							
L-leut	A	93	107	52	0.49	0.00966	8
	B	128	192	60	0.31	0.00837	8
	C	83	134	35	0.26	0.01465	8
	D	94	80	24	0.30	0.02140	8
S-leut	A	78	301	69	0.23	0.00731	8
	B	86	93	29	0.31	0.01740	8
	C	51	168	25	0.15	0.02061	8
	D	92	117	19	0.16	0.02641	8
Brazil[3]		2,136	4,834	540	0.11	0.00093	32

N=sample size of parental population.
N'=sample size of filial population.
N_e=estimated variance effective number.
f_r=the inbreeding coefficient due to random genetic drift.
1) Fujiki *et al.* (1968), 2) Steinberg *et al.* (1967), 3) Morton (1964).

number is thus obtained from

$$N_e = 1/8\, V_{\delta\theta} .$$

The rate of fixation or the change of inbreeding coefficient (f_r) due to random gene frequency drift is then $1/2N_e = 4V_{\delta\theta}$.

Application of the method is made to some human populations and pertinent figures are summarized in the table. Although the numbers obtained (N_e) are underestimates due to migration and other possible causes of fluctuation in gene frequencies between two generations, the results clearly show that: the smaller the size of population (N'), the more random drift of gene frequency takes place as indicated by small N_e. The ratios of effective to actual numbers are 0.11~0.90. It is of interest that isolated populations give smaller ratios than a relatively large population. For details, see Yasuda, N. (1969. Jap. J. Hum. Genet. 14(1): 10-16).

VI. EXPERIMENTAL STUDIES ON POPULATION GENETICS

Characteristics of Japanese Natural Populations of *D. melanogaster*

Chozo OSHIMA and Takao K. WATANABE

The mean total frequencies of lethal and semilethal chromosomes were 21.5 per cent in coadapted genetic background and 36 per cent in Samarkand isogenic background. The isogenic background decreased the viability of homozygotes for semilethal and subvital chromosomes and the coadapted background, on the contrary, increased it. A clear negative correlation between the frequency of deleterious chromosomes and relative viability of quasinormal homozygotes was ascertained.

Complete allelism tests were carried out between lethal genes extracted from the same or from different populations. The frequencies of allelism between lethals extracted simultaneously from a population were fluctuating, but the average rate was 3.03 per cent. The rate of elimination (IQ^2) of lethal genes was estimated to be 0.0014 which was about two times higher than in the American populations.

On the other hand, allelism tests were performed between lethal genes extracted in successive two or three years, and a fairly large proportion of all lethal genes was found to have been maintained for a long time in the same population. About 40 per cent of all lethals were persistent (one year) and about 22 per cent were long-term lethals (persistent for over two years). Consequently, about 60 per cent lethals have newly arisen. The mean viability of persistent lethal heterozygotes was estimated to be equal to or rather better than that of normal heterozygotes with coadapted genetic background.

Recessive Autosomal Sterility Genes Concealed in Natural Populations

Chozo OSHIMA and Takao K. WATANABE

The frequency of sterile chromosomes among non-lethal chromosomes extracted simultaneously from a natural population was reported to be 12.6 per cent in the previous Annual Report (No. 19). Thereafter 36 male

sterile chromosomes and 19 female sterile chromosomes have been maintained by *Cy*-balanced system. The frequencies of allelism between male and female sterility genes were determined by half diallel crosses between *Cy*-sterility lines. Among 630 crosses, double heterozygotes for two male sterility genes found in 95 crosses were sterile when they were crossed with virgin Oregon-R females. The frequency of allelism of male sterility genes was 15.1 per cent and that of female sterility genes was 10.5 per cent. Such high frequencies were due to a frequent appearance of a few kinds of sterility genes, for example, 13 male sterile chromosomes, 6 male sterile chromosomes and 6 female sterile chromosomes were found to carry allelic genes as shown in Table 1.

Table 1. Frequency of sterile chromosomes extracted from a natural population in Katsunuma and allelism between sterily genes

No. of second chromosomes tested		475	
Sex		Male	Female
No. of sterile chromosomes		44	25
Frequency of sterile chromosomes (Q)		0.093	0.053
No. of chromosomes tested for allelism		36	19
No. of crosses		630	171
No. of allelic crosses		95	18
Frequency of allelism (I)		0.151	0.105
No. of different loci of sterility genes		17	12
Frequency of appearance	1	13	10
	2	2	—
	3	—	1**
	6	1*	1
	13	1	—

* Allelic group A.
** Allelic group B represented in Table 2.

From the observation of salivary gland chromosomes, those male sterile chromosomes were found to have a heterotic inversion *C* on the right arm and one of them had additionally a heterotic inversion *B* on left arm, as shown in Table 2.

The loci of those male and female sterility genes could not be exactly determined because they were located on inversion *C* and were assumed to be different. Although most of the sterile chromosomes manifested semilethality in homozygous state, they were assumed to exist frequently and persistently in the natural population. Such as assumption could be supported by linkage with heterotic inversion *C*. However, it remains to

Table 2. Linkage relationship between frequent sterility genes and inversions

Numerical symbol of chromosome	Group A Male	Group B Female	Inversion 2L	Inversion 2R	Viability of homozygote
372	st.	+	*B*	*C*	semilethal
594	st.	+	+	*C*	semilethal
597	st.	+	+	*C*	subvital
27	st.	st.	+	*C*	semilethal
277	st.	st.	+	*C*	semilethal
549	st.	st.	+	*C*	semilethal

be proved whether sterility and semilethality are the result of pleiotropic actions of one gene or are individual actions of two different genes involved.

Frequency of sterile flies collected from natural populations was assumed to be about three per cent in both sexes as shown in Table 3.

Table 3. Frequency of sterile flies in natural populations in Kofu and Katsunuma locality

Sex	Population	Month of collection	No. of flies tested	No. of sterile flies	Frequency (%)
Male	Katsunuma	Oct. '68	604	18	3.0
	Kofu	Oct. '69	226	6	2.7
	Katsunuma	Dec. '69	42	1	2.4
	Total		872	25	2.9
Female	Katsunuma	Oct. '69	236	10	4.2
	Katsunuma	Nov. '69	242	4	1.7
	Katsunuma	Dec. '69	83	2	2.4
	Total		561	16	2.9

On the other hand, the rate of elimination of male sterility genes by homozygosis in a population could be calculated by using the values of I and Q of Table 1. If both second and third chromosomes were assumed to have the same values for I and Q, the rate of elimination of male sterility genes by homozygosis could be calculated to be $0.151 \times 0.171 = 0.00258$. The rate of elimination of female sterility genes by homozygosis could be calculated similarly to be 0.0006. From the results, about one tenth of sterile male flies and one fiftieth of sterile female flies were caused by autosomal recessive sterility genes and most of them could be attributable to some physiological or environmental factors.

A New Mutant Gene '*tra-b*' on the Second Chromosome

Takao K. WATANABE

When the viabilities of homozygotes for many second chromosomes extracted from a cage population were estimated by *Cy*-method, a chromosome carrying a mutant gene, termed '*transformer-b*' was found.

In the F_1 offspring of *Cy*/*tra-b* heterozygous flies, *Cy*/*tra-b* heterozygous flies and *tra-b*/*tra-b* homozygous flies emerged as expected in the ratio of 2 : 1. However, all *tra-b* homozygous flies were male. Female flies, whose genotype was *X*/*X*; *tra-b*/*tra-b*, were evidently transformed into phenotypic male flies and their mating ability was normal, but they were completely sterile due to their reduced testes.

A. H. Sturtevant (1945. Genetics 30: 297–299) reported a *transformer* gene (*tra*: 3–45) on the third chromosome. The functions of both *tra* and *tra-b* gene were very similar, but male flies, whose genotype was *X*/*Y*; *tra*/*tra*, were fertile.

The locus of *tra-b* gene was assumed to be close the *Lobe* gene (2–72.0) on the right arm and it remains to be proved whether the sterility of *tra-b* homozygous male flies is due to the pleiotropic action of that gene or not.

A Heterotic Inversion on the Second Chromosome of *D. melanogaster*

Takao K. WATANABE and Taishu WATANABE

A second chromosome inversion, *In*(*2L*)*B*, has been maintained in the natural populations of Kofu and Katsunuma at the frequency of about 30 per cent during the past 7 years. Two cage populations, containing the heterotic inversion at 25 per cent, were started in the laboratory. One has been kept in a constant 25°C environment and the other in an environment fluctuating 20–30°C two times a day. After about 15 months, 200 flies were sampled from each population, and their salivary chromosomes were examined. From the results, the equilibrium frequency of the inversion was assumed to be about 20 per cent in the constant and about 11 per cent in the fluctuating environment.

The viability of random heterozygotes was estimated by using *Cy*-method, and the random heterozygous female and male productivities were estimated by the number of their offspring. There relative values (mean) of normal and inversion *B* heterozygotes and inversion homozygotes are given in Table 1.

Table 1. Relative viability, female and male productivity of *In(2L)B* heterozygotes.

Environment	Genotype	No. of crosses	Viability	Productivity ♀	Productivity ♂
Constant	+ / +	125	1.00	1.00	1.00
	+ / *B*	81	0.99	1.11**	0.96
	B / *B*	8	0.98	0.96	1.02
Fluctuating	+ / +	152	1.00	1.00	1.00
	+ / *B*	38	0.93	1.21**	1.21**
	B / *B*	3	1.03	0.71	1.02

** Highly significant

Although the viabilities in both environments were not statistically different, the productivity of inversion female and male heterozygotes showed a significant superiority over normal heterozygotes. This result reveals one of the mechanisms by which the inversion, *In(2L)B*, has been maintained in natural and cage populations.

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN ANIMALS

Studies on Strain Differences in Radiosensitivity in the Silkworm. VII. Strain Differences in Sensitivity to Germ Cell Killing

Yataro TAZIMA and AKIO ONUMA

Strain differences in radiosensitivity to cell killing have been studied with regard to spermatogenic cells using fertility decline as a ctiterion. The materials used were seven representative strains which had been selected for embryonic killing; two resistant, two intermediate and three sensitive.

It has been known that spermatogenic cells are extremely sensitive to killing by radiation during the larval period from late fourth instar to early fifth instar, when most of the germ cells are just before the first meiotic metaphase. Hence, γ-irradiation was administered to the larvae at fifth instar day one (V-1) with several different doses. The killing effect on spermatogenic cells was expressed per batch basis as an index of the average number of fertilized eggs relative to control, when irradiated males had been crossed to non-irradiated females. From dose-reaction curves LD_{50} values were calculated.

The results of our first experiment showed remarkable differences among the strains with the following LD_{50} values: Aojuku, 2,359 R; Kojiki, 1,650 R; Kansen, 1,000 R; and Sekko, 300 R. The maximum difference in sensitivity among strains were about eight fold.

Surprisingly enough, the sensitivity to germ cell killing did not run in parallel with that to embryonic killing. Sekko, which belongs to the intermediate group with regard to embryonic killing showed extremely high sensitivity to cell killing. In contrast, Kojiki, which was classified as most sensitive to embryonic killing was found to be fairly resistant to cell killing. A further experiment revealed that strain rb was more sensitive than strain Sekko with respect to cell killing, although LD_{50} values became a little higher for both strains than in the first experiment.

In conclusion, there was no parallelism between the sensitivity to germ cell killing and the sensitivity to embryonic killing.

Studies on Strain Differences in Radiosensitivity in the Silkworm. VIII. Strain Specificity at Various Response Levels

Yataro TAZIMA and Kimiharu ONIMARU

A comparative study of various kinds of radiosensitivity among silkworm strains has yielded several informations. Above all it has been made clear that each strain has its own specific response level in radiosensitivity.

First, using embryonic killing as a criterion for selection, we have extracted several radiosensitive and radioresistant strains from the world-wide collection of silkworm strains maintained at the Sericultural Experiment Station.

Second, using those strains and an already established radiosensitive strain rb and a standard type strain C108, we have carried out a comparative study of sensitivity to cell killing and sensitivity to mutation induction by radiation. The results are summarized in Table 1.

Table 1. Radiosensitivity at various levels for seven representative strains

Strain	Embryonic killing	Germ cell killing	Mutation induction in			Spontaneous mutation
			Spermato-gonia	Spermatids	Sperm	
Kansen	+	++	+	+	+	+
Aojuku	+	−	++	++	+	
C108	++		++		+	
Sekko	++	+++	+++	++	+	++
Kojiki	+++	+	±	+	+	
Ascoli	+++		++		+	
rb	+++	+++	+++	++	++	++

In this table the strains are classified into three groups, sensitive, intermediate and resistant, according to the sensitivity to embryonic killing. As pointed out previously, sensitivity to embryonic killing, sensitivity to germ cell killing and sensitivity to mutation production do not run parallel to another among the strains except for the hypersensitive rb, which showed always the highest sensitivity. The most noteworthy is Sekko. Although it is placed in the intermediate group with regard to embryonic killing, it showed extreme sensitivity to germ cell killing and to mutation induction in spermatogonia. Another extreme case is Aojuku. It was highly resistent to germ cell killing, but intermediate with regard to mutation induction.

Regarding mutation response a marked difference is observed among strains when spermatogonia were irradiated. The difference, however, diminishes with advancing germ cell stage.

The above results seem to indicate that sensitivity to radiation is not only strain specific but also developmental phase specific.

Studies on Strain Differences in Radiosensitivity in the Silkworm. IX. Relation between Mutation Induction and Cell Killing

Yataro TAZIMA and Yosoji FUKASE

A contrasting mode of dominance has been discovered between mutation induction and germ cell killing by radiation in F_1 hybrids produced between sensitive strains.

Strains used for this study were rb and Sekko. It has been already reported with respect to embryonic killing that the F_1 between two sensitive strains, Kojiki and Ascoli, became resistant showing far higher LD_{50} values than both parental lines. A similar phenomenon of overdominance was observed with respect to germ cell killing, when F_1 hybrids between rb and Sekko were compared with the parental strains. Contrarily, the same F_1 hybrids exhibited intermediate sensitivity with respect to mutation induction. The intermediate sensitivity in F_1 regarding mutation induction was also confirmed in another cross produced between rb and Kojiki.

In this study the sensitivity to mutation was estimated in terms of the mutation frequency at the specific loci, $+^{pe}$ and $+^{re}$, after irradiation of early spermatogonia and sensitivity to cell killing was studied by irradiating spermatocytes using fertility decline as a criterion. Since visible mutations at the specific loci represent chromosomal events incurred in the germ cell and cell killing is estimated from the fertility decline produced in the same cell line, both results could be reasonably compared.

The intermediate type of dominance observed in F_1 with regard to mutation induction suggests that a modification of chromosomal lesions by several intracellular and/or extracellular factors is rather slight. Whereas, the overdominance exhibited in the same F_1 cross with regard to cell killing indicates that the possibility to such modification is fairly large in the case of cell killing. Increased vigor in F_1, for instance, seems to relieve radiation lesions to an appreciable extent leading to increased survival. It is concluded from these results that factors other than chromosomal lesions are also concerned with cell death.

Studies on Strain Differences in Radiosensitivity in the Silkworm. X. Spontaneous Mutation Frequencies Observed in Different Radiosensitive Strains to Mutation Induction

Yataro TAZIMA and Kimiharu ONIMARU

Since remarkable differences in radiosensitivity were discovered among silkworm strains with regard to mutation induction, we have examined whether such differences could be detected with respect to spontaneous mutation frequency.

Seven representative strains for differential sensitivity were used for this purpose; *i.e.* rb and Sekko as the most sensitive, Aojuku, C108 and Ascoli as intermediate and Kansen and Kojiki as the least sensitive. Among them the best analysed were rb, Sekko and Kansen. Mutation frequencies were examined only for the male. In order to minimize environmental variables influencing mutation frequency, all strains were raised simultaneously. The mutation frequency was assesed by utilizing specific loci method.

Spontaneous mutations can occur at any time of germ cell development. Therefore, it is impossible to guess exactly the stage of their occurrence. However, whether they had occurred at pre-meiotic stage or post-meiotic stage could be surmised from their appearance. Should a mutation occur in germ cells at premeiotic stage, it could be detected as whole type mutation in the next generation. Whereas, would it occur after the meiosis in one of the DNA double strands, it might be manifested as a mosaic mutant in the next generation. Furthermore, mutations arising at pre-meiotic stage are sometimes characterized by their occurrence in clusters.

The data obtained for the past three years are given in Fig. 1. The difference is clear among strains at $+^{pe}$ locus. Mutation frequencies were higher in strains rb and Sekko than in Kansen. The difference was, however, less clear at $+^{re}$ locus, owing to lower mutation responses. The highest peak which appeared in the score for 692 was due to the cluster-like appearance of the mutants. When more than two mutants appearing in the same batch were counted as one mutation, the peak fell down to approximately average level. Nevertheless, the relative order in mutation frequency among strains remained unchanged.

These results suggest that strains sensitive to mutation induction by radiation show higher mutation frequency even under non-irradiated condition.

As to the incidence of mosaics no clear difference was observed among

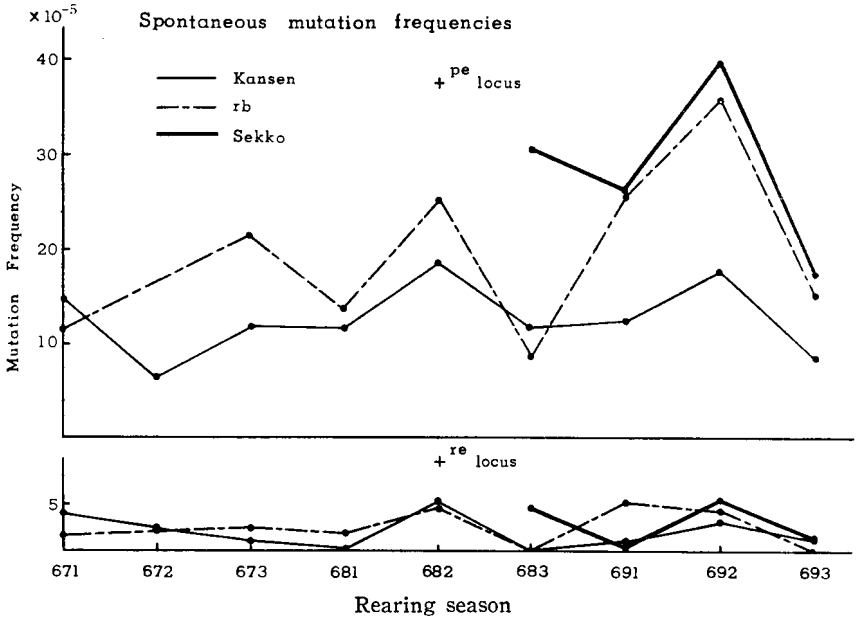

Fig. 1. Spontaneous mutation frequencies observed for three silkworm strains during past three years.

strains, suggesting that the occurrence of spontaneous mutations at postmeiotic stages does not differ appreciably among strains.

A Comparison of Mutagenicity of 14 MeV Fast Neutrons on Primordial Germ Cells among Different X-ray Sensitive Silkworm Strains

Akio MURAKAMI

A comparison of the mutagenicity of 14 MeV neutrons among strains having different radiosensitivities may give a better insight intot he factors affecting relative biological effectiveness (RBE) values of the two kinds of radiation. The present study has been carried out with primordial germ cells of the silkworm in order to compare the mutation rates of neutrons to those of X-rays among five different strains. Treatments were given to newly hatched larvae. Mutation rates were estimated by the egg-color-specific-locus method using the *pe* and *re* genes.

For X-rays, there was a remarkable difference in mutation frequencies between the most and the least sensitive strain. The difference was, however, less pronounced for neutrons. Comparing the mutation rates of the two radiations (or RBE of neutrons to X-rays), it has been shown that the RBE values of resistant strains are higher than those of sensitive strains. The result suggests that the efficiency of neutrons in inducing mutations varies according to radiosensitivity perhaps due to interaction between germinal selection and reparability of biological damage.

Effects of Ultraviolet Light on the Oocytes of the Silkworm with Special Regard to Strain Sensitivity

Akio MURAKAMI

Sensitivity to ultraviolet light was studied in order to compare strain sensitivity of different X-ray sensitive strains with regard to killing and mutagenic effects.

After a UV irradiation was delivered to mature oocytes and/or eggs in the early cleavage stages of the silkworm, their sensitivities to killing and mutagenic effects were measured in terms of embryonic mortality and scored by the *pe*:*re* egg-color-specific-locus method, respectively.

The results showed the occurrence of distinct differences for both biological effects on oocytes and eggs: for the killing effect there was found a difference of 4–5 times or more and for mutagenicity a difference of 10 times or more between the most and the least resistant strain. The results further indicated that strains sensitive to UV radiation killing are also sensitive to mutation-induction and *vice versa*. There was often no positive correlation between UV and X-ray sensitivities for both biological effects. In the treated oocytes many more fractional than whole-body mutations were induced with UV, while X-rays gave the opposite result.

Comparison of Stage Sensitivity to X-rays during Meiosis in Silkworm Oocytes

Akio MURAKAMI

Meiosis comprises two different types of division, a reductional and an equational division. Comparison of the radiosensitivity of cells these two kinds of divisions may provide some clews for a better understanding of the mechanisms underlying the effects of X-ray in the higher organism.

Such consideration prompted the present experiment.

The radiosensitivity was determined by LD_{50} value from the dose-hatchability curves. The strain used was wild type C108. Six doses of X-rays (180 kVp, 25 mA, 1.0 mm Al filter and a dose-rate at 1,000 R/min) were used: 1,000, 2,000, 3,000, 4,000, 5,000 and 6,000 R. For the irradiation of prophase I oocytes the X-rays were administered to eggs in the ovaries of late stage pupae or moths which were mated after irradiated with male moths having *pe* and *re* markers. This scheme allowed for a simultaneous check for induced mutations and cell killing. For the irradiation of post-oviposition eggs (oocytes at metaphase I through the time of syngamy), eggs were used of wild type moths mated with the marker strain. Samples were collected every 10 min. X-irradiations were applied to eggs at 10 min intervals from immediately after oviposition until first mitotic stage.

The results indicated that cells in the dividing phase were more sensitive to X-rays than those in any other phases for both first and second meiosis, and that cells in the first meiosis were more resistant than those in the second meiosis. Furthermore the sensitivity of the second meiotic cells was almost similar to that of the early mitotic cells. The differences in sensitivity may be interpreted as being due to (1) whether cells are associated with DNA synthesis or not and (2) the difference in chromosomal structure at the time of irradiation.

Changes in Radiosensitivity during the Second Meiotic Stage of Silkworm Oocytes

Akio MURAKAMI

In the previous report, we have demonstrated that in cleavage cells the radiosensitivity to cell killing and to mutation varied with the progress of the division cycle (this Annual Report, No. 16, 1967). The most sensitive phase to cell killing was found to be the dividing phase, whereas that to mutation production was late dividing phase and/or early interphase.

The present work was undertaken in order to extend the observation to the second meiotic oocytes.

Mutation frequencies were estimated by the specific-locus method using the *pe* and *re* genes. Radiosensitivities for cell killing were measured by the reduction of hatchability in comparison with the control. Materials were oocytes and/or eggs laid by the wild type female moths mated with the marker male moths. Samples were collected every 10 min at

25°C. They were irradiated with 2,000 R X-rays (180 kVp, 25 mA, 1.0 mm Al filter and a dose-rate of 1,000 R/min) at 10 min intervals from their age of 60 until 140 minutes. They corresponded to eggs from meiotic division of oocytes to the stage of syngamy.

Mutations most frequently found in this work were fractional-body mutations and whole-body mutations were scarce. The peak stage for cell killing was observed to be at 110 min age corresponding to the dividing phase of the second meiotic oocytes, whereas for mutation production to be at 120 min age corresponding to the end of the second meiotic division or early pro-nuclear stage. These findings indicate that the sensitive phases for both biological events do not coincide, suggesting that the mechanisms involved in both events are different. The mutation rate observed for the oocytes around the end of the second meiosis was almost similar to that observed for the early cleavage stage cells showing approximately 10 times higher value than the other stages of oogenesis.

The above results could be interpreted, at least in part, on the assumption that cells around the end of the second meiotic division stage are in the course of DNA synthesis or chromosomal duplication.

Radiation-Dependent Loss of Sorting-Out Activity of HeLa Cells

Yukiaki KURODA

It has been previously found that HeLa cells has a characteristic sorting-out property when intermixed with embryonic chick and quail liver cells in rotation culture. In the present experiment changes in this sorting-out activity of HeLa cells were quantitatively examined after X-irradiation of HeLa cells. HeLa S3 cells were irradiated with various doses of X-ray (175 KVp, 25 mA, distance 40 cm, filter 1.0 mm Al, dose rate 300 R min). After irradiation 3×10^5 HeLa cells were intermixed with 6×10^5 liver cells dissociated from 7-day quail embryos. Mixed cell suspensions were rotated at 37°C at 70 rpm on a gyratory shaker for 24 or 48 hours, then the formed aggregates were fixed, sectioned, stained and histologically examined.

Non-irradiated HeLa cells which were intermixed with quail liver cells formed aggregates in which a small number of liver cells was randomly engulfed by overwhelming HeLa cells. HeLa cells which were irradiated with various doses of X-ray formed aggregates in which quail cells engulfed by HeLa cells gradually increased in number with increasing X-ray doses.

Changes in the number of quail cells in aggregates of HeLa cells after 24 hours of rotation culture are shown in Table 1.

Table 1. The number of quail liver cells engulfed in aggregates of X-irradiated HeLa cells

Dose (R)	No. of total cells in aggregate	No. of HeLa cells in aggregate	No. of quail cells in aggregate	Per cent of quail cells
0	112	102	10	8.9
400	143	125	18	12.6
1,200	131	109	22	16.8
1,600	113	78	35	31.0
2,000	139	90	49	35.3

It was also found that the number of X-irradiated HeLa cells engulfed in aggregates of quail liver cells increased with gradually increasing doses of X-ray. The result of counts for each type of cells in these aggregates formed after 24 hours of rotation culture is shown in Table 2.

Table 2. The number of X-irradiated HeLa cells engulfed in aggregates of quail liver cells

Dose (R)	No. of total cells in aggregate	No. of quail cells in aggregate	No. of HeLa cells in aggregate	Per cent of HeLa cells
0	475	467	8	1.7
400	155	147	8	5.2
1,200	416	384	32	7.7
1,600	346	317	29	8.4
2,000	220	195	25	11.4

The figures given in Table 2 show that the percentage of HeLa cells engulfed in quail cell aggregates was relatively lower than the percentage of the latter engulfed in the aggregates of the former. The difference in these percentages suggest that the formation of discrete aggregates of each type of cells which were brought about by the mutual sorting-out property of both types of cells may depend upon a chasing out activity of the aggregated cells on the intermingled unlike cells and escaping activity of intermingled cells from aggregates of unlike cells.

VIII. RADIATION GENETICS IN MICROORGANISMS AND PLANTS

Correlations between Mutation, Transformation Efficiency and Radiosensitivity in Mutants of *Bacillus subtilis*

Yoshito SADAIE and Tsuneo KADA

Bacillus subtilis strain H-17 (*try arg*) was treated with N-methyl-N'-nitro-N-nitrosoguanidine and surviving cells were screened for the increase in radio-sensitivity. Among mutants isolated and purified, seven were sensitive to both gamma-rays and UV.

All the mutant strains had reduced rates of gamma-induced resistance to Kanamycin (1 μg/m*l*) in comparison with the original strain H-17. While strain H-17 is genetically stable as an efficient recipient for transforming DNA carrying arginine and tryptophane markers, the efficiency of transformation was remarkably low in radiosensitive mutants. It is evident that a recombinational event between extracellular DNA and the recipient chromosome is required for marker incorporation in transformation.

It has been supposed by different authors that simultaneous occurrence of enhanced γ-ray- and UV-sensitivities may be due to genetic lack in repair involving the recombinational process. Thus the reduction of induced mutations in our radiosensitive mutants would posseses a similar molecular basis.

Gamma-Ray Mutagenesis, Repair and Chemical Radiosensitization in Bacteria

Tsuneo KADA

One of the possible mechanisms involved in the radiosensitization with certain chemical reagents may be an inhibition of repair of radiation damages on the level of cellular DNA. If it were so, it is possible that radiation-induced mutagenesis might be modified in some way. Reversions to prototrophy in *Escherichia coli* B/r *try* WP2 were studied under mild sensitizing conditions using KIO_3 as a radiosensitizer (Kada, T. 1969. Int. J. Radiat. Biol. 15: 271).

When bacteria were irradiated at 0°C with gamma-rays from ^{137}Cs in phosphate buffer (0.067M) containing a non-toxic level of KIO_3, many more cells were inactivated as compared to the control without the re-

agent. To observe the effect of the reagent at the time of irradiation on the yield of mutations, cellular suspensions ($\sim 5\times10^8$/ml) containing increasing concentrations (1~5 mM) of KIO_3 were exposed to gamma-rays (1~5 kR) at 0°C, then the cells were washed twice with cold phosphate buffer and resuspended in the same solution of 1/10 volume. Plating was carried out after appropriate dilutions to determine the number of viable bacteria on minimal glucose agar enriched with 0.2% Difco liquid broth and revertant cells on the same minimal agar with or without broth enrichment. All plates were incubated at 37°C for 3 days.

Results of some typical experiments are shown in Table 1. It is clear

Table 1. Effect of KIO_3 (PI) addition during gamma-irradiation on radiation-induced reversions of *Escherichia coli* B/r WP2 *try*

Dose of γ-rays (kR)	Number of viable cells per ml		Frequency of induced mutants per 10^8 survivals			
			Minimal Med.		Broth (0.2%) enriched MM	
	−PI	+PI	−PI	+PI	−PI	+PI
0	2.4×10^8	2.4×10^8	0	0	0	0
1.4	1.7×10^8	1.7×10^8	1.9	0.9	3.4	4.4
2.7	1.5×10^8	1.2×10^8	2.9	0.7	4.0	3.0
4.0	1.3×10^8	6.9×10^7	3.5	0.9	7.1	3.1

that, while some decrease of viable cells was observed under above sensitizing conditions, the frequency of reversions decreased also markedly.

Recent studies carried out in this laboratory on macromolecular synthesis in bacteria that had been irradiated with gamma-rays in the presence of KIO_3 have shown that *de novo* DNA synthesis as well as rejoining of radiation-induced DNA strand-breaks were prevented in the course of post-irradiation incubation. It is thus supposed that potential mutant cells might be inactivated more efficiently than the non-mutant population because of lacking in rejoining repair or that some specific mutagenic lesions might be more preferentially repaired than lethal lesions in the absence of DNA synthesis.

Macromolecular Synthesis in Bacteria Radiosensitized with Iodine Compounds

Takehiko NOGUTI and Tsuneo KADA

In order to elucidate the biochemical mechanisms underlying increase of cellular death by radiosensitization with iodine compounds such as iodoacetic acid ($I \cdot CH_2 \cdot COOH$), potassium iodide (KI) or potassium iodate (KIO_3), DNA, and protein synthesis were studied in cells of *Bacillus subtilis* strain H-17.

Bacteria exponentially grown in TF medium were irradiated with 7 kR gamma-rays from ^{137}Cs in the presence of 0.8 mM KI, 0.25 mM KIO_3 or 0.2 mM $I \cdot CH_2 \cdot COOH$, washed in cold and reincubated in the same medium. Cellular incorporations of ^{3}H-thymidine or ^{14}C-amino acids into acid-insoluble materials were measured in parallel cultures. We observed in repeated experiments that, while irradiation or drug treatment alone had little effect on the macromolecular synthetic pattern as compared to that of the control culture, the incorporation of radioactive precursors into DNA was more inhibited than into protein (Table 1).

Above results indicated a close correlation between increased cellular inactivation and inhibition of DNA synthesis in radiosensitized cells. Similar relationships are well known concerning the effect of ultraviolet

Table 1. Effect of the presence of iodine compounds during gamma-irradiation on incorporation of radioactive precursors into DNA or protein in cells of *Bacillus subtilis*. Treated cells were washed and reincubated for 40 minutes. Radioactivities were measured at this stage by precipitating and washing cells by 5% TCA and expressed in percentage as comparedto the control.

Treatment of cells			^{3}H-thymidine	^{14}C-amino acids
γ-ray (kR)	Drug (mM)	Survival fraction (%)	(DNA)	(Protein)
0	None	100	100	100
7	None	53	133	104
0	KI (0.8)	100	88	84
	KIO_3 (0.25)	100	108	96
	$I \cdot CH_2 \cdot COOH$ (0.2)	100	85	62
7	KI (0.8)	7	3	16
	KIO_3 (0.25)	0.03	0.2	6
	$I \cdot CH_2 \cdot COOH$ (0.2)	5	1	2

irradiation of bacteria. However, the radiosensitization with iodine compounds was not due to the increase of *in situ* radiation damage in cellular DNA (Kada, T., T. Noguti and M. Namiki. 1970. Int. J. Radiat. Biol. 17: 407), unlike the case with ultraviolet light that directly modifies DNA molecules. We suppose that some transient products containing iodine atoms might be produced by irradiation and that might react with certain enzymes involved in DNA metabolisms or modify the cellular membrane site thus affecting preferentially DNA synthesis.

Rejoining Inhibition of Radiation-Induced DNA Strand-Breaks in Radiosensitized Bacteria with Iodine Compounds

Takehiko NOGUTI and Tsuneo KADA

Evidences have been accumulated recently in various studies on radiation-induced cellular inactivation indicating that the radiation-induced lethality and its repair are closely related to the production of strand-breaks in cellular DNA molecules and their rejoinings in post-irradiation growth. Radiosensitization by cellular incorporation of 5-bromouracil into DNA was correlated to the inability of strand-break rejoinings (Kaplan, H. S. 1966. Proc. Nat. Acad. Sci. 55: 1442). Extensive studies were carried out to see if such a mechanism might be also involved in the case of radiosensitization by extracellular presence of iodine compounds such as potassium iodate (KIO_3), potassium iodide (KI) or iodoacetic acid ($I \cdot CH_2 \cdot COOH$) at the time of gamma-irradiation.

Cells of *Bacillus subtilis* strain H-17 were labeled with ^3H-thymidine for several generations during growth in TF-medium. Exponential bacteria were washed, and exposed to gamma-rays (usually 10 kR) from ^{137}Cs in suspension in cold phosphate buffer (pH 7.0) containing increasing concentrations of reagent. Cells were washed immediately after treatment and inoculated in TF-medium. Sedimentation analysis in alkali-sucrose gradint of cellular DNA was carried out by techniques already described (Kada, T., T. Noguti and M. Namiki. 1970. Int. J. Radiat. Biol. 17: 407) on samples collected 0 minutes and 40 minutes after aerobic incubation under shaking at 37°C.

Results obtained have shown following points (Table 1). (a) Irradiation provoked *in vivo* single-strand breaks in DNA molecules of smaller sizes. The presence of drug did not affect the number of breaks produced *in situ* by irradiation. (b) Cellular incubation followed by irradiation without reagent allowed broken molecules to rejoin. However, such an

Table 1. Effect of the presence of iodine compounds during gamma-irradiation on production of strand-breaks in cellular DNA and their rejoining. Treated cells were washed, lysed and analysed by sedimentation (38,000 rpm for 80 min) in alkali-sucrose gradient (5~20%). In this table, the position of main peak of DNA is shown as distance (cm) from the bottom. Total length of the gradient was 4.0 cm.

Treatment	None	KI (1 mM)	KIO_3 (0.3 mM)	$I \cdot CH_2 \cdot COOH$ (0.2 mM)
Not irradiated. Contact with drug for 30 minutes at 0°C.	1.1	1.1	1.1	1.1
Irradiated (20 kR at 0°C, 30 minutes required) in the presence of drug. No incubation.	2.4	2.3	2.3	2.3
Irradiated (20 kR) in the presence of drug and incubated for 40 minutes.	1.2	—	2.5	2.4

event was strongly inhibited or totally prevented in cells irradiated in the presence of the reagent.

These results favor the view that rejoining inhibition of radiation-induced DNA strand-breaks may be rosponsible at least for part of the enhanced cellular death under mild radiosensitization conditions.

Genetic Resistance to Radiosensitization with Iodine Compounds

Tsuneo KADA

A question could be posed if inactivation of a specific cellular metabolism could be involved in radiosensitization. One way to answer it may be by isolating mutant strains possessing modified responses to radiosensitization and by determing their genetic characters. Previous studies (Kada, T., T. Noguti and M. Namiki. 1970. Int. J. Radiat. Biol. 17: 407) have indicated that the sensitization might not be due to the increase of radiation-induced *in situ* damage in cellular DNA. Therefore it is possible to suppose that some metabolisms may be involved in the repair of radiation damages in cellular DNA in the radiosensitization process, in addition to other possible target metabolisms.

A F^- strain PA309 of *Escherichia coli* K12 was treated with N-methyl-N′-nitro-N-nitrosoguanidine, and the cells were grown overnight. The

bacteria were then exposed to 1 mM solution of iodoacetic acid, pH 5.4, that had previously been exposed to 6.7 kR of gamma-rays from ^{137}Cs, and the surviving cells were screened for genetic resistence.

Non-mutant bacteria are usually killed very efficiently at 0°C in the presence of persistent toxic products from the reagent induced by irradiation. However, the above isolated mutant strains were fairly resistant to radiation-induced toxicity from iodoacetic acid. They were also resistant to radiation-induced toxic products from potassium iodide or potassium iodate, but not to unirradiated iodoacetic acid or iodine dissolved in potassium iodide solution. We observed that typical three mutant strains resistant to irradiated iodine compounds were also more resistant to radio-sensitization with these compounds, that is, simultaneous treatment with gamma-irradiation and the reagent in neutral buffer. Genetic analysis is under way in order to determine the location of genes responsible for the resistance

These observations indicate: (a) Important part of the enhancement in radiation lethality by irradiation of cells in the presence of iodine compounds may be due to the action of toxic transient products whose life may be pH-dependent. (b) The cellular sites responsible for radiosensitization were similar in all experiments with the three iodine sensitizers studied and/or similar radiochemical products were involved in the radiosensitization with these iodine compounds (details reported in Int. Radiat. Biol. 1970. 17: 419).

Possible Toxic Effects of Halogen Atoms in Radiosensitization with Some Halogenated Pyrimidines

Tsuneo KADA, Yoshito SADAIE and Takehiko NOGUTI

It has been well established that cells or viruses containing 5-bromouracil or 5-iodouracil in their DNA are more sensitive to ionizing radiation or ultraviolet light (Zamenhof, S., R. De Giovani and S. Greer. 1958. Nature 181: 827; Opera-Kubinska, Z., Z. Lorkiewicz and W. Szybalski. 1961. Biochem. Biophys. Res. Comm. 4: 288; Stahl, F. W., J. M. Grasemann, L. Okun, E. Fox and C. Laird. 1961. Virology 13: 98). These observations offered a very important argument for the assumption that primary lesions for radiation-induced cellular lethality may reside in DNA molecules.

Radiation chemical studies on DNA possessing 5-bromouracil or 5-iodouracil substituents strongly suggest that dehalogenation may occur by both

ionizing and ultraviolet radiations of brominated or iodinated bases in cellular DNA (Rupp, W. D. and W. H. Prusoff. 1965. Biochem. Biophys. Res. Comm. 18: 158; Zimbrick, J. D., J. F. Ward and L. S. Myers, Jr. 1969. Int. J. Radiat. Biol 16: 525). It is very likely that such an event will produce abnormal characters in DNA molecules and might weaken the DNA backbone structure or cause irreparable strand-scissions (Beer, J. Z., J. T. Lett and P. Alexander. 1963. Nature 199: 193; Aoki, S., R. P. Boyce and P. Howard-Flanders. 1966. Nature 209: 686; Kaplan, H. S. 1966. Proc. Nat. Acad. Sci. 55: 1442).

We have been carrying on studies on mechanisms of radio-sensitization with iodine compounds such as potassium iodide, potassium iodate or iodoacetic acid (Kada, T. 1969. Int. J. Radiat. Biol. 15: 271; Kada, T., T. Noguti and M. Namiki. 1970. Int. J. Radiat. Biol. 17: 407; Kada, T. 1970. Int. J. Radiat. Biol. 17: 419). Because all these agents produced by irradiation at pH 5.4 persistent toxic products inactivating bacteria efficiently at 0°C and that mutant strains of *Escherichia coli* K12 resistant

Table 1. Radiosensitization with 5-iodouracil (IU) and toxic effect of γ-irradiated drug solution in different microbial strains. Bacteria grown exponentially in broth were washed and resuspended in phosphate buffer (pH 7.0 or 5.4). (a) They were irradiated at 0°C with γ-rays from ^{137}Cs, with or without supplementation by 1 mM IU. (b) Phosphate buffer solution containing 1 mM IU was irradiated at 0°C ,then combined with bacteria. The mixture was then kept at 0°C for 30 minutes.
Survivals were determined by plating on broth agar.

Conditions of treatment				Number of colony forming cells per ml		
Irradiation	pH	mM of IU	Minutes of contact	*Escherichia coli* B	*Salmonella typhimurium* TM2	*Bacillus subtilis* H-17
None	7.0	0	—	8.3×10^5	1.1×10^6	8.0×10^5
	7.0	1	30	9.0×15^5	1.0×10^6	7.8×10^5
Simultaneous treatment with γ-rays (10 kR) and IU	7.0	0	—	4.7×10^4	3.2×10^5	2.2×10^5
	7.0	1	—	9.0×10^3	2.0×10^5	5.1×10^3
None	5.4	0	—	8.0×10^5	1.3×10^6	8.1×10^5
	5.4	1	30	7.5×10^5	1.0×10^6	8.0×10^5
Treatment by γ-irradiated (10 kR) IU solution	5.4	0	30	8.1×10^5	9.3×10^5	7.5×10^5
	5.4	1	35	<1	<1	<1

to the above products were also resistant to radiosensitization, that is, simultaneous treatment with gamma-rays and the reagent in neutral buffer, it is supposed that an important part of cellular death under sensitizing conditions may be due to the above transient toxic products very probably containing iodine atoms.

Studies were carried out to examine if halogenated purines or pyrimidines might produce toxic products. Table 1 indicates that 5-iodouracil produces by gamma-irradiation at pH 5.4 highly toxic persistent toxicities that killed very efficiently at 0°C different kinds of microorganisms such as *Escherichia coli*, *Salmonella typhimurium* as well as *Bacillus subtilis*. The enhancement of radiation lethality was also observed under usual radiosensitizing conditions, that is, when they were irradiated in neutral phosphate buffer solution containing the reagents.

The above results strongly suggest the possibility that toxic products could be also produced from cellular DNA possessing iodouracil substituents. If this were the case, the toxic effects of halogen atoms produced under irradiation might play a certain role in radiosensitization by cellular incorporation of iodinated bases. (Related results were reported, Int. J. Radiat. Biol. 18: 281. 1970).

Repair Systems for UV Damage in Phage T6

Etsuo AMANO and Mutsuo SEKIGUCHI[1]

Bacteriophage T4 is resistant to ultraviolet (UV) irradiation in comparison with T6. UV resistance of T4 may be mainly due to a repair system or systems active in the dark. It has been domonstrated that an extract of T4-infected cells contains an enzyme whose action excises pyrimidine dimers from UV irradiated DNA (Sekiguchi, M. *et al.* 1970. J. Mol. Biol. 47: 231–242). Lack of this specific excision activity in strains of UV-sensitive phages such as T4v_1 and T2 supports the view that it is instrumental in the repair of T4 (Yasuda, S. and M. Sekiguchi. 1970. J. Mol. Biol. 47: 243–255). In the present experiments, similar biochemical methods were used to investigate repair activities in T6 and T5 phages.

To prepare the ^{14}C-thymine labeled DNA, cells of *E. coli* strain B3 (*thy*$^-$) were infected with T4 or T6, and phage DNA was extracted by phenol treatment from purified particles. Cell-free extracts were prepared by means of sonication from phage-infected cells of *E. coli* strain

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1100, an endonuclease I-less mutant of K12. A germicidal lamp was used as UV source. UV-irradiated radioactive DNA was incubated with the cell extract, and the specific excision activity of the cell extract was measured using Dowex I ion-exchange column chromatography. T4-infected cell extract showed specific excision of thymine dimers from UV irradiated T4 or T6 DNA. Cell extract from T6-infected cells showed none at all or only very weak activity in excising dimers when irradiated T4 or T6 DNA was used as substrate. Lack of the specific excision activity may be the reason for increased UV sensitivity of phage T6 in comparison to T4.

In T6, photoreactivation of UV damage was observed when appropriate host bacteria were used. Under photoreactivation conditions, the survival rate of UV-irradiated T6 phages was comparable to that of T4. In addition to these two repair systems tested, a liquid holding effect was observed in the course of incubation of phage-infected cells in the presence of chloramphenicol. Some preliminary experiments indicated that this effect may be independent of UV sensitivity, photoreactivation ability or host cell reactivation of bacteria, although the effect seemed to depend on host cell strains used.

In T5 phage, none of the above three repair systems was detected. However, some technical difficulties in phage adsorption did not allow a definite conclusion.

This study was carried out at Dept. of Biology, Fac. Sci., Kyushu Univ., while one of the authors (E. A.) received a support from Kagaku Gijutsu-cho (Science and Technology Agency).

Wave Length Dependence of Photoreactivation of UV Irradiated Maize Pollen

Etsuo AMANO

The mutagenic effect of ultraviolet light (UV) on the linked endosperm marker genes (*C sh bz wz*) in maize, and its photoreactivation was studied. Within the range tested the mutagenic effect was found only at 250 mμ and no significant effect was noted at 306 mμ or more. Photoreactivation could be observed from the decrease of mutant frequency after post-irradiation with light of longer wave length. The same methods as in a previous experiment (Amano, E. 1968. this Annual Report 19: 75) were used in the present work which deals with a wider wave length range and a larger number of maize kernels. Since mutant spectra in terms

of entirely *vs.* fractionally mutated kernels, or stable type *vs.* B. F. B. mosaics, did not differ from single locus mutation *vs.* multiple loci, the results are represented in Table 1 according to their classification. Kernels which were affected by mutation in more than 1/8 of surface area were scored as mutant kernels. Multiple loci mutations which include B. F. B. mosaics may be brought about by chromosome breakage taking place on some point of the mutation process.

To produce damages by UV, four germicidal lamps set in a plane were used as UV light source, and irradiations were made at an intensity of 100 erg/mm²/sec for 60 sec. As a standard PR condition, postirradiation by visible light from a high presure mercury fluorescent lamp at 6,000 lux for 30 min was used and the treatment reduced the mutation frequencies very effectively. Immediate pollination after UV irradiation in an open field on a sunny afternoon without aluminium foil light shielding also reduced the frequencies. Postirradiation by monochromatic lights, with a constant dose of 3×10^5 erg/mm², revealed that the most effective wave length was around 400 mμ producing a decrease in both mutation categories, single locus or multiple loci. Severe damage at 290 mμ and

Table 1. Photoreactivation of UV irradiated maize pollen. Kernels with 1/8 surface area or more affected by mutant characters were scored as mutated. Multiple loci mutants include B.F.B. mosaics.

UV irradiation (erg/mm²)	PR condition*	No. of observed kernels	No. of kernels per pollinated ear	Frequency of mutant kernels (%)		
				Single locus	Multiple loci	Total
0	dark	14,548	175.28	0.00	0.2062	0.2062
0	6,000 lux	5,202	80.03	0.00	0.0577	0.0577
6,000	dark	3,393	54.73	0.4715	4.2735	4.7450
〃	6,000 lux	1,467	20.96	0.0681	0.0681	0.1363
〃	field PR	3,300	44.59	0.0909	1.6060	1.6969
6,000	290 mμ	0	0.0	—	—	—
〃	313 mμ	1,522	19.27	0.9855	6.0446	7.0302
〃	334 mμ	1,995	28.10	0.6516	3.3082	3.9598
〃	365 mμ	4,614	57.68	0.3467	2.7308	3.0775
〃	405 mμ	2,893	36.62	0.3801	2.8689	3.2492
〃	436 mμ	3,625	51.06	0.2757	3.0344	3.3103
〃	546 mμ	2,227	29.69	0.9878	4.6699	5.6578

* For details see text.

very high mutation frequencies at 313 mμ might be due to the results of very high dose used at these wave lengths or to the effects of contaminating light of shorter wave length. 540 mμ light (yellow green) was ineffective in reducing the UV damages. Photoreactivation was observed in both single locus and multiple loci mutations, and this may suggest that UV damages may be reparable in the early state regardless of their consequences, point mutation or chromosome breakage.

Preliminary Intragenic Mapping of Induced *wx* Mutants in Maize

Etsuo AMANO

The structural relationship among *wx* mutant genes in maize can be analyzed by scoring *Wx* recombinant pollen produced in F_1. Normal *Wx*

Table 1. Recombination frequencies between standard *wx*s and *wx* mutants of maize induced by ethyl methanesulfonate

wx mutant (64A-)	leaky mutant	*Wx* pollen frequency (%)	
		Cross with wx^{H21}	Cross with *wx*-639
94	−	0.0376	0.025
92	−	0.0420	0.005
84	+	0.0067	0.00
82	−	0.0099	0.03
89	+	0.0018	0.035
5	+	0.0019	0.04
76	−	0.0195	0.04
93	−	0.0127	0.075
3	+	0.0182	0.14
90	+	0.0223	0.06
74	−	0.0216	0.055
67	+	0.0240	0.065
4	+	0.0322	0.06
79	−	0.0336	0.075
wx-639	−	0.0258	<0.0003
wx^{H21}	−	<0.0006	0.0311
wx^{90}	−	0.0152	0.0500

pollen grains observed in F_1 might be mostly the results of intragenic recombination and their frequencies might depend on the distance between the mutant sites tested (Nelson, O. E. 1962. Genetics 47: 737–742).

14 *wx* mutants independently induced by EMS treatments were studied. All F_1 endosperms expressed waxy phenotype, and the cross with *wx*-639 showed that they are all in the same cistron. In all tests, more than 1.8×10^5 pollen grains were surveyed in each cross, and *Wx* pollen frequencies in homozygous standard stocks were much lower than those usually found in F_1. In the present tests, only wx^{H21} was used as standard *wx* and the results are shown in Table 1. Previous results with *wx*-639 are also included in the table. Since the tests with wx^{H21} and *wx*-639 were made in different years, the results may not represent precise three point tests, but may by comparison give an idea on the locations of the mutated sites. Of the 14 EMS induced *wx* genes, two might be located outside of *wx*-639, two between *wx*-639 and wx^{H21} and ten outside of wx^{H21}.

Artificial induction of *wx* mutation by radiation treatments have been tried but only a few established *wx* mutants were obtained. Very low survival frequencies of the three fast neutron induced *wx* mutants and other evidences of deletion type mutations by radiation suggest that this difficulty may mostly be due to structural damages by radiation.

Fractionation and Dose Rate Effects of Gamma-rays on Mutation Frequency in Maize

Taro FUJI

Mutation frequency at 1,400 rad exposure to gamma-rays by two fractions with 2 hr interval is lower than that delivered by single dose, and it is imaginable that some of premutational damage induced by gamma-rays could be repaired during this interval (Fujii, T. 1968. Ann. Rep. Natl. Inst. Genet. 18: 78–79). Under this assumption, the relation between mutation frequency and dosage by fractionation was examined and the dose rate effect was also investigated.

Pollen grains with *Bz*-gene were irradiated by gamma-rays in a single treatment or in two fractions with the intensity of 40 kR/h, and low dose rate with 2 kR/h. Irradiated pollen grains, when used on the recessive stock, allow to observe mutation from *Bz* to *bz* in F_1 seeds with bronzy aleuron color. Two types of mutations, whole and partial, could be recognized by this method. Table 1 shows that total mutation frequency (sum of whole and partial) increased with the increasing dosage from

500 to 2,000 R, both in single and fractionation series, but it was lower in fractional than in single treatments. When the mutation frequency between the single and fractionation series was compared, the frequency of partial type mutation did not show any significant difference between the two series, while the frequency of whole type mutation at total dosage of 1,000 and 2,000 R lots was significantly lower in fractionation lots than in single lots. Similarly, lower mutation frequencies in low dose rate lots in comparison with those of acute lots also depended on the frequency of whole type only, those of partial type showing a non-significant difference between the acute and the chronic series.

These results suggest that the repair of gamma-ray induced premutational event under fractionated or chronic irradiation occurred merely for whole mutation. Although similar kinds of mutation could also be induced by ultraviolet treatment, the trend of recovery was incompatible with that of gamma-rays; almost equal extent of recovery was observed in those two types when photoreactivation treatment was applied after

Table 1. Relation between mutation frequency and dosage or dose rate

Treatment (R)	Number of seeds examined	Mutation frequency (%)		
		Total	Whole	Partial
Single (acute) irradiation (40 kR/h)				
500	6,994	0.66	0.35	0.31
1,000	9,611	2.55	1.42	1.13
2,000	7,391	6.21	3.72	2.49
Fractionated (acute) irradiation (40 kR/h, 2 hr interval)				
250+ 250	4,459	0.70	0.27	0.43
500+ 500	5,914	1.82	0.81**	1.01
1,000+1,000	7,934	5.47	2.79**	2.68
Chronic irradiation (2 kR/h)				
1,000	7,489	1.92	1.03*	0.89
2,000	7,611	3.89	1.72**	2.17
Control	8,890	0.04	0.01	0.03

*, ** Significant at 5 and 1% levels, respectively.

UV-exposure. Photoreactivation occurred in neither of these two types of gamma-ray induced mutations (Fujii, T. 1969. Rad. Bot. 9: 115–123). Namely, the kind of premutational event and the fixation of the event must differ according to various mutagens, since reparability is thus

different while gamma-ray and UV-induced mutation is phenotypically the same.

A Green Revertant Obtained from a *Chrorina* Mutant in Diploid Wheat by Gamma-Irradiation

Taro FUJII

A recessive *chlorina* mutant induced by X-rays in *Triticum monococcum flavescens* has been maintained for a number of years in homozygous condition and has never shown any kind of spontaneous mutation. Its pigmentation was uniform and it remained yellowish green from seedling stage to maturity (Fujii, T. 1955. Proc. Japan Acad. 31: 88–92). Dry dormant seeds of this *chlorina* mutant were irradiated by 5 and 10 kR of gamma-rays derived from ^{137}Cs source. Germination and survival rates, as well as seed sterility in the irradiated lots showed no difference from the non-irradiated control lot. All plants of the control and 10 kR lots had the same *chlorina* coloration. However, one plant out of 185 survivals in the 5 kR lot gave revertant tillers, namely among 72 tillers 61 showed normal green color. Ten *chlorina* tillers and ten green were selfed. Average seed fertility was 75% for the *chlorina* tillers and 92% for the green ones, their fertility being similar to that of the original *chlorina* and green strains.

The seeds were sown and the offspring in the next generation were examined. All seedlings derived from 10 selfed and 1 open-pollinated *chlorina* tiller had the original *chlorina* coloration, and all seedlings (234 seedlings from 10 selfed and 288 seedlings from 7 open-pollinated tillers) derived from the green tiller had normal green coloration. As it is known that the cells for tiller formation are differentiated already in the embryo of the dormant seed, a mutational event which takes place in response to a mutagen among the innumerable cells of a ripe embryo is usually of chimeric kind. Some of these cells have further developed to initials of other tillers. It was at first assumed that the normal green tillers which arose due to irradiation of the *chlorina* plant are back mutations of one of the *chlorina* alleles of the homozygote. If so, segregation of *chlorina* seedlings (at least about 130 on the basis of 3 : 1) was expected in the seedling population derived from normal green tillers. This unexpected result remained of a somewhat similar investigation by Tullen *et al.* (1968. Genetics 59: 45–55) who assume a mutation of a suppressor in which the phenotypic effects of a given mutation are modified toward

the wild type by the presence of a second, non-allelic mutation. But there is an essential difference between the two cases. In their case the *chlorina* shows a tendency to greening and the newly arisen green plants have a somewhat weaker pigmentation than the normal greens. Therefore, further experiments are necessary before deciding how to interpret my findings.

Relative Biological Effectiveness of Monoenergetic Fast Neutrons on Oilseeds

S. S. RAJAN[1)]

It is well known that seeds which are rich in vegetable oils are relatively highly resistant to damage by ionizing radiations when compared to seeds not so rich, as for instance, the cereals. In an attempt to throw more light on this radio resistance property of oilseeds, the EBR of monoenergetic fast neutrons (14.1 MeV from a Toshiba NT 200-2S neutron generator employing D-T reaction) were estimated with reference to gamma-rays (^{137}Cs-6 kCi, at the rate of 70 kR/h), with germination, cotyledonaly abnormalities and seedling height as the biological end points. The seed material chosen for the study were, one variety of *Brassica campestris*, and two of *Sesamum indicum*. These were chosen since their chromosome numbers ($2n$=20 and 26 respectively) as also their nuclear volumes are comparable while their oil contents were different, viz., ca. 40% in *Brassica* and ca. 50% in the *Sesamum* material. Further, the oil in the *Brassica* seeds contain a high percentage of erucic acid, an unsaturated C_{22} compound and a relatively higher content of unsaturated fatty acids viz., 94% as compared to 85% in *Sesamum*. The neutron doses were first measured by the sulphur activation method but later estimated as the first collision dose using the chemical analyses (courtesy Kyushu University) of the elements H, C, N and O, in the seed. Treated seeds along with the controls were raised in the pots in the glass house and observations were recorded on germination, cotyledonary abnormalities and seedling height on the 10th, 20th and 30th day after sowing.

The LD_{80} estimates for neutrons and gamma-rays and the RBE value for the neutrons are given in the Table 1 for germination as well as for seedling heights at three stages of growth. The LD_{80} values for both the

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radiations were higher in the case of *Brassica* seeds than for *Sesamum*. This was so for all the four parameters studied. This is contrary to expectations based on their relative contents of oil. The RBE values in the case of the *Brassica* were in the neighbourhood of 10, while they were less for *Sesamum*. Thus, while the neutrons were found to be ten times as effective as gamma rays, for the *Brassica*, they were only 5 to 8 times as effective for *Sesamum*. This indicates the *Sesamum* to be more radio resistant of the two with reference to neutron irradiation. Another point of interest was that while in the case of *Brassica*, the RBE values were almost the same for germination as well as for seedling heights at all the stages studied, (10.00 to 10.40), those for *Sesamum* showed a wider variation (4.57 to 9.21). In the latter the RBE for seedling height was almost half of that for germination particularly in the variety 45 BON. This points to the necessity of specifying the stage and nature of measurement in the estimation of RBE values.

It is fairly well established that organic peroxides play an important role in the radiation induced biological damage. In seeds with a high degree of unsaturated fatty acids the oxidation products are mainly from

Table 1. The LD_{80} estimates for fast neutrons and gamma-rays and the RBE values for fast neutrons for *Brassica* and *Sesamum* seeds

Biological end point	Radiation	Dose in krads	LD_{80}			RBE		
			Brassica campestris	*Sesamum indicum* 45 BON	*Sesamum indicum* 219-3QA	*Brassica campestris*	*Sesamum indicum* 45 BON	*Sesamum indicum* 219-3QA
Germination	Fast neutrons	10^3	12.6	7.7	9.5	10.33	8.90	9.21
	Gamma-rays	10^4	13.1	6.7	8.8			
Seedling height								
10 days	Fast neutrons	10^3	11.2	8.4	9.3	10.00	5.41	6.89
	Gamma-rays	10^4	11.2	4.6	6.5			
20 days	Fast neutrons	10^3	11.7	9.3	7.5	10.17	4.57	9.06
	Gamma rays	10^4	11.9	4.2	6.8			
30 days	Fast neutrons	10^3	12.5	6.8	8.2	10.40	5.36	8.18
	Gamma-rays	10^4	13.0	3.6	6.7			

the unsaturated fatty acids. The extent of unsaturated fatty acids in *Brassica campestris* is 94% of the total oil content as compared to 85% in *Sesamum indicum*. But in terms of unsaturated carbon atoms, 5.5% and 7.1% of carbon atoms respectively are unsaturated. If the extent of unsaturation of the carbon atoms determine the sensitivity to radiations, the *Sesamum* material should be more sensitive to radiations than the *Brassica* seeds. While the LD_{80} values lend support to this, the RBE values do not. In both the seeds the fatty acids are stored in the cotyledons and very little is found in the embryo. If we score the cotyledonary abnormalities like, early senescence, cleft cotyledons and tricotyledons, these were met with only in the *Brassica* material and not in the *Sesamum*. This is again contrary to expectations on the degree of unsaturated carbon atoms these seeds contain. It thus appears that neither the total oil content nor the degree of unsaturation of carbon atoms of the fatty acids is related to radio resistance of oilseeds and one should look for this property in other components of the seed. It is probable that native antioxidants like tocopherols in the seed play an important role in this system. (The author is grateful to the Japan Society for the Promotion of Science for sponsorship under their Visiting Professorship Programme.)

Callus Formation in Wheat Anthers

Taro FUJII

Anthers containing pollen grains at tetrad stage in diploid, tetraploid and hexaploid wheats were used as the materials. They were planted on modified White's medium supplemented with 20 mg/l of 2, 4-D and solidified with 0.9% agar in a test tube. They were incubated under dark condition and constant temperature of 25°C. The pH of medium was adjusted to 5.8. Callus formations started about 4 weeks after planting. Differences in the production of callus among the species were observed and the results are given in Table 1. Among diploid species, about 3% of explants formed callus in the wild *T. aegilopoides*, while the tendency to callus formation was very low in the cultivated *T. monococcum*. A similar tendency was also observed in tetraploid species, namely, wild *T. dicoccoides* showed a relatively high callus production of about 18%, and no callus at all was found in about 600 explants of cultivated *T. durum*. It is interesting that no callus was observed in two cultivated hexaploid wheats, *T. aestivum* and *T. spelta*, so far examined. Callus tissues obtained from the above diploid and tetraploid species could be successfully

Table 1. Ability to form callus in di-, tetra- and hexaploid wheats

Strain		Number of explants	Number of anthers with callus (%)
Diploid wheats			
T. aegilopoides		698	23 (3.3)
T. monococcum		2,410	0
〃	(5030)*	979	0
〃	(5031)*	1,733	2 ?
〃	(5074)*	509	0
〃	(5075)*	1,001	0
Tetraploid wheats			
T. dicoccoides		492	86 (17.5)
T. durum		592	0
Hexaploid wheats			
T. spelta		638	0
T. aestivum		631	0

* Homozygotic strain of X-ray induced recessive mutant.

subcultured with the same or other kinds of synthetic medium and have been maintained over several passages up to present. Although the ability to form callus is lower in diploid than in tetraploid species, its growth was better in the former. The use of haploid or polyhaploid callus cells for mutation experiments is under way. In spite of the very slow growth, modified White's medium supplemented with 2,4-D was the best for wheat anthers, among three types of medium, namely, Erickson's, and Murashige and Skoog's.

Callus induction was also tried in roots of diploid wheats, maize and *Arabidopsis* which are our materials for mutation experiments. Calli were formed easily in these materials, and their growth was more vigorous than of those obtained from anthers. Roots were formed from *Arabidopsis* callus on modified White's medium while wheat and maize did not form roots on the same medium. Requirements for callus formation and differentiation must be different by different materials. (The guidance in culture techniques of Dr. Hiroo Niizeki is gratefully acknowledged.)

IX. MICROBIAL GENETICS

Correlation between the Number of Flagella and the Efficiency of Locomotion in Peritrichously Flagellate Bacteria

Tetsuo IINO

In peritrichously flagellate bacteria such as *Salmonella*, several flagella appear from all over the surface of a cell. When a bacterium moves, they are bundled winding around each other. In order to estimate the correlation between the number of flagella per bacterium and the efficiency of bacterial locomotion, the distribution of moving speed was compared between two bacterial populations differing in flagellar number per bacterium. The experiment was carried out on a wild strain of *S. typhimurium*, TM2, and its leaky *fla* mutant, SJ772, which produces flagella about half the number of flagella per cell generation as compared with the wild strain. The speed was measured by the motility track method of Vaituzis & Doetsch (1969. Appl. Microbiol. 17: 584). The result indicated that the decrease in the number of flagella is paralleled to the decrease in the speed of bacterial locomotion. It was also shown that the fraction of cells showing no translational movement exceeds the cell fraction having less than three flagella in both strain. Of the cells showing no translational movement, one third to one fourth of them wobbled. These results may mean that a flagellar bundle must be composed from more than three flagella for a peritrichously flagellate cell to move and that the increase in the number of flagellar filaments in a bundle enhances the speed of bacterial movement. When the number of flagella per bacterium is less than four, rotation exerted by the flagellar bundle may not be strong enough to drive forward the cell body. It may be worth noting here that in *Pseudomonas* and the related genera, which are characterized by the presence of a single or a small number of polar flagella, can move translationally as efficiently as the movement of peritrichously multiflagellate cells of *Salmonella* in liquid media. However, the movements of the former fall off more steeply with the increase of viscosity of the media than the movements of the latter. (The detail submitted to Recent Progress in Microbiology, 1970)

Genetical Studies of Flagellation and Motility in *Pseudomonas aeruginosa*

Tetsuo INO

Fifteen mutants which fail to spread on semisolid media were isolated from a motile strain, M10, of *Pseudomonas aeruginosa*. They were classified into three groups as regards flagellar characteristics and cellular motility. Group-1 comprised ten mutants, group-2 four mutants and group-3 a single mutant. The mutants in group-1 (*fla*$^-$) are non-flagellate. The mutants in group-2 (*spr*$^-$) produce flagella both morphologically and antigenically indistinguishable from those of the parental strain, and they are motile in liquid media. Differing from chemotactic mutants (*che*$^-$), they show positive chemotaxis to oxygen and glucose. The mutant in group-3 produces straight flagella, consequently, it is non-motile.

Transductions were carried out with bacteriophage F116 between these mutants and *P. aeruginosa* PJ33, which produces flagella antigenically distinct from M10. As the results, mutant sites of *fla*$^-$ and *spr*$^-$ were classified into three and one complementation groups respectively. The mutant site of straight flagella was mapped in *H*, *i.e.* the structural gene of flagellin. It was further shown that *H*, *fla* and *spr* disclosed in this study cluster on a chromosome and they are capable to be transduced simultaneously by F116. By mating experiments between PJ3 (*FP*$^+$ *ade*$^-$ *fla*$^-$) and PJ38 (*FP*$^-$ *trp*$^-$ *his*$^-$), this gene cluster was mapped between *leu* and *ade*.

Extraordinary Polymerization of Salmonella Flagellin *in vitro*

Hirokazu HOTANI[1], Sho ASAKURA[1] and Tetsuo INO

A cellular component which promotes polymerization of Salmonella flagellin under physiological environmental conditions was fractionated from an autolysate of the deflagellated bacterial cells by differential centrifugation. This component is membranous in nature and probably originating from the cell wall and/or cytoplasmic membrane. Filaments produced in this polymerization are straight and are thinner than flagella. They are highly stable against heat (boiling for 5 min), acid (0.1 M HCl) and alkali (0.1 M NaOH) and never depolymerize under the environmental conditions where flagella completely depolymerize. These filaments are named P-filaments. Serological comparisons between P-filaments and flagella

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showed that the two types of filaments have common specific antigenicities (The detail published in Biochim. Biophys. Acta, 1969).

Chlorate-Resistant Mutants of *Salmonella typhimurium*

Masatoshi ENOMOTO

Chlorate-resistant (*chl*) mutants were isolated on nutrient medium supplemented with $KClO_3$ (0.2% w/v) and glucose (0.2% w/v) in the prolonged incubation under anaerobic condition. A frequency of mutations was approximately one per 10^6 cells. The *chl* mutants were classified into three groups (*chlA*, *chlB*, and *chlC*) by transductions with phage P 22 and matings with an Hfr strain, SW 1391. The order of *chl* and other loci used in the experiment was inferred to be *his-trp-chlC-chlB-chlA-bio-gal*. The *chlC* mutants showed no growth on minimal medium, which was restored by the addition of NAD (nicotinamide-adenine dinucleotide) or a commercial vitamin complex, Panvitan (Takeda). The *chl* mutants lost the activity of nitrate reductase, because of the defect of an enzyme system capable of using both chlorate and nitrate as a common substrate. Specific activity of nitrate reductase in the wild-type strain, TM2, was 148 (mμmole NO_2/mg protein/min) in a soluble extract and 1045 in an insoluble one and that of the *chl* mutants was less than 0.7% of TM2. Details will be published elsewhere.

Transductional Analysis of the Gene Controlling Arginine Sensitivity in *Salmonella typhimurium*

Jun-ichi ISHIDSU

It has been reported that the site of an arginine sensitive mutation in *S. typhimurium*, *ars-1*, is linked to *pyrA* locus by P 22 phage mediated transduction at the co-transduction frequency of 30 to 60% (Eisenstark, A. 1967. Nature 213: 1263). Linkage relationship between various *ars* and *aus* (sensitivity to both arginine and uracil) mutants and mutants whose map position scatter around all over the range of the *pyrA* locus was analyzed. *PyrA* mutants were used as recipients and P 22 L4 phage grown on *ars* or *aus* mutants were employed as donors. Washed cell suspensions of 4 to 5×10^9/ml were mixed with phage suspensions in broth (10^{10}/ml) at the ratio of 1 to 1, and 0.1 ml aliquots of each mixture were plated on minimal agar plates. After about 48 hour incubation at 37°C,

transductant colonies were scored. There found two types of colonies with respect to their sizes, namely large and small. After their characteristics were examined by transferring each colony onto minimal and minimal with 10^{-3} M arginine plates using toothpicks, it can be said that small colonies are consist of transductants which have received *ars* or *aus* allele from the donor, while cells forming large colonies have not received such allele. By this indication, co-transduction frequency was calculated for each combination. They are listed in Table 1.

Table 1. Co-transduction frequency (%) in crosses between *ars* or *aus* and *pyrA* mutants

Recipient* / Donor	*pyrA 39*	*pyrA127*	*pyrA102*	*pyrA 78*	*pyrA 81*	*pyrA103*	*pyrA109*
ars-1	75.1	82.9	86.4	89.0	89.8	91.5	98.7
ars-2	77.3	87.4	86.4	91.9	87.7	91.2	94.4
ars-3	73.8	87.2	89.5	93.5	90.6	91.9	95.5
aus-1	34.8	11.8	59.7	58.8	50.0	65.3	79.8
aus-2	41.4	30.8	66.3	75.1	71.1	73.8	88.2
aus-3	47.4	33.8	72.2	83.9	81.1	84.4	89.9
aus-4	43.0	37.1	62.3	69.2	61.9	73.1	83.5
aus-5	49.6	39.1	72.5	84.8	74.2	80.6	93.8

* Arranged in accordance with the results of genetical analysis made by Yan and Demerec (Yan, Y. and M. Demerec. 1965. Genetics 56: 643).

From these results, eight *ars* or *aus* mutants are arranged in the order shown below, taking each *pyrA* mutant as standard.

pyrA 39—ars-2—ars-1—ars-3—aus-5—aus-3—aus-4—aus-2—aus-1
pyrA127—ars-2—ars-3—ars-1—aus-5—aus-4—aus-3—aus-2—aus-1
pyrA102—ars-3—ars-2—ars-1—aus-5—aus-3—aus-2—aus-4—aus-1
pyrA 78—ars-3—ars-2—ars-1—aus-5—aus-3—aus-2—aus-4—aus-1
pyrA 81—ars-3—ars-1—ars-2—aus-3—aus-5—aus-2—aus-4—aus-1
pyrA103—ars-3—ars-1—ars-2—aus-3—aus-5—aus-2—aus-4—aus-1
pyrA109—ars-1—ars-3—ars-2—aus-5—aus-3—aus-2—aus-4—aus-1

Although some discrepancies still remain in detail, it is clear that both *ars* and *aus* are linked to *pyrA* by transduction, that their order is —*pyrA*—*ars*—*aus*— and that they are located on the right hand side of *pyrA*

between *pyrA* and *ara* because *pyrA39* is known to map farmost and *pyrA109* nearmost from *ara* gene (Yan, Y. and M. Demerec. 1965. Genetics 52: 643) and it is evident from Table 1 that both *ars* and *aus* are linked to *pyrA109* much closer than to *pyrA39*.

Some Properties of Azide Resistant Mutants of *Salmonella typhimurium*

Jun-ichi ISHIDSU

Sixty-eight spontaneous sodium azide (NaN_3) resistant mutants (*azi-501*~*568*) were isolated from *S. typhimurium* wild type strain LT2 and some mutants derived from LT2 (*pyrA81*, *ars-1* and *aus-1*) grown on nutrient agar plates containing 4×10^{-3} M NaN_3. Thirty-nine of them formed large colonies and the rest formed minute colonies. Surprisingly, neither of them could grow on minimal medium containing the same concentration of NaN_3, even when requirements were supplemented in case the parent was an auxotroph. By auxanography, it was found that, when NaN_3 is present, they all require methionine or cysteine and in addition minute colony formers grow only anaerobically. In either case methionine was a better supplement. Many sulfur containing compounds were tested for their ability to support growth of the mutants under the presence of NaN_3. NaSH (about 2×10^{-2} M) was found to support the growth of both types quite effectively and Na_2SO_3 the growth of large colony formers. Physiological and genetical analyses of these mutants are being continued.

High Frequency Reversion and Modified Efficiency of Amber Suppression Induced by λ-lysogenization in *Escherichia coli* K12

Tsuneo KADA

High frequency spontaneous reversions in a threonineless strain of *Escherichia coli* K12 have been previously reported (Kada, T. 1970. Mutation Res. 10: 91 and 103). This frequency was increased more than 10^4 fold by introduction of a gene named *mod*$^+$ linked to the *leu* locus and the site of frequent mutations resided at a locus named *fgr* locating between *gal* and *try*. It has been supposed that the presence of *mod*$^+$ permitted to detect frequent genetic changes taking place at *fgr* locus, bringing about suppressor functions on the level of ribosomes.

Frequent reversions also took place at many auxotrophic loci by λ-lysogenization of *E. coli* K12 S-1014 (*F*⁻, *thr*-1014 *leu mod fgr try his arg thi str-r*). Two categories of strains S-1014 (λ) were obtained: category I remained apparently unchanged except for the possession of λ; lysogenic strains of category II had the frequency of reversions promoted $10^3 \sim 10^4$ fold at all auxotrophic loci studied (*thr*, *leu*, *try*, *his* and *arg*) and became sensitive to streptomycin (this Annual Report 18: 118. 1967).

In order to examine if the phage λ might have introduced a gene working similarly to *mod*⁺ into S-1014 having the mutable *fgr* locus, strain S-1014 was lysogenized using phage particles liberated from non-mutable lysogenic strains of category I. No transfer of the hypothetic gene was expected in these experiments. However some of isolated S-1014 (λ) strains were mutable. These observations are against the supposition that λ transduced specifically a gene that makes bacteria mutable.

In other experiments, mutable lysogenic strains were cured of λ by means of superinfection with phages φ170 kindly provided by Dr. Iijima (Osaka). It was found that some bacteria remained mutable even after curing and were sensitive to streptomycin. These results rather indicate that a physical integration event may be responsible for the genetic change responsible for high mutability.

A remarkable genetic modification other than mutability and streptomycin sensitivity accompanied with lysogenization was the change concerning the efficiency of amber suppression. The parental strain S-1014 has an amber suppressor gene (*su II*⁺) and permits the growth of mutants of T4 phages possessing amber nonsence codons. It was observed that the lysogenic mutable strains (category II) was not permissive at all (Table 1). On the other hand, the parental S-1014 and all the lysogenic derivatives were lacking completely a permissive capacity for an ochre

Table 1. Plating efficiencies of mutants of phage T4 possessing nonsense codons on different *F*⁻ strains of *Escherichia coli* K12

Strains	T4 mutants			
	Amber			Ochre 427
	Am 52	B 17	N 69	
S-1014 (λ−, *str-r*)	1.0	1.0	1.0	6.3×10^{-7}
I-A (λ⁺ catogory I, *str-r*)	1.2	0.97	1.4	5.0×10^{-7}
II-B (λ⁺ category II, *str-s*)	3.4×10^{-5}	4.4×10^{-7}	4.2×10^{-6}	7.5×10^{-6}
B/r *try*⁺ 4 (Permissive indicator for ochre 427)	—	—	—	1.0

T4 mutant.

Ribosomal mutations affecting efficiency of amber suppression were recently reported by Strigini and Gorini (J. Mol. Biol. 1970. 47: 517). To interpret our observations we may suppose that the chromosomal integration of λ phage might cause mutations at a mutator or specific suppressor gene residing at the integration site.

X. HUMAN GENETICS

Sexual Variation in Finger Pattern Types and Ridge Counts in Germans

Ei MATSUNAGA, Ei MATSUDA and Heinrich SCHADE[1)]

The dermal ridge patterns on finger tips vary in many respects between males and females. Among various racial samples hitherto investigated, males have generally more whorls and radial loops than females, while the females have more ulnar loops and arches than males. There is also a sexual variation in the pattern size as measured by ridge count. The total ridge count, *i.e.*, the sum of the ridge counts for each of ten fingers of a given individual, is on average larger in males than in females. Relevant data from a Japanese sample consisting of 200 males and 200 females were reported previously (Ann. Rep. Nat. Inst. Genet. 18: 120, 1967). An interesting finding in our study revealed a significant sexual difference in the mean ridge count per digit for a whorl but not for a loop. This was rather unexpected because the larger total ridge count had been thought to imply a larger pattern size in males than in females irrespective of the pattern type, whorl or loop.

In order to see whether the above finding may have a general significance, we examined finger prints of a German sample consisting of 243 males and 220 females, which had been collected by one of us (H. S.).

Table 1 shows the total ridge counts as well as the percentages of four major pattern types according to sex. As compared with the Japanese, the Germans have on average in both sexes smaller total ridge counts, less

Table 1. Total ridge counts and percentages of different pattern types among Germans

Sex	No. examined	Total ridge count		Pattern types (%)			
		Mean	S. D.	Arch	Loop		Whorl
					Radial	Ulnar	
M	243	142.4	51.00	5.2	5.6	57.8	31.4
F	220	130.8	53.32	7.5	3.4	61.7	27.5
Diff. (M−F)		11.6*		−2.3	2.2	−3.9	3.9

* $P<0.02$.

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whorls and more loops and arches. However, the sexual variations in the frequency of pattern types and the total ridge count are in the same direction as those found among Japanese.

Table 2 represents the mean ridge count per digit according to sex

Table 2. Mean ridge count of a whorl and a loop, based on 243 males and 220 females in Germany

Pattern type	Sex	Digits (left and right combined)					All digits
		I	II	III	IV	V	
Whorl	M	21.3	16.9	18.3	19.8	17.7	19.2
	F	20.2	16.9	19.0	18.5	16.8	18.5
	Diff. (M−F)	1.1	0	−0.7	1.3	0.9	0.7*
Ulnar loop	M	15.5	10.1	11.7	14.4	13.4	13.2
	F	13.9	9.8	11.7	14.2	12.1	12.4
	Diff. (M−F)	1.6	0.3	0	0.2	1.3	0.8**
Radial loop	M	—	9.4	—	—	—	9.6
	F	—	9.0	—	—	—	8.4
	Diff. (M−F)		0.4				1.2

* $P<0.02$, ** $P<0.001$.

and pattern types for the Germans. Here, the males have on average larger ridge count than females for all pattern types, although there are more or less variations according to digits which may be due to chance fluctuation. This finding apparently differs from the one obtained from our Japanese sample for which the sexual difference was seen only for a whorl. Further studies are needed to see whether the discrepancy is due to a real ethnic difference or to some sampling error.

Polymorphic Hematological Traits of the Japanese

Tomotaka SHINODA

Polymorphic genetic systems, particularly of red cell enzymes recognized in man have revealed striking differences between the populations, not only in specific gene frequencies but also in the presence or absence of particular genes.

In order to estimate gene frequencies of several red cell enzymes and to make a survey of regional variations, if any, in the distribution of

various phenotypes, an extensive investigation has been carried out using blood samples collected from three different areas, Tokyo, Mishima and Nagoya. Parts of this work were already published in Jap. J. Hum. Genet., 14: 316-323, 1970 and Jap. J. Genet. 45: 147-152, 1970.

Acid phosphatase: Of 405 samples, collected in Tokyo and examined, 18 (4.4%) were of type A, 129 (31.9%) of type AB and 258 (63.7%) of type B. Accordingly, the frequencies of the genes P^a and P^b may be estimated as 0.204 and 0.796, respectively. In Mishima, 685 samples were so far analyzed. Of those 28 (4.1%) were of type A, 231 (33.7%) of type AB and 426 (62.2%) of type B. The frequencies of the genes P^a and P^b may be estimated as 0.209 and 0.791, respectively.

Adenosine deaminase: A total of 1,499 samples, collected in Tokyo, Mishima and Nagoya were analyzed for ADA phenotypes. In our samples ADA 1 type was the most common and occurred in roughly 94% of the populations. ADA 2 type was rare and was found only once in each population. Table 1 summarizes the results.

Table 1. Distribution of ADA phenotypes and estimates of ADA^1 and ADA^2 gene frequencies among unrelated Japanese

Area	No. examined	ADA phenotypes			Gene frequency	
		1	2-1	2	ADA^1	ADA^2
Tokyo	286 (%)	268 (93.7)	18 (6.3)	0	0.969	0.031
Mishima	645 (%)	606 (94.0)	38 (5.9)	1 (0.1)	0.969	0.031
Nagoya	586 (%)	549 (93.7)	36 (6.1)	1 (0.2)	0.968	0.032
Total	1,517 (%)	1,423 (93.8)	92 (6.1)	2 (0.1)	0.969	0.031

As far as the frequencies of the genes ADA^1 and ADA^2 are concerned, no marked differences were found among the populations.

Phosphoglucose isomerase: Analysis of 933 blood samples was made for PGI types. So far, three cases of type 3-1, 2 of type 4-1 and one rare type, tentatively designated as PGI 6-1 J, were found. Further survey for the enzyme system is under progress on blood samples collected from different areas.

Phosphoglucomutase: Nearly 2,900 samples were collected during this year in two different areas. Of those 2,742 samples were so far analyzed. Table 2 summarizes the result. The variation found in zymograms was

Table 2. Distribution of PGM_1 phenotypes among unrelated Japanese

Area	No. examined	PGM_1 phenotypes							
		1	2-1	2	3-1	3-2	7-1	8-1	unidentified
Tokyo	965	585	320	54		1	3	1	1
	(%)	(60.6)	(33.2)	(5.6)		(0.1)	(0.3)	(0.1)	(0.1)
Shizuoka	1,677	1,001	576	89	1		5	1	4
	(%)	(59.7)	(34.4)	(5.3)	(0.06)		(0.3)	(0.06)	(0.24)
Total	2,642	1,586	896	143	1	1	8	2	5
	(%)	(60.0)	(33.9)	(5.4)	(0.04)	(0.04)	(0.3)	(0.08)	(0.2)

concerned in every instance with PGM_1 but not with PGM_2 phenotypes. In addition to three common phenotypes, 19 cases in all of rare variants were observed, some of them were similar to PGM_1 types 3-1, 3-2, 7-1, 7-2 and 8-1. On the basis of the incidence among 2,642 unrelated persons, the frequencies of genes PGM_1^1, PGM_1^2, PGM_1^3, PGM_1^7, and PGM_1^8 have been estimated to be 0.7720, 0.2239, 0.0004, 0.0015 and 0.0004 respectively, with no difference between the two areas.

6-Phosphogluconate dehydrogenase: Three phenotypes designated as types A, AC and C were found throughout the survey. Type A was the most common in our samples and occurred in roughly 87% of the population with no difference among the areas investigated. So far 1,078 samples were analyzed for PGD types. Of those 936 (86.8%) were of type A, 137 (12.7%) of type AC and 5 (0.5%) of type C, respectively. Accordingly, the frequencies of the genes PGD^A and PGD^C were estimated to be 0.933 and 0.067, respectively.

Fumarase: As to variation in fumarase, 600 samples were tested, but all samples except one case were of the same type, designating as FUM 1. The enzyme exhibited two isozyme components, one major with faster mobility and one minor with slower mobility. The only variant consisted of two equally intense isozyme bands, one occupying the same position as the major component of type 1, the other migrating at the same rate as the minor component of type 1. The variant was tentatively designated as FUM 2-1.

Nucleoside Phosphorylase: Red cell nucleoside phosphorylase exists in two isozyme bands, one major with faster mobility and a minor with slower mobility. The enzyme was stable for months, if stored at −22°C. Of 960 samples tested, all but two were of the same type, designated as NP 1. The two variants showed two equally intense isozyme bands, one of which had the same migration rate as the major component of NP 1

and the other was its minor component. The variants are tentatively designated as NP 2-1. Further analysis of larger samples is under progress.

Besides the enzymes mentioned above, several other enzymes such as adenylate kinase, citrate dehydrogenase, glycerokinase, pyruvate kinase and enolase are under investigation.

Structure Analysis of Human Immunoglobulins

Tomotaka SHINODA

Bence-Jones proteins, now generally recognized as a light chain of immunoglobulins carrying either Inv or Oz serological factor, were isolated from urine of patients with multiple myeloma and were purified by several procedures such as $(NH_4)_2SO_4$ precipitation, chromatography on DEAE-cellulose column and gel filtration in Sephadex. By these procedures specimens (referred to as HBJ-1 and HBJ-2) were found to be pure, not only in starch gel electrophoresis but also in amino-terminal analyses by DNP-method. They were of κ-type, but were not tested for the Inv serological factor. Molecular weight determination revealed the figure 22,000–23,500 for either specimen. An aspartic acid residue was found to be the amino-terminal for both proteins. Pepsin digests of intact HBJ-1 and HBJ-2 were prepared in 2% by weight of pepsin using 0.01 N HCl at 37°C for 8 hours. After lyophilization, the digest was separated on a filter paper (60×80 cm) by electrophoresis at pH 6.5 and 2,500 volts for 90 min. The "diagonal" maps, prepared in a similar way for the purification step, were compared for both samples. Of four pairs of cysteic acid containing peptides, three were found to be common to two samples, but the remaining pair was different in mobility in electrophoresis at pH 6.5. The common peptides may have derived from the constant part and the carboxyl-terminal cystine of Bence-Jones protein, whereas the remaining pair derived from the variable part of the protein.

Sequence analysis for the variable part is under progress.

Partial Purification of Genetically Different Phosphoglucomutase of Human Red Cells

Tomotaka SHINODA

In order to investigate the relationship between phenotypic variation and biochemical nature of human red cell enzymes, partial purification

has already been carried out of acid phosphatase in the preceding year (Shinoda, T. 1968. this Annual Report 19:35). In this year, attempts were made to purify genetically different types of phosphoglucomutase (PGM) [α-D-glucose-1, 6-diphosphate : α-D-glucose-phosphate phosphotransferase EC. 2.7.5.1] isolated from normal human red cells. As well as on starch gel electrophoresis heterogeneity was observed on column chromatography 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 hemolyzate, except that faster cathodal minor components disappeared from the purified material. Heat stability of PGM was carried out at various temperatures, and was found to be different according to the phenotype of the enzyme, the order of their stability at 50°C being PGM 2 > PGM 1 ≈ PGM 2-1. A large scale purification was made using bottles of blood purchased from a Blood Center. By combination of several procedures, such as $(NH_4)_2SO_4$ precipitation, DEAE- and CM-cellulose column chromatography and gel filtration with Sephadex, specific activities of 35-140 units/mg for PGM 1 and of 22-135 units/mg for PGM 2-1 were attained. Blood samples of PGM 2 type were not available in large quantity, since the incidence of PGM 2 was less than 8% among Japanese.

When purified material and stroma free hemolyzate were submitted to gel filtration in Sephadex, the enzyme, regardless of its type, was eluted as a single, and nearly symmetrical peak. In contrast to starch gel electrophoresis, no clear-cut separation among the isozymes of PGM was observed on gel filtration, presumably because they are similar in molecular sizes.

Recombination experiment was carried out without success using the intact enzyme and a partially trinitrophenylated material.

A New Variant of α_2-globulin Fraction in Human Serum

Yoshito OGAWA and Norio ODAKI

A new polymorphism of subfraction of α_2-globulin in human serum was found by two-dimensional electrophoretic analysis (Cellulose acetate - Disc) and immunochemical examination.

Three autosomal codominant alleles, designated by symbols $\alpha_2'^F$, $\alpha_2'^M$ and $\alpha_2'^S$ are responsible for the observed five phenotypes ($\alpha_2'^M/\alpha_2'^M$, $\alpha_2'^F/\alpha_2'^M$, $\alpha_2'^F/\alpha_2'^S$, $\alpha_2'^F/\alpha_2'^F$ and $\alpha_2'^M/\alpha_2'^S$) on the basis of the family and population data. Occurrence of phenotypes associated with $\alpha_2'^S$, gene, seems to be

very rare in the Japanese population. No phenotype corresponding to $\alpha_2'^S/\alpha_2'^S$ was therefore found in one thousand and eleven cases.

Details of this work had been published in Physico-Chemical Biology Vol. 14 (1969).

Mutation Rates of Translocation Down's Syndrome

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

It is now known that G trisomy is responsible for the overwhelming majority of the cases with Down's syndrome, while translocation and mosaicism account for a very small number. The purpose of the present study was to estimate the frequency of patients with chromosome translocation within the general population as well as the rate of particular chromosomal mutations leading to translocation Down's syndrome.

Since the last report of 1966, we have examined chromosomes of further 673 cases of Down's syndrome. Whenever a patient was found to have 46 chromosomes which included a presumptive translocation the chromosomes of the parents and other close relatives were also examined. For estimating the frequency of translocation, other data reported in Japan were combined with the present one.

Among the total of 902 patients with Down's syndrome, 41 or 4.5% were of translocation type. Among them 23 or 56.1% were of t(DqGq) and 18 or 43.9% were of t(GqGq) type. These two groups of 23 and 18 cases contained 3 and 4 families respectively, where neither parent was studied. If we apportion sporadic and inherited classes according to the proportions worked out for the families where both parents were studied chromosomally, then the number of sporadic and inherited cases among the t(DqGq) patients was 13.8 (60%) and 9.2 (40%), respectively. The corresponding figures for the t(GqGq) patients were 15.4 (86%) and 2.6 (14%), respectively.

The incidence of Down's syndrome among newborns in Japan has recently been estimated to be about 0.134%. Using this figure, the frequency of translocation Down's syndrome among all live births may be estimated on the assumption that they have the same mortality rate as the primary trisomics, to be 6.03×10^{-5} or 1 in 16,600 live births, as compared

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with the incidence of Down's syndrome of 1 in 750 live births.

From the above estimate and the proportion of sporadic cases, the figure of 2.15×10^{-5} was obtained for the mutation rate of gamete with t(DqGq) or t(GqGq) in unbalanced form, resulting in sporadic cases of translocation Down's syndrome in each generation. However, this represents an underestimate, probably by a factor of 4, if early embryonic deaths due to chromosome mutation are taken into account. Upon the consideration of the proportion of inherited cases, the mutation rate for the production of translocation heterozygotes carrying t(DqGq) or t(GqGq) was estimated to be 0.87×10^{-5}. It was noted that our figures are remarkably close to those reported by European workers. The details of this work were published in the Jap. J. Human Genet. 14: 93-106, 1969.

Clinical Conditions of Patients with Apparently Normal Chromosomes, VI

Hidetsune OISHI and Yasumoto KIKUCHI

Based on chromosome analysis performed on cultured leucocytes, patients with various pathological conditions were found to have apparently normal chromosome assortments of 46 chromosomes. Cases of some interest are listed below.

	Name	*Age*	*Legal sex*	*Clinical conditions*
a) Multiple deformations with mental retardation				
105	H.W.	2 years	M	Arachnodactyly; transverse palmar creases; external rotation of lower extremity; hypertonia
106	T.K.	3 years	M	Hypertelorism; high-arched palate; micrognathia; transverse palmar creases; logopathy
107	D.T.	3 years	M	Strabismus; high-arched palate; epilepsy; logopathy
108	M.I.	6 years	F	Arachnodactyly; congenital cataract; nystagmus
109	M.I.	4 months	F	Congenital heart disease; hypertelorism; high-arched palate; small deformed lobes of the ear; low-set ears; short neck; cubitus valgus; genu varum; pes abductus; alopecia; hemangioma

110	M.F.	4 months	M	Low-set ears; hypertelorism; epicanthus; high-arched palate; short neck; brachycephaly
111	M.Y.	10 months	F	Microcephaly; pseudohydrocephalus; high-arched palate; hemiatrophy; incurved fifth fingers
112	K.H.	3 months	F	Microcephaly; craniostenosis; exophthalmos; high-arched palate; low-set ears; micrognathia; polycystic kidney
113	N.U.	2 months	M	Microcephaly; trigonocephalus; narrow and oblique palpebral fissures; exophthalmos; cleft palate; short neck; umbilical hernia
114	M.I.	3 months	M	Microcephaly; high-arched palate; micrognathia; retentio testis abdominalis
b) Sex anomalies				
115	K.S.	1 year	F	Male pseudohermaphroditism; short neck; shield chest; cubitus valgus; vaginal atresia
116	K.N.	3 years	F	Female pseudohermaphroditism
117	H.G.	29 years	M	True hermaphroditism
118	K.A.	2 years	M	Female pseudohermaphroditism
c) Hereditary diseases				
119	K.K.	6 years	F	Marfan's syndrome
120	I.Y.	16 years	M	Pachydermoperiostosis
121	M.N.	4 years	M	Familial vertebral dysplasia

Qualitative Relationships between X Chromosome and Leucocyte Drumstick

Hidetsune OISHI and Akira TONOMURA[1]

It is generally considered that the so-called drumstick found in polymorphonuclear leucocytes of normal females is an expression of one of the X chromosomes being inactivated. This interpretation may be extended to subjects with structurally abnormal X chromosomes, although the qualitative correlation between the X chromosome and the drumstick is still obscure.

Comparison of the sizes of drumsticks has been made for four normal females and four patients with abnormalities of the X chromosome. Of

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the four patients three showed a sex-chromosome constitution of an X and an isochromosome for the long arm of the X (46,XXqi) and the remaining one had a deletion of the short arm in one of the two X chromosomes (46,XXp–). The mean value of the drumstick/nucleus (D/N) ratio estimated from normal female cells in terms of chromatin mass showed 3.00±0.39%, whereas in cells with 46,XXqi and with 46,XXp– the mean values were 4.22±0.49% and 2.22±0.20%, respectively. These results strongly suggest that the proportional changes involved in the amount of X chromosome material are reflected in the size of the drumsticks, since the mean value of the D/N ratio in cells with 46,XXp– was comparable to half the value in cells with 46,XXqi. Furthermore, if we assume that in normal female cells one of the two X chromosomes is totally heterochromatic and forms a drumstick, a part of the drumstick manifested by the chromatin of the short arm may be expressed as:

drumstick (X) – drumstick (Xp–) or 1/2 drumstick (Xqi) .

The estimation from the above data gives 0.78 or 0.89% for this value. Based on this assumption, the ratio of the short arm to the long arm of the X chromosome can be calculated as 0.35 or 0.42. However, according to a note on length measurements of human chromosomes in Penrose's report (1964), the arm ratio of the X chromosome is represented as 0.58. Since the estimates of the arm ratios in terms of area measurements are closely related to those based on length measurements (Oishi and Tonomura, 1965), the difference may indicate that a part of the X chromosome (about 10.1 or 14.6%) is euchromatic and remains within the nucleus.

Pattern of DNA Synthesis in Human X Chromosomes with Structural Abnormalities

Yasumoto KIKUCHI and Hidetsune OISHI

In normal female cells one of the X chromosomes synthesizes most of its DNA at the end of DNA synthesis period (S period). This late replicating X chromosome is most easily identified by autoradiographic terminal labeling of the chromosome complement. Recently, internal asynchrony of this late replicating X chromosome has been observed. In this study we examined the pattern of DNA synthesis of structurally abnormal X chromosomes as compared to that of a normal X chromosome.

The studies were performed on cultured leucocytes obtained from the following two female patients with Turner's syndrome; one with 46,XXp–,

and the other with 45,X/46,XXqi. DNA synthesis in the chromosomes at the end of the S period was analysed by means of ^{3}H-thymidine autoradiography.

An Xp- chromosome was morphologically similar to the members of group D except that its short arm and the satellite region were missing. Therefore the cells with 46,XXp- had 7 large acrocentrics. After developing the autoradiographic film, 43 labeled metaphase plates were observed. In most cells one of the 7 large acrocentrics was heavily labeled, being presumably an Xp-.

Sixty-five metaphases with 46, XXqi were formed to be labeled. An Xqi resembling in size and arm ratio the autosome no. 3 was the most heavely labeled chromosome of the entire complement during the end of the S period. No such late replicating chromosome was observed in the cells with 45,X.

Internal asynchrony of DNA synthesis was observed frequently in those two abnormal X chromosomes. In some Xqi chromosomes the labeling patterns on their both arms seemed to be asymmetrical. These findings indicate that in the Xp- and Xqi chromosomes DNA synthesis is apparently completed first in the centromeric region, followed by the distal part of the arm, and the proximal part of the arm is the last to complete synthesis.

The results may indicate that in those patients with one normal and one abnormal X, the structurally abnormal X chromosome was always inactivated in preference to the structurally normal X chromosome in the cells. Evidences from the labeling pattern of the Xp- and Xqi chromosomes suggest that both behave in a fashion similar to the long arm of a normal X chromosome.

Details of this study have been published in the Jap. J. Human Genet. 15: 114-123, 1970.

XI. APPLIED GENETICS

Further Study on Competition in Sugi-Tree, *Cryptomeria japonica*, with Special Reference to the Age of the Forest

Kan-Ichi SAKAI and Shigesuke HAYASHI

A part of results of this study has been reported in the last issue of this Annual Report (No. 19, pp. 95-96). Further data are added in the present report. Investigated were two forests, both raised from seedlings. Two hundred disks from one, and one hundred disks from the other forest were collected, and stem growth was measured for yearly ring width in eight directions in order to estimate the mean annual growth. Both forests were about 50 years old, though in one of the two it was impossible to measure growth older than the age of 25 years, because the disks were collected from the bottom of stumps remaining on the cleared area and swelling by roots of yearly rings disturbed correct measurements of growth older than 25 years. Correlation of stem growth between paird adjacent trees was calculated. When the correlation was positive and high, effect of intertree competition was understood to be none or slight, whereas low and positive, zero or negative correlation was assumed to show the occurrence of competition.

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 during the first six or seven years correlations were positive and high, and no competition appeared to occur in both forests. The value of correlations, however, decreased thereafter, indicating that competition started among the trees. The intensity of competition was increasing year after year probably up to a certain stage of maturity, so far as neither spontaneous nor artificial thinning was done. When thinning was performed, competition appeared to weaken for several years, though it was again recovered later. It appeared that the start of competition was associated with the start of vigorous growth of trees. Results of this study will be reported in a journal of forestry.

Genetic Variability on Enzymatic Level in Natural Forests of *Thujopsis dolabrata*

Kan-Ichi SAKAI, Yasusada MIYAZAKI and Takashi MATSUURA

Two natural forests of *Thujopsis dolabrata* growing in two promontories in Aomori prefecture were investigated to find out whether they were genetically different. The two promontories are isolated from each other by about 10 kilometer wide straits. Needle leaves were collected from the thickest bough of each tree of both forests for the study of leaf characters on one hand, and electrophoretic analysis on the other. The measurements concerned length and width of main leaf, width of compound leaf, length and vertical length of lateral leaf and leaf thickness. The electrophoretic study was performed by the technique developed by Smithies (1955) and Endo (1968).

In an early stage of our study of electrophoretic peroxidase variability, 53 bands were counted as the maximum number. However, after a statistical treatment of the material, the number was reduced to 42. With those 42 isoperoxidase bands, two comparisons were made between the two forests. The first was a comparison of the average number of bands exhibited per tree. In one of the two forests, called Masukawa, the average number was 16.29 ± 2.17, while in the other called Ohata, it was 15.61 ± 2.76. The *t*-test failed to reveal a statistical significance of the difference between the two forests. The second was a comparison of incidences of various bands in the two forests. It was found by statistical comparison that among 42 bands, there were 15 pairs which were statistically significant. This amounted to 36% of 42 pairs, which was far more than 5% expected by chance only. In other words, the two populations were considered to be significantly different so far as isoperoxidase distribution is concerned.

As to the measurements concerned with leaf characters, it was found that two of them, namely vertical length of lateral leaf and leaf thickness, were significantly different between the two forests. The vertical length of lateral leaf in Masukawa forest was 4.51 ± 0.52 mm while it was 4.12 ± 0.50 mm in Ohata. The leaf thickness was in the former 1.25 ± 0.24 mm and 1.42 ± 0.30 mm in the latter. It was thus concluded that the two natural forests of *Thujopsis dolabrata* which were ecologically not much different but geographically isolated by straits were genetically differentiated from each other.

Family Analysis in a Natural Forest of *Thujopsis dolabrata*: A New Method for Quantitative Genetic Studies

Kan-Ichi SAKAI and Yasusada MIYAZAKI

The aim of our study was to develop a method for genetic investigation of quantitative or physiological traits of forest trees in a natural stand where individuals of different age are growing at various densities. For this study, it was necessary to detect sib-trees constituting families in the forest. The detection of sib-trees was made possible by a comparative study among trees with respect to their isoenzyme patterns of peroxidase. The approach started first with accurately mapping on a section paper the numbered individual trees of the forest. Then a few measurements concerned with growth as well as leaf characters of those trees were taken. Leaf samples from each tree were investigated for electrophoretic variation of peroxidase isozymes.

Two natural forests of *Thujopsis dolabrata*, one with 45 and the other with 83 trees, were investigated. The electrophoretic study was conducted by the technique developed by Smithies (1955) and Endo (1968). The number of isozyme bands was 42 in total. Comparison of isozyme bands among different trees within the same forest was made by measuring the number of azygous bands or the "disagreement count". During this comparative study of peroxidase among trees, we noticed that there was every indication that the disagreement counts between trees growing in the neighborhood were often very small in comparison with those between trees growing far apart in the same forest. The lower disagreement count or higher similarity in the isozyme pattern among trees growing in the neighborhood might suggest that genetically related individuals are standing rather in proximity than being scattered.

It has been considered that trees which differed from each other by a low disagreement count might, in some way or other, be sib-trees. In order to examine if this hypothesis is correct, variance of a few vegetative characters within each group of trees classified according to low as well as high disagreement count was investigated. It was then found that the within group variance of those vegetative characters were very small when the disagreement count among trees was so low as 0, 1, 2 or 3. In other words, trees which are comparatively similar in isozyme patterns resemble each other also in vegetative characters, and they may most probably be genetically related members of the same family.

Sometimes, trees having higher disagreement count than 4 might be sibs, but we have conservatively concluded that disagreement count 0

would indicate vegetative propagules while the disagreement counts 1, 2 and 3, seed-propagated sib-trees. We have been thus able to make a genetic study in a natural forest of *Thujopsis dolabrata* on the basis of family analysis as outlined above. Details of this study will be published in a forestry journal.

Genetic Parameters of Organ and Body Weights in Japanese Quail

Takatada KAWAHARA and Katsumi SAITO

Genetic and phenotypic correlations among whole body weight and those of various organs and their heritability were analyzed. The weights recorded were those of total body (X_1), bones (X_2), heart (X_3), lungs (X_4), liver (X_5), gizzard (X_6), intestines (X_7), pancreas (X_8), spleen (X_9), kidneys (X_{10}), ovary or testes (X_{11}), oviduct (X_{12}) and eviscerated body (X_{13}) collected from 25 week old birds. Sinistral and dextral sides of lungs, kidneys and testes were pooled and weighed. Data were collected from 584 progenies (305 females and 279 males) originated from 68 pair matings each of one sire with one dam. The estimates of heritability and the coefficients of genetic and phenotypic correlations among various traits are given in Tables 1 and 2. It is found from the two tables that the heritabilities of body weight and eviscerated body weight of females are low in comparison with the males. Comparison between male and female with regard to genetic and environmental variances of eviscerated body weight showed that the environmental variance was approximately the same between both sexes, whereas the genetic variance was different. The same seemed to hold true for total body weight, though it was less apparent than in eviscerated body weight. This fact may suggest a dosage effect of sex-chromosomes on body weight, particularly on muscle weight.

Table 1. Heritability of and genetic and phenotypic correlations among various traits in males 25 weeks after hatching.

h^2	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{13}	
X_1	0.693	0.186**	0.440**	0.097	0.614**	0.367**	0.315**	0.354**	0.118	0.459**	−0.027	0.992**	
X_2	0.174	0.777	0.123	0.265**	0.023	−0.040	−0.043	0.027	0.001	0.081	0.105	0.154*	
X_3	0.690	0.309	0.553	0.231**	0.272**	0.192**	0.138*	0.210**	0.084	0.354**	0.061	0.412**	
X_4	−0.025	0.832	0.332	0.306	0.044	−0.016	−0.130*	−0.037	0.024	0.190**	0.171**	0.069	
X_5	0.650	−0.229	0.301	−0.184	0.346	0.404**	0.451**	0.411**	0.142*	0.425**	−0.095	0.529**	
X_6	0.399	−0.327	0.249	−0.286	0.264	0.648	0.267**	0.261**	0.151*	0.306**	−0.175**	0.294**	
X_7	0.596	−0.461	0.406	−0.628	0.404	0.260	0.174	0.373**	0.118	0.296**	−0.080	0.244**	
X_8	0.523	−0.210	0.181	−0.220	0.385	0.126	0.690	0.282	0.123	0.327**	0.032	0.312**	
X_9	−0.049	−0.046	0.268	0.270	−0.390	0.087	0.046	−0.163	0.515	0.065	0.022	0.100	
X_{10}	0.778	0.194	0.680	−0.236	0.453	0.283	0.549	0.582	−0.156	0.379	0.086	0.416**	
X_{11}	−0.056	0.129	0.028	0.177	−0.215	−0.243	−0.233	−0.313	0.006	−0.031	0.737	−0.081	
X_{13}	0.998	0.164	0.674	−0.026	0.695	0.399	0.610	0.537	−0.037	0.781	−0.105	0.693	
	Genetic correlation coefficients												h^2

Phenotypic correlation coefficients

*, **: Exceed the 5% and 1% significance levels, respectively.

Table 2. Heritability of and genetic and phenotypic correlations among various traits in females 25 weeks after hatching.

h^2	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	
X_1	0.300	0.383**	0.288**	0.236**	0.473**	0.170**	0.381**	0.337**	0.109	0.261**	0.399**	0.378**	0.934**	
X_2	0.399	0.748	0.227**	0.125*	0.074	0.088	0.163**	0.152**	0.031	0.163**	0.147*	0.081	0.405**	
X_3	0.082	0.601	0.232	0.206**	0.135*	0.024	0.164**	0.222**	0.070	0.201**	0.072	0.196**	0.223**	
X_4	0.089	0.371	0.651	0.184	0.145*	0.113*	0.145*	0.193**	0.048	0.034	0.158**	0.115*	0.178**	
X_5	0.463	0.084	−0.077	0.069	0.469	0.076	0.321**	0.279**	0.140*	0.280**	0.395**	0.303**	0.327**	
X_6	−0.165	0.022	−0.007	−0.084	−0.176	0.400	0.048	0.104	0.149**	0.002	0.051	0.007	0.099	
X_7	0.388	0.536	0.375	0.480	0.458	−0.235	0.175	0.157**	−0.043	0.165**	0.294**	0.230**	0.220**	
X_8	0.583	0.193	0.198	0.448	0.438	−0.079	0.164	0.426	0.050	0.186**	0.229**	0.324**	0.280**	
X_9	0.088	−0.173	0.191	−0.083	0.207	0.347	−0.274	0.023	0.501	0.085	0.102	0.180**	0.075	
X_{10}	0.205	0.292	0.487	−0.099	0.506	−0.463	0.989	0.084	0.183	0.294	0.223**	0.245**	0.232**	
X_{11}	0.606	0.104	0.124	0.381	0.428	0.333	0.046	0.617	0.338	0.300	0.455	0.413**	0.264**	
X_{12}	0.260	0.169	0.362	0.272	0.335	0.069	0.152	0.676	0.613	0.480	0.710	0.542	0.239**	
X_{13}	0.900	0.209	−0.180	0.024	0.234	−0.318	0.234	0.346	0.138	−0.210	0.304	−0.129	0.348	
	Genetic correlation coefficients													h^2

Phenotypic correlation coefficients

*, **: Exceed the 5% and 1% significance levels, respectively.

Variance and Covariance Analysis of Some Traits of Japanese Quail

Takatada KAWAHARA and Ryo KUSAKA

Genetic and phenotypic correlations among various traits and their heritability were investigated in a random bred population of Japanese quail. Measurements on birds were taken at several developmental stages. Traits examined at sexual maturity were body weight (X_9), egg weight (X_{10}) and age (X_{11}). Hen-day egg production rate was recorded up to 120 days after the first egg (X_{12}). Eight traits were measured in 23 week old birds. They were egg weight (X_1), albumen weight (X_2), yolk weight (X_3), shell weight (X_4), shell thickness (X_5), egg length (X_6), egg width (X_7) and body weight (X_8). The birds were killed at the age of 25 weeks in order to take weight of oviducts (X_{13}) and ovaries (X_{14}). Data on eggs were obtained from measuring three eggs per quail, they were collected from 305 female descendants originated from 68 pair matings each of one male with one female. The ovary data were collected from 289 birds, since the remaining 16 females had no egg material in their uteri.

The estimates of heritability and the coefficients of genetic and phenotypic correlation among various traits are given in Table 1. It is found from Table 1 that the genetic correlations between age at sexual maturity on one hand and ovary and oviduct weights on the other were -0.448 and -0.211, respectively. The genetic correlation between egg production rate and weight of those two sexual organs was close to zero. This may indicate that egg laying performance is genetically independent from the size of sexual organs. Sexual maturity and size of sexual organs were, however, found to be genetically associated. In the present study, it was found that the genetic correlation between egg number and egg size in quails was as low as -0.053 or zero. This is of interest in view of the widely accepted opinion that the same correlation in chickens is rather high in negative direction. It should be noticed here that yolk weight and egg production rate were negatively correlated either genetically or phenotypically.

Table 1. Heritability of and genetic and phenotypic correlations among various traits in quails.

h^2	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	
X_1	0.452	0.907**	0.762**	0.627**	0.035	0.702**	0.861**	0.478**	0.292**	0.371**	−0.054	−0.048	0.439**	0.347**	
X_2	0.982	0.632	0.524**	0.530**	−0.009	0.649**	0.831**	0.405**	0.280**	0.397**	−0.081	0.021	0.495**	0.247**	
X_3	0.666	0.390	0.355	0.459**	−0.025	0.603**	0.709**	0.388**	0.233**	0.232**	−0.001	−0.170**	0.209**	0.424**	
X_4	0.712	0.628	0.298	0.586	0.444**	0.493**	0.498**	0.351**	0.200**	0.230**	−0.006	0.010	0.341**	0.271**	
X_5	0.040	0.041	−0.149	0.422	0.484	0.087	0.037	0.050	−0.033	0.026	0.045	0.147*	0.027	−0.036	
X_6	0.705	0.582	0.550	0.582	0.412	0.535	0.427**	0.413**	0.237**	0.288**	−0.021	−0.016	0.326**	0.280**	
X_7	0.985	0.853	0.560	0.538	0.095	0.400	0.538	0.360**	0.226**	0.335**	−0.075	−0.081	0.334**	0.260**	
X_8	0.412	0.392	0.257	0.192	0.057	0.459	0.210	0.370	0.521**	0.170**	−0.053	0.145*	0.372**	0.370**	
X_9	0.373	0.287	0.386	0.196	0.037	0.235	0.172	0.824	0.315	0.419**	0.267**	0.114*	0.235**	0.202**	
X_{10}	0.635	0.580	0.444	0.315	0.109	0.401	0.592	0.226	0.567	0.514	0.310**	−0.095	0.265**	−0.012	
X_{11}	−0.212	−0.186	−0.115	−0.234	−0.106	−0.127	−0.100	0.159	0.125	0.451	0.410	−0.215**	−0.051	−0.120	
X_{12}	−0.053	0.030	−0.239	0.006	0.286	−0.074	0.152	0.248	0.290	0.033	−0.371	0.429	0.009	0.078	
X_{13}	0.909	0.816	0.634	0.545	0.071	0.695	0.619	0.442	0.334	0.217	−0.211	−0.082	0.542	0.345**	
X_{14}	0.366	0.184	0.551	0.425	−0.159	0.289	0.063	0.463	0.316	0.234	0.448	−0.064	0.681	0.425	
	Genetic correlation coefficients														h^2

Phenotypic correlation coefficients

*, **: Exceed the 5% and 1% significance levels, respectively.

Early vs. Sustained Vigor Type in Growth and their Bearing on Yielding Potential in Rice

H.I. OKA and Hiroko MORISHIMA

To examine genetic variation in the growth curve parameters and their contribution to factors determining grain yield, 40 randomly chosen F_7 lines of a cross between two contrasting rice varieties were measured for dry matter weight and for other yielding characters during the growing period. From the dry weight data, growth curves were computed using linear and quadratic (Pearl-Reed) logistic equations, and various values derived from them were compared among lines. The results showed that: 1) The growth curves of the lines varied genetically between the "early-vigor" and "sustained-vigor" types. 2) Total panicle length per unit area, representing the potentiality for carbohydrates to be produced in grains, seemed to depend on the growth rate at floral initiation, and panicle weight per unit length, representing the rate of carbohydrate supply to the panicles, seemed to depend on the growth rate at heading. These two values were negatively correlated. 3) "Early-vigor" types tended to produce more panicles but had a low rate of carbohydrate supply than "sustained vigor" types. Either "potentiality" or "supply" was a limiting factor in grain production, depending upon the pattern of growth being either "early vigor" or "sustained vigor" type. This experiment was carried out in the International Rice Research Institute by Dr. T.T. Chang and Mr. Oscar Tagumpay, and the authors were engaged in data processing. This paper will be published in "Theoretical and Applied Genetics" 40: 50–55, 1970.

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ABSTRACTS OF DIARY FOR 1969

January	30	166th Meeting of Misima Geneticists' Club
February	28	167th Meeting of Misima Geneticists' Club
May	6	168th Meeting of Misima Geneticists' Club
	26	169th Meeting of Misima Geneticists' Club
June	27	170th Meeting of Misima Geneticists' Club
July	14	86th Biological Symposium
	26	87th Biological Symposium
September	19	171st Meeting of Misima Geneticists' Club
October	30	172nd Meeting of Misima Geneticists' Club
November	5	173rd Meeting of Misima Geneticists' Club
	6	88th Biological Symposium
	15	Public Lectures on Genetics (at the National Science Museum, Ministry of Education, Tokyo)
	21	174th Meeting of Misima Geneticists' Club
December	19	175th Meeting of Misima Geneticists' Club

FOREIGN VISITORS IN 1969

April	7–9	LONG, T. Swansea University, Great Britain
June	19	MESHCHEROV, M.
	20	STUKALIN, B. Scientific journalist, USSR
		KOLSTOVOI, B. 〃 〃 〃
		BESLOV, S. 〃 〃 〃
		BRITANS, Y. 〃 〃 〃
		GOLOVACHEV, V. 〃 〃 〃
		KULIKOV, F. 〃 〃 〃
		PEKELIS, V. 〃 〃 〃
		TOLSTIK, A. 〃 〃 〃
		KHANG, K. 〃 〃 〃
July	14	TATUM, E. L. The Rockefeller University, U.S.A.
	26	MATTE, R. University of Montreal, Canada
August	7	VALENZUELA, R. G. WHO, Geneve, Switzerland
October	29	SHALIN, Y. P.
		CHUMARK, A. G.
		MARKARJYANTS, S. B.
	30	DASS, M. Member of the Parliament, Vice-chairman, Central Silk Board, India
		SONI, K. L. Director of Sericulture, Jammu & Kashmir, India
		SHARMA, R. Director of Sericulture, Assam, India
		MAHADEVAPPA, D. Assistant Director of Sericulture, Mysore, India
November	6	TOMACH, L. J. Washington University, U.S.A.
	13	SZABOLCSI, G. Institute of Biochemistry, Hungarian Academy of Sciences, Hungary
	15	SESHADRI, V. S. Indian Agricultural Research Institute, India

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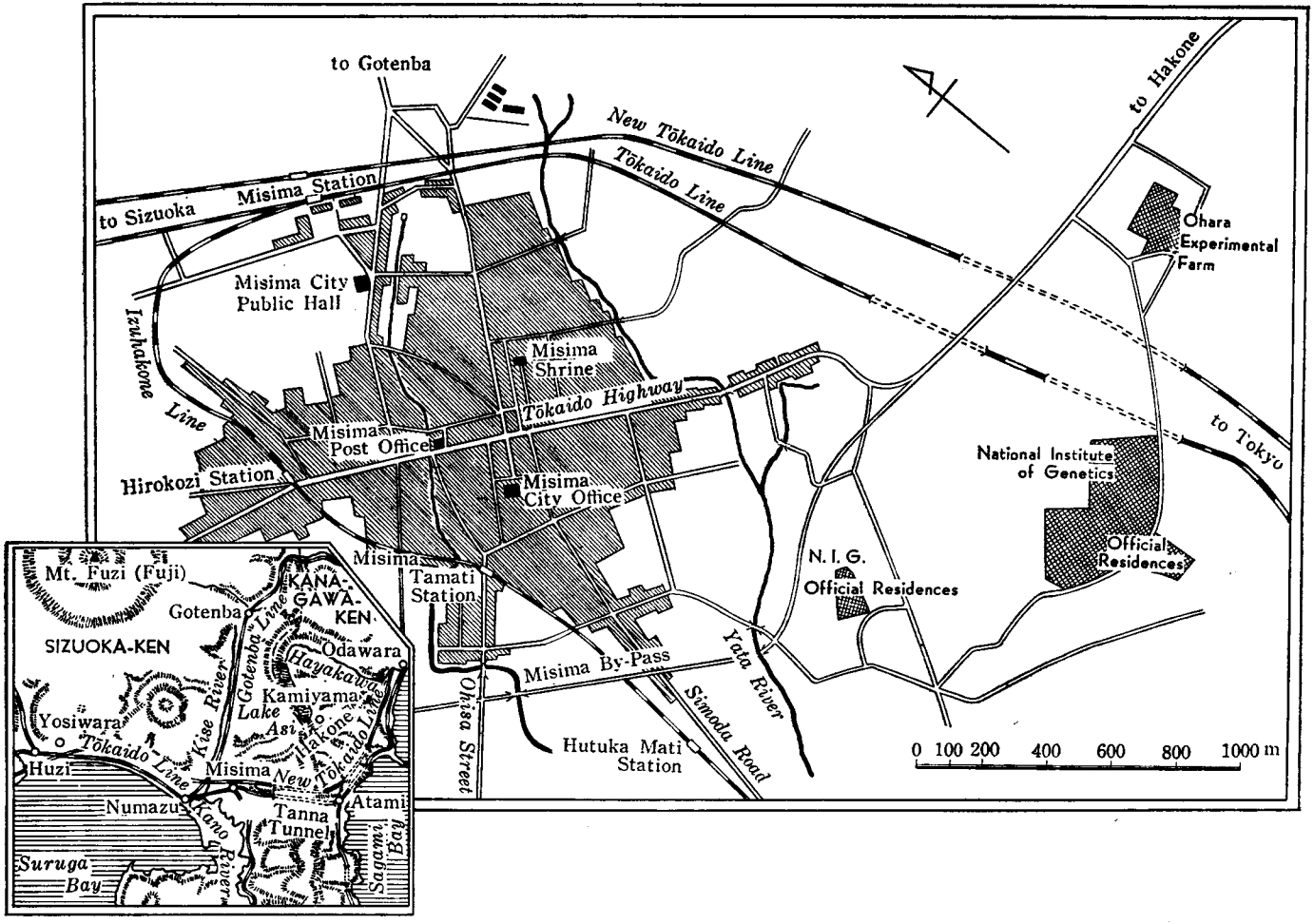
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